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ADAPTATION OF THE SIGNAL TRANSDUCTION SYSTEM OF DIFFERENT SKELETAL MUSCLES DURING EXERCISE*

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Abstract

The purpose of this review is to present a brief overview of the effects of acute and chronic exercise on the ß-adrenergic-G-protein-adenylyl cyclase-cAMP-protein kinase signal transduction system in different skeletal muscles. Special attention is given to such components as ß-adrenergic receptors characteristics, cAMP content, adenylyl cyclase, and cAMP-dependent protein kinase activity in different fibers of the skeletal muscle in relation to duration and intensity of exercise and to the adaptation of the system to chronic exercise.

Key words: G-proteins, cAMP, adenylyl cyclase, cAMP-dependent protein kinase, skeletal muscle, exercise

Introduction

Cell communication occurs through chemical signals and cellular receptors by either the direct contact of molecules on cell to cell surfaces, or the release of a „chemical signal" recognized by another cell (near or far). Prior to 1970, little was known of hormonal mechanisms of action. Since then, numerous advances in the area of signal transduction have occurred. As evidence of this, more than one quarter of all Nobel Prizes for Physiology and Medicine (from 1970 to 1998) have been awarded to scientists whose work revealed some aspect of hormonal action at the molecular level. Before the work of Earl W. Sutherland, scientists knew that the adrenal gland produces a hormone called epinephrine, which travels to the body's cells and causes a breakdown of stored carbohydrates (glycogen) and triggers other energy producing events to cope with different stressful situations. However, it was not apparent how this hormone and other hormones produced such regulatory effects. In particular, Sutherland investigated the effect of epinephrine on hepatic tissue. He discovered that the hormone—the „first" messenger—stimulates formation of a „second messenger" within cells. It is this second substance, cyclic adenosine monophosphate (cAMP) that stimulates the breakdown of glycogen to glucose. Sutherland suggested that the actions of many other hormones could be explained in the same fashion. Sutherland had discovered a new biological principle, a general mechanism for the action of many hormones. Sutherland was awarded the Nobel Prize for Physiology and Medicine in 1971 for isolation of cAMP and demonstrating its involvement in various metabolic processes that occur in animals.

It is of interest to present some of the biographical data of this accomplished scientist: Sutherland graduated from Washburn College (Topeka, Kansas, USA) in 1937. He earned a medical degree in 1942 from Washington University Medical School (St. Louis, MO). In 1953, he became director of the department of medicine at Case Western Reserve University in Cleveland, Ohio, where in 1956 he discovered cyclic AMP. In 1963, he became professor of physiology at Vanderbilt University (Nashville, Tennessee). From 1973 until his death he was a member of the faculty of the University of Miami Medical School. The discovery of G-proteins by American biochemists Rodbell and Gilman were soon to follow. Martin Rodbell thought it was unlikely that so many receptors could interact directly with a single adenylyl cyclase (AC) molecule. He predicted that there must be a "*go-between"* messenger that carries the signal from each receptor to the AC molecule. Rodbell found that each hormone acts through a specialized receptor, but all hormones stimulate the same AC molecule. Rodbell used the word „transducer" for this *go-between* messenger, adopting a term from

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computer science. Rodbell's experiments established that a molecule called guanosine triphosphate (GTP) was necessary for the transmission of a message from a glucagon-bound receptor to AC.

Rodbell was born in 1925. He attended John Hopkins University and later earned his PhD in biochemistry from the University of Washington (Seattle, WA). He then spent the remainder of his career at the National Institutes of Health (NIH) where he worked as a research biochemist (1956-1998). Although Rodbell did not identify and isolate the transducer, he found that it existed and was dependent on GTP, and was an integral feature of all receptors that increase the activity of AC. „It was a period in which my life experiences had kaleidoscoped into a wonderful sense of creativity shared with not only my immediate colleagues but with scientists from all over the world. We are working hard in the lab these days with a sense of excitement that we may shortly have some important answers to the question of how hormones activate adenylyl cyclase systems" Martin Rodbell wrote in his letter to his colleague Mr. Anil Joshi on November 15, 1973. In 1994, the Nobel Prize in Medicine was awarded jointly to Martin Rodbell and Alfred G. Gilman of the University of Texas Southwestern Medical Center for the discovery of G-proteins and their role in signal transduction in cells. Alfred G. Gilman shared the Nobel Prize with Rodbell for finding the transducer, called the „G-protein." Gilman provided a critical piece of the puzzle. He isolated the transducer molecules and defined their structure. Because they were proteins that bind GTP, Gilman called them G-proteins.

