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SPORTS & EXERCISE | RESEARCH ARTICLE

The effect of exercise in some sport branches on urinary second messenger cyclic nucleotide levels

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Abstract: In this study, the possible effects of exercise on hormonal secretions in some sport branches were investigated on the basis of urinary cyclic nucleotide (cAMP and cGMP) excretion, since it was thought that the total, ultimate effects of secreted hormones, under the effect of exercise stress, may be estimated by means of the changes in the second messenger, cAMP, and cGMP, concentrations in urine. A total of 60 subjects from different sport branches were included in the study, and pre- and post-training urinary cyclic nucleotide levels of them were determined by EIA method. The differences in cAMP and cGMP between pre-training and post-training periods were statistically significant ($p < 0.05$ for both), with the post-training levels being higher. The increased post-training cyclic nucleotide concentrations in urine showed that a complex hormonal response occurs as a result of both metabolic state and the exercise stress in sporters. It was concluded that the complicated hormonal status of the sporters may be explained partly by determining the second messenger concentrations. Since, the present study looking at this perspective is a preliminary one in this area, the determination of all second messenger changes for the evaluation of the total hormonal status of sporters is needed in future.

Subjects: Medical Genetics; Nephrology; Physiology

Keywords: exercise; urinary cAMP; urinary cGMP

1. Introduction

There are some situations in which the body needs to call on its fuel stores and with which it may have to cope. Accordingly, physical injury, severe infection, strenuous exercise, and starvation can be considered generally as stress. In the case of strenuous exercise, in which the requirement for energy is suddenly increased, more vigorous changes in metabolic regulation take place (Bergström & Hultman, 1972; Cahill et al., 1966; Coyle, 1995; Spurway, 1992). In stress, the role of the sympathetic nervous system and adrenal medulla may become dominant, resulting in gradual changes in plasma substrate and hormone concentrations. Other hormones respond to exercise and are involved in the regulation of fuel availability. The increases in adrenaline, glucagon, growth hormone, and cortisol concentrations are typical "stress" responses (Houmard, Cox, MacLean, & Barakat, 2000; Kraemer et al., 2001).

ABOUT THE AUTHORS

The main research activities of research group is investigation of routine and some special biochemical analytes (i.e. copeptin, brain natriuretic peptide, ischemic-modified albumin) in exercise-related situations. Clinical laboratory studies and reference ranges are the other research areas of our study group.

PUBLIC INTEREST STATEMENT

Sporting person may have significant hormonal status changes during exercise. When evaluating the hormonal tests of sporting person, the substantial effects of exercise on hormonal status should be kept in mind and all parameters affecting hormonal status should be considered in order to determine the net hormonal status.

The cyclic nucleotides adenosine monophosphate (cAMP) and its guanine analog guanosine monophosphate (cGMP) are two of second messengers that help transmit signals from external sources to intracellular enzymes. The cAMP is produced from ATP by the action of adenylyl cyclase, and cGMP from GTP in a similar reaction by the action of guanylyl cyclase. Many hormones that regulate intracellular metabolism exert their effects on target cells by activating these two enzymes (Houslay & Milligan, 1997; Patel, Du, Pierre, Cartin, & Scholich, 2001; Towle, 2001).

When the cytosolic concentration of cAMP is increased, it exerts its functions through the activation of several effectors. In short, hormones that bind to stimulatory receptors activate adenylyl cyclase and raise intracellular cAMP levels. Hormones that bind to inhibitory receptors inhibit adenylyl cyclase activity via receptor interaction with the transducer. Hormones increasing the intracellular cAMP levels include ACTH, alpha-MSH, calcitonin, FSH, Gastrin, glucagon, GnRH, Histamin, LH, norepinephrine (beta1 adrenergic), PGE1, PTH, secretin, TSH, vasopressin, and VIP (Houslay & Milligan, 1997).

If one wants to see the metabolic perspective of cAMP, it is a central intracellular "second messenger" that influences many cellular functions, such as gluconeogenesis, glycolysis, lipogenesis, muscle contraction, membrane secretion, learning processes, ion transport, differentiation, growth control, and apoptosis (Krauss, 2003), many of which can be affected by the physical activity or stress.