Researchers have discovered hundreds of different receptors that act through G-proteins (Rodbell's transducer molecules). It was estimated that more than 65% of all prescription drugs act on such receptors. Defective G-proteins cause a number of diseases. McCune Albright syndrome is an unusual type of disease with varied symptoms. In this disease, a mutation occurs sometime after conception, affecting only some of the body's cells. The number and type of symptoms depend on which cells, and thus which organs, are affected. Scientists have found that the mutation affects the gene that codes for he same G-protein involved in cholera. This G-protein gets trapped in the „on" position. In skin cells, this causes darker than normal pigment. If the mutation affects bone cells, it causes weakness and fractures. In hormone-producing cells, the mutation causes the release of excess hormones. "When we know the whole diagram (of signal transduction in all types of cells), we'll know how every cell is controlled. You'll be able to design a drug that works only on the molecule you want and on no other molecule in the body. It will happen. I just can't tell you when," alleged Alfred G. Gilman.

The effects of exercise on *β*-adrenergic-G-protein-adenylyl cyclase signal transduction system in skeletal muscle

Physical training induces many adaptive changes in skeletal muscles. Muscle adaptation to endurance training involves qualitative changes in intrinsic properties of mitochondria. This results in an improved control of aerobic energy production (1). It is also well known that skeletal muscle of trained animals and humans shows an increase in mitochondrial density and oxidative capacity, content of metabolic enzymes, involved changes in sensitivity to hormones and the composition of contractile elements (2). Training-induced adaptations in oxidative enzyme activity are blunted if the exercise is carried out under *β*-adrenergic blockage. *β*-adrenergic agonist administration can maintain many of the physiological effects of physical training in trained human subjects submitted to bed rest.

Currently, the information on the adaptation of the signal transduction system in different skeletal muscles is limited and controversial. Many complications arise in an attempt to compare different exercise studies, because investigators have used different modes, intensities, frequency and durations of exercise training. The results of studies conducted on the effects of acute exercise on AC and cAMP content in skeletal muscle vary. Acute exercise involving a 20-min run on a treadmill at 20 m/min did not alter AC activity in type IIa [red fast-twitch, red vastus (RV)], type IIb [white fast-twitch, white vastus (WV)], and type I [red slow-twitch, soleus (S)] muscles (3). The same group (4) reported that cAMP content increased in type IIa and IIb fibers of rats that ran for 5-30 min at a similar intensity. Interestingly, cAMP levels in type IIb fibers were independent of both intensity and duration of exercise in this study. Type IIa muscle cAMP levels were higher from 10 to 30 min at the 30-m/min intensity compared with the 15-m/min intensity. Palmer, however, found no detectable changes in rats cAMP concentration of slow-twich red soleus or fast-twich red vastus lateralis muscle following two-hours of swimming, whereas there was a slight increase in cAMP in fast-twich vastus lateralis muscle (5).

To gain further insights into cAMP recovery after a bout of long-term exhaustive exercise, it was determined that immediately following a prolonged, exhaustive treadmill run, the AC activity and cAMP content in skeletal muscle were decreased, however, returned to baseline values by the 48^{th} and 36^{th} h, respectively (6,7,8). Further, it was observed that the administration of isobutyl methyl xanthine inhibits PDE activity in the skeletal muscles, thus prevents endurance exercise-induced reductions in cAMP (9). It was determined that exercise training increases re-

sting cAMP levels, AC activity, and influences the activity of cAMP-phosphodiesterase (PDE) in skeletal muscle (3,10,11,15).

A limited number of laboratories have examined the changes in the density of β-adrenoreceptors (β-AR), AC activity and G-proteins in different skeletal muscle fiber types in response to endurance training (12). Muscle tissue consists of differing fiber types distinguished by their contractile properties, metabolic mechanisms, and structure. Depending on the frequency, duration, and intensity of exercise, different metabolic pathways are utilized for energy metabolism within the muscle fibers. Predominantly, signal transduction system during exercise was investigated in the soleus (the superficial white portion of the vastus lateralis, the deep red portion of the vastus lateralis, and in the gastrocnemius muscle). Armstrong and Laughlin (13) reported that the composition of these muscle fibers were as follows: soleus (87% Type I fibers, 13% Type IIa fibers, slow-twitch, high oxidative, low glycolytic), the deep red portion of the vastus lateralis (9% Type I fibers, 56% Type IIa fibers, 35% Type IIb fibers, fast-twitch, low oxidative, high glycolytic), the superficial white portion of the vastus lateralis (3% Type IIa fibers and 97% Type IIb fibers) and the gastrocnemius muscle (approximately 6% type I and 94% type II fibers). Functionally, red vastus muscle relies on the glycolytic pathway for intense, acute exercise and oxidative metabolism during prolonged exercise, while soleus muscle (red, slow-twitch fibers) uses oxidative metabolism for prolonged exercise. White vastus muscle mainly uses glycolytic mechanisms for intense, short-duration exercise.

Nieto et al. (12) used male Wistar rats to determine the effects of endurance training on β-AR density and affinity, AC activity, and G-proteins (Gs,α) content in the gastrocnemius muscle. A trained group of animals exercised on a motor driven treadmill for 12 weeks using an incremental protocol. Importantly, the animals were decapitated 64 hours after the last training session to dissociate the effects of training from post-exercise events. The results from this study showed that the number of binding sites in the gastrocnemius decreased in the trained rats.

Regulation of AC activity is accomplished by two distinct G-proteins: Gs - mediates AC activation and Gi - mediates AC inhibition. This study showed that the density of Gs-proteins was lower in trained vs sedentary. In addition, the amount of α-subunit of stimulatory G*s,* α-protein in the gastrocnemius decreased in trained vs sedentary. Nieto et al. (12) measured the AC activity in a procedure that shows changes in enzyme specific activity and enzyme levels. β-adrenergic agonist isoproterenol, induced a dose-dependent increase in cAMP. The stimulatory effect of isoproterenol on AC activity was lower in trained vs sedentary. Forskolin produces a direct stimulation of the catalytic subunit of AC, bypassing Gs protein. Catalytic activity in the gastrocnemius of AC was diminished with training when stimulated by forskolin. Therefore, according to Nieto et al. (12), β-AR density, was lower in trained vs controls, G-proteins density, were lower in trained vs controls, stimulatory effect of agonist (isoproterenol) on AC activity was lower in trained as well. Catalytic activity of AC was diminished with training when stimulated by forskolin. Interestingly, Dohm et al (14) reported that exercise training augmented basal AC activity in the gastrocnemius. Increased basal AC activity in Wistar rats' skeletal muscles were reported as a result of 6 and 18 weeks of endurance training on a treadmill by (3, 15). Buckenmeyer et al. (3) determined the effects of endurance training on cAMP, β-AR receptor binding characteristics and AC activity in type I, type IIa, and type IIb skeletal fiber types. After the final training run, all rats rested for 48 hours to control for any acute effects from the last exercise bout, compared to 64 hours in Nieto et al. (12). Binding characteristics and AC activity were examined in type IIa [red fast-twitch, red vastus (RV)], type IIb [white fast-twitch, white vastus (WV)], and type I [red slow-twitch, soleus (S)] muscles. Endurance training consisted of a progressive treadmill protocol that involved increasing intensity and duration of exercise for 18 wk. β-AR density increased in skeletal muscle fiber types, which were primarily recruited during submaximal training (types I and IIa), whereas nonreceptor-mediated AC activity was altered in the three fiber types. Endurance training significantly increased β-AR density in RV and in S whereas in WV β-AR density was not altered. Basal AC activity in RV was increased approximately 2.5 fold by endurance training. Nonreceptor-mediated AC activity, stimulated by NaF and forskolin, increased by approximately twofold in both RV and WV as a result of endurance training. The data showed a greater effect of endurance training in types I and IIa fibers with respect to alterations in β-AR density and alterations in AC activity in each fiber type. Acute exercise did not alter these parameters in the trained rats either. Plourde et al. (16) concluded that training caused a significant increase in β-AR density in the soleus, as well as in the red portion of the vastus lateralis, however, β-AR density did not change in the white portion of the vastus lateralis, which corroborates the results of Buckenmeyer et al (3). Yet there were inconsistencies amongst the different studies examining the rat soleus. Fell et al. (17) and Mazzeo et al. (18) reported no changes in β-adrenergic density.