Guanylyl cyclase receptors, on the other hand, are receptor enzymes and convert GTP to the second messenger cGMP. Elevated cGMP activates protein kinase G (PKG), which then phosphorylates target proteins to propagate the response. The second messenger function of cGMP is directed toward several targets (Hofmann, Ammendola, & Schlossmann, 2000; Sakamoto & Goodyear, 2002). Only one receptor class, those for the natriuretic peptides, has been shown to be coupled to the production of intracellular cGMP. The receptors for the natriuretic factors are integral plasma membrane proteins, whose intracellular domains catalyze the formation of cGMP following natriuretic factor binding. Intracellular cGMP activates the PKG, which phosphorylates and modulates enzyme activity, leading to the biological effects of the natriuretic factors. Amylin is a 37 amino acid peptide that is secreted from β-cells of the pancreas simultaneously with insulin in response to nutrient intake. The key second messenger system associated with the amylin receptors appears to be cGMP. Nitric oxide also binds to an intracellular, soluble guanylyl cyclase, producing cGMP.

For a sport-making person to manage the energy stores during training, the hormone epinephrine and glucagon increase the synthesis of cAMP in a number of tissues (Hofmann et al., 2000; Sakamoto & Goodyear, 2002). On the other hand, it has been mentioned that the urinary cAMP is considered to be a reflection of hormonal action in renal tissue that is due to increased production and secretion by the kidneys (Crajoinas et al., 2011).

It is well known that the exercise stress in general results in changes in some metabolites and hormones in body fluids. However, when starting the present study, we preferred a short cut way to partially estimate the effect of exercise stress on cyclic nucleotide signaling pathway, and it was thought that the effect of training for some sport branches on hormonal secretions associated with cyclic nucleotides could be explained on the basis of urinary cyclic nucleotide excretion, since the total, ultimate effects of secreted hormones under the effect of exercise stress may be estimated by means of the changes in the second messenger concentrations. For this purpose, pre- and post-training urinary cyclic nucleotide levels of subjects from different sport branches were determined.

2. Materials and methods

A total of 60 sportsmen were included in the study, who were composed of wrestling (*n*: 20), boxing (*n*: 20), and kick-boxing (*n*: 20) sport, were healthy professional sporting subjects, and had no medication. After pre-training urine samples were taken in a period of time (randomly, neither in fasting nor in postprandial state), they were subjected to training and competition, and immediately after

this physical activity period, the post-training urine samples were obtained. The pre-training samples were taken as control or basic samples for the subjects, and the results of these samples were compared with those of post-training samples.

The training and competition program of the subjects included a training of 50 min and competition of 2 × 3 min for wrestling, a training of 60 min and competition of 3 × 3 min for boxing, and a training of 75 min and competition of 3 × 2 min for kick-boxing. The both pre- and post-training urine samples were collected into proper containers and stored at −80°C up to the analysis day.

The urinary cyclic nucleotide, cAMP, and cGMP, concentrations were determined by EIA method using Cayman's kits (Cayman Chem. Co. 2013; cAMP item no: 581001 and cGMP item no: 581021) and ELISA reader (Power Wave XS; Bio Tek). The experimental steps were conducted by following the kit inserts. In the same samples, the urinary creatinine levels were also determined in order to calculate analyte concentration per g creatinine, since the urinary creatinine is considered to be a good measurand for glomerular filtration rate.

The statistical analyses were performed by statistical software program SPSS for Windows (version 20.0) (SPSS, Chicago, IL, United States). Results were given as mean ± standard deviation (SD). Since variables showed normal distribution by Kolmogorov–Smirnov test, *t*-test (paired *t*-test) was used in the comparison of the means of the numeric values of two dependent groups, and $p < 0.05$ was considered statistically significant.

This study was approved by the ethical board of Ataturk University, informed consent was obtained from each participant and patient anonymity was preserved. The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 1983.

3. Results

Table 1 summarizes the demographic properties of the selected subjects in addition to the results obtained. Since there was no statistical difference in sub-sporting groups with respect to urinary cyclic nucleotide levels, all subjects were taken as one group, and the statistical analyses were made accordingly. In order to make comparisons with the previous reports, cAMP, and cGMP concentrations were given with three different units: ng/ml, nmol/l, and mg/g Cre.

The statistical analyses of the results obtained showed that the difference in cAMP and cGMP between pre-training and post-training periods were statistically significant at the level of $p < 0.05$ for two parameters (95% CI [(−9.03) − (−5.55)] and [(−3.6) − (−2.4)]), respectively). The post-training levels of both cyclic nucleotides were increased when compared with the pre-training ones. In addition, no statistically significant correlation was found between cAMP and cGMP ($p > 0.05$).