Plourde et al. (16) revealed a relationship between the NAD-ICDH activity (in the gastrocnemius) and

the density of β-AR in muscle membranes of the untrained and trained rats and showed that there was a correlation between the gastrocnemius NAD-ICDH activity and the density of β-AR in the soleus. There was a correlation between the gastrocnemius NAD-ICDH activity and the density of β-AR in the red portion of the vastus lateralis as well. It has been reported that the oxidative capacity is higher in rat muscle rich in Type I or Type IIa fibers and it is important to note that Plourde et al. (16) showed that the density of the β-AR is closely related to the fiber type and oxidative capacity of the skeletal muscle. Endurance training results in systemic (cardiac output and maximal O_2) and peripheral (increase in the density of the mitochondria, activity of the oxidative mitochondrial enzymes, and blood flow) adaptations. Plourde et al. showed an increase in the activity of enzymes of the citric acid cycle (NAD-ICDH) and that there was a relationship between the activity of this enzyme in the gastrocnemius and the β-AR density in the soleus and in the red portion of the vastus lateralis. Plourde et al. hypothesized that β-AR plays a role in the training induced increase in the oxidative capacity of skeletal muscle. In conclusion, Plourde et al. showed a positive correlation between the activity of NAD-ICDH and the β-AR density in the soleus and in the red portion of the vastus lateralis. This study showed that the density of the β-AR is closely related to the fiber type and oxidative capacity of the skeletal muscle. Taken all together, this data suggests that different fiber types of skeletal muscles responded in a different way to physical training in terms of β-AR density. β-AR density was different in red and white portions of vastus lateralis. β-AR density can be increased through endurance training.

In Plourde et al. report, AC activity was measured in the absence or presence of: isoproterenol, NaF, and forskolin. β-adrenergic agonist isoproterenol stimulates AC and induces increase in cAMP production. Male Wistar rats were exposed to 10 weeks of a running endurance program. Such training programs produce a training effect documented by the increase in activity of the NAD-linked isocitrate dehydrogenase (NAD-ICDH - enzyme of the citric acid cycle) in trained rats. No significant difference between trained and sedentary rats in red vastus lateralis in isoproterenol stimulated AC activity was observed (16). However, Buckenmeyer et al. (3) reported that isoproterenol stimulated AC in the red portion of the vastus lateralis muscle in response to exercise, which was significantly higher in the trained. Buckenmeyer et al. also found a 2.5 fold increase in basal adenylate activity in the red vastus lateralis muscle and surprisingly no significant changes in the soleus muscle. Therefore, there is a striking disagreement between the data from these two different laboratories. Conversely, the basal AC activity and AC activity to isoproterenol stimulation did not change in the trained white portion of the vastus lateralis. However, in the soleus trained vs control, the AC activity to isoproterenol stimulation was increased. Stimulatory effects of agonist isoproterenol on AC activity was different in distinct fiber types in the trained. Discrepancies existed in regards to red vastus lateralis. The effects were different in the same type in red and white portions of the vastus lateralis.

Forskolin-stimulated AC activity was significantly higher in response to exercise training in the red and white portions of the vastus lateralis muscle according to Buckenmeyer et al. (3). These results suggest that AC activity in some muscle fiber types can be increased by non-receptor mediated effects. On the other hand, the response of AC to forskolin stimulation in the soleus fibers was not influenced by physical training (16). By contrast, forskolin-stimulated AC activity in the gastrocnemius was diminished with training (12). Therefore, the response of forskolin-stimulated AC activity to exercise training likely is tissue specific.

The effects of exercise on cAMP-dependent protein kinase in skeletal muscle

The data on the effects of exercise and the activity of cAMP-dependent protein kinase (PKA) in skeletal muscle are scarce (7,11,19,20,21,22). Two different fractions of rat muscle PKA were isolated, partially purified in a buffer concentration gradient at normal state and after prolonged exercise and studied. The differences were observed in some kinetic parameters and in the cAMP stimulated activities between skeletal muscles PKA after acute prolonged exercise to exhaustion (22). During a 6-week training period using rats, the activity of isoenzymes of skeletal muscles PKA increased significantly (20). An acute exercise does not cause any significant changes in the activity of PKA in skeletal muscles of trained rat. The maximal activity of the isoenzymes from skeletal muscles of the control and experimental rats is observed at the same concentrations of cAMP and pH of 6,0-6,5. During training and under acute physical loads, the apparent Km values for ATP of PKA do not change significantly, whereas that of V shows an increase. The apparent Km and V values for the histone increase for isoenzyme I obtained from skeletal muscles of trained rats both at rest and under physical load. In case of isoenzyme II the Km value for the histone decreases, while that of V remains unchanged. The changes in the properties of isoenzymes I and II of PKA from skeletal muscles suggest the participation of the enzyme in adaptation to systematic muscular activity (20).

These studies ultimately led to identification of the role of PKA in the regulation of glycolysis and lysosomal membrane functioning, as well as other aspects of skeletal muscle metabolism. Acute prolonged physical exercise was found to lower the total and bound activity of cathepsin D in the lysosome fraction and to enhance the nonsedimentary activity of the enzyme dissolved fraction of the skeletal muscles lysosome. Exercise training increases the ability of PKA to regulate the yield of cathepsin D from liposome's (7).

As the result of rats engaged in exercise training, the resting phosphorylase *ß*-kinase activity in skeletal muscles were increased. Physical load causes an increase in the phosphorylase *ß*-kinase activity in skeletal muscles of untrained and trained rats. The degree of phosphorylase *ß*-kinase phosphorylation by a homologous PKA from the muscles of trained rats at rest was increased by 1.9 times compared to the control group. The cAMP-dependent phosphorylation of phosphorylase b kinase of untrained and trained rats under physical load was increased 2.5-fold. The data obtained are indicative of the regulatory role of cAMP-dependent phosphorylation in biochemical adaptation of skeletal muscles when their function is increased (21).

It should be noted that many questions concerning the effects of acute exercise and exercise training on the signal transduction systems remain unanswered.