Table 1. Demographic properties and the results of the selected subjects

(*n* = 60)	Mean ± SD	(*n* = 60)	Pre-training (Mean ± SD)	Post-training (Mean ± SD)
Age (years)	25.6 ± 6.79	cAMP (ng/ml)	2.41 ± 1.97*	4.85 ± 2.57
Height (cm)	172,3 ± 6.38	cGMP (ng/ml)	0.70 ± 0.44*	1.44 ± 0.8
Weight (kg)	72.78 ± 11.79	cAMP (nmol/l)	7.32 ± 5.98*	14.74 ± 7.81
BMI	24.43 ± 2.78	cGMP (nmol/l)	2.10 ± 1.21*	5.22 ± 2.41
		cAMP (mg/g Cre.)	8.95 ± 3.53*	21.56 ± 1.92
		cGMP (mg/g Cre.)	4.50 ± 3.85*	7.5 ± 4.8

Notes: SD: Standart deviation; BMI: Body mass index; cAMP: cyclic AMP; cGMP: cyclic GMP; Cre: creatinin.

*$p < 0.05$.

4. Discussion

A strenuous endurance exercise represents a different type of extreme situation or stress for the human body. In this situation, energy need extremely increases, and the metabolic map must be modulated suitably, which could not be achieved without coordinated physiological changes (Coyle, 1995).

Despite the complexity of the signaling mechanisms in the body, it is well documented that G protein-coupled receptors (GPCR), a kind of signaling way receptor, may be affected by mechanical stress or stretch in sporting subjects (Wojtaszewski, Nielsen, & Richter, 2002). The majority of the signaling molecules, the ligands, cannot pass through the plasma membrane, and thus they use second messengers for signal transmission. The changes in intracellular second messenger status must not be sustained and must be counterbalanced by antagonistic tools. In the case of cyclic nucleotide second messengers, phosphodiesterase (PDE) activation, phosphatase activation, and removal of excess cyclic nucleotides into extracellular fluid counterbalance the hormonal action. Cyclic nucleotide removal is a reflection of their urinary excretion. It has been reported that PDEs may be important in cGMP/cAMP cross-talk. The interplay between cAMP and cGMP signals through PDEs has been suggested to occur in a number of cell types (Gotz et al., 2014; Lee et al., 2013; Maurice et al., 2003).

While a number of hormones, some of which are stress-associated hormones, increase the intracellular cAMP levels (Zaccolo & Movsesian, 2007), such hormones as atrial natriuretic peptide and nitric oxide use the cGMP as second messenger (Hofmann et al., 2000). As mentioned above, while some external stimuli increase the intracellular cAMP and cGMP levels, the others may stop their syntheses. Some signaling elements, on the other hand, can make cross-talking in transduction, rendering the hormonal effect difficult to understand and to correctly estimate the net, total effect of the respective second messengers (Maurice et al., 2003). Although the effects of a few hormones are clearly known under the exercise and stress conditions, it is very difficult to correctly estimate which hormones are secreted and to evaluate the effect of any individual hormone on the body metabolism. As result, we have postulated that because the total, ultimate effects of secreted (agonistic and antagonistic) hormones under the effect of exercise stress may be estimated in general by means of the changes in second messenger concentrations, the effect of some sport activities on hormonal secretions in general can be explained on the basis of urinary cyclic nucleotide (cAMP and cGMP) excretion. Only in this way can one easily consider the effect of exercise stress situation on the general hormonal secretion and on ultimate second messenger concentrations by taking consideration of cross talks and second messenger cross effects. In fact, we have to handle the subject with two aspects. First, the energy needs during exercise stress conditions in sporting person necessitate some metabolic changes, which is provided by suitable hormonal responses. Secondly, the exercise stress itself during sporting may result in different hormonal responses. Both the events together with each other render the hormonal status of the sporting person confused. On the basis of these considerations, we can easily explain the finding that the differences in cAMP and cGMP between pre-training and post-training periods are significant as determined in this study. While the pre-training levels of both cyclic nucleotides were within the normal resting ranges (Marks & Mesko, 2002), their levels increased when compared with the pre-training values, which shows an overt hormonal response by means of sporting activities. The effect of treadmill exercise on plasma and urinary cyclic AMP levels was studied in 12 healthy subjects by Lin (1978), who reported high but insignificant urinary cAMP levels, which may be caused by less number of subjects.

We can easily conclude from these considerations that the complexed hormonal status of the sporting person may be explained partly by determining the second messenger concentrations. In the present report, only two of these messengers could be considered with this respect. Therefore, our findings related to the increased post-training cyclic nucleotide concentrations in urine showed that a complex hormonal response occurs as a result of both metabolic state and the exercise stress in sporting persons. Since the present study looking at this perspective is a preliminary one in this area, one needs the determination of other second messenger changes in any way for the evaluation of the total hormonal status of sporting person.2013/65

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Competing interests
The authors declare no competing interest.

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