References

1. Fernstrom M, Tonkonogi M, Sahlin K. Effects of acute and chronic endurance exercise on mitochondrial uncoupling in human skeletal muscle. *J Physiol* 2004; 554(3): 755-63.
2. Booth FW, Thomason DB. Molecular and cellular adaptation of muscle in response to exercise: perspectives of various models. *Physiol Rev* 1991; 71: 541-85.
3. Buckenmeyer PJ, Goldfarb AH, Partilla JS. et al. Endurance training, not acute exercise, differentially alters ß-receptors and cyclase in skeletal fiber types. *Am J Physiol* 1990; 258: E71-E7.
4. Goldfarb AH, Bruno JF, Buckenmeyer PJ. Intensity and duration of exercise effects on skeletal muscle cAMP, phosphorylase, and glycogen. *J Appl Physiol* 1989; 66(1): 190-4.
5. Palmer WK. Effect of exercise on cardiac cyclic AMP. *Med Sci Sports Exerc* 1988; 20(6): 525-30.
6. Kotsuruba VN, Kalinski MI, Chalmuradov AG. Changes in adenylate cyclase system of skeletal muscle and myocardium in period of recovery after exercise, *Journal of the Academy of Science of Ukraine* 1983; (10): 79-81.
7. Kalinski MI. Influence of 3':5'-AMP-dependent protein kinase on cathepsin D release from lysosome of skeletal muscle and heart of rats during exercise. *Ukr Biokhim Zh* 1984; 56(4), 408-13.
8. Kalinski MI, Kotsuruba VN, Chalmuradov AG. et al. Effect of immobilization of adrenaline on adenylate cyclase system of cardiac and skeletal muscle in recovery period after exercise. *Ukr Biokhim Zh* 1984; 56(1), 52-7.
9. Kalinski MI, Kotsuruba VN, Chalmuradov AG. Effect of inhibitor on adenylate cyclase system of myocardium and skeletal muscle of rats in recovery period after exercise. *Ukr Biokhim Zh* 1984; 56(2): 153-7.
10. Kalinski MI, Kursky MD, Osipenko AA. Biochemical Mechanisms of Adaptation During Exercise. Higher Education Publ., Ukraine, Kyiv, 1986.
11. Kalinski MI, Antipenko AYe, Dunbar CC, Michielli DW. Exercise and Intracellular Regulation of Cardiac and Skeletal Muscle. Champaign, IL, Human Kinetics Publishers, 1995.
12. Nieto JL, Diaz-Laviada I, Malpartida JM. et al. Adaptations of the beta-adrenoceptor-adenylyl cyclase system in rat skeletal muscle to endurance physical training. *Pflugers Arch* 1997; 434 (6): 809-14.
13. Armstrong RB, Laughlin MH. Influence of training on rat muscle blood flow distribution during exrcise. *Am J Physiol* 1984; 246 (*Heart Circ Physiol* 15): H59-H68.
14. Dohm GL, Pennington SN, Barakat H. Effect of exercise training on adenyl cyclase and phosphodiesterase in skeletal muscle, heart, and liver. *Biochem Med* 1976; 16(2): 138-42.
15. Kalinski MI, Zemtsova II. Metabolism of cyclic AMP in skeletal muscle during acute exercise and training. *Vprosi na Phizicheskata Cultura* (Sophia, Bulgaria) 1981; 4: 241-6.
16. Plourde G, Rousseau-Migneron S, Nadeau A. Effect of endurance training on beta-adrenergic system in three different skeletal muscles. *J Appl Physiol* 1993; 74(4): 1641-6.
17. Fell RD, Lizzo FH, Cervoni P, Crandall DL. Effect of contractile activity on rat skeletal muscle beta-adrenoceptor properties. *Proc Soc Exp Biol Med* 1985;180(3): 527-32.
18. Mazzeo RS, Podolin DA, Henry V. Effects of age and endurance training on beta-adrenergic receptor characteristics in Fischer 344 rats. *Mech Ageing Dev* 1995; 84(2): 157-69.
19. Kalinski MI, Osipenko AA, Kondratyuk TP, Kurski MD. Characterization of protein kinase from myocardium and use for study of content of cAMP in tissue during exercise. *Ukr Biokhim Zh* 1977; 49(3): 99-103.
20. Kalinski MI, Kurski MD, Zemtsova II, Osipenko AA. Changes of some properties of 3': 5'-AMP-dependent protein kinase of skeletal muscle of rats during endurance exercise training. *Biokhimiia* 1981; 46(1): 120-5.
21. Kalinski MI, Kondratyuk TP, Kurski MD. cAMP-dependent phosphorylation of kinase phosphorylase „b" of skeletal muscle during acute exercise and training. *Biokhimiia* 1982; 47(12): 1988-92.
22. Kurski MD, Osipenko AA, Kalinski MI, Kondratyuk TP. Some properties of 3': 5'-AMP-dependent protein kinase of skeletal muscle during exercise to exhaustion. *Biokhimiia* 1978; 43(10): 1776-81.

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