Neurotransmitter and vasoconstrictor peptide response in elite athletes upon workload through a treadmill test

Abstract of the PhD Thesis

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Introduction

From the beginning of the high-performance sports, it is well-known that efficacy of performance can be influenced only by training. Besides athletes and trainers, researchers have been always interested the boundaries of the human performance. The singularity of Sport Science, as an interdisciplinary research field, also consists of the fact that applies the knowledge of Medicine although it does not deal with diseases. Among its different branches, Sport Physiology deals with the recognition of morphological and functional characteristics of the performance, as well as the determination of the training adaption and factors limiting the performance. Nowadays, elite sport activities require a constant preparedness, the maximal use of the individual capabilities and a constant performance. Besides creating a precise physiological picture, there is an increasing demand on complementary tests such as mapping of the genetic background of athletes.

In high-performance sports, it is important to choose the most adequate sport activity for the individuals. Results of the modern biological research have now shown that the former *one hormone – one function* theory is not correct anymore. Nowadays, it is well known that certain cellular functions are influenced simultaneously by several impacts (e.g., humoral, hormonal, neural and environmental ones). Moreover, one hormone exerts diverse influences on different cells. The hormonal effects are chained up independently of their origin. The hormonal network is extended onto the full body and it forms a system found in a constant, fluctuating change. Their effect aim at the maintenance and stabilization of homeostasis. The competitiveness of the laboratories monitoring athletes would be enhanced if trainers could have the most exhaustive picture on the athlete according to the newest progresses in science. In order to achieve the maximal physical performance, the use of treadmill ergometers is recommended. Therefore, it would be of paramount importance to perform complex studies on elite athletes for the interpretation of the physiological and biological responses occurring upon stress.

Objectives

In scientific reports, the stress responses in athletes are contradictory presumably due to the scarcity of complex hormonal tests. Moreover, there are few studies performed with identical protocol on elite athletes of different sports. Therefore, the objective of my PhD work was to determine simultaneously the concentration change of neuroendocrine and vasoconstrictor peptides for the highest number of compounds upon maximal workload for individual and team sport athletes, namely cyclic and ball game athletes by applying the same protocol. Therefore, adrenaline (*A*), cortisol (*C*), dopamine (*D*), noradrenaline (*NA*), big endothelin-1 (*big ET-1*) and angiotensinogen (*AGT*) have been chosen to be monitored in blood samples taken from elite kayakers, triathletes, as well as handball and soccer players upon acute stress by spiroergometric load before and after the workload test. The cardiorespiratory and anthropometric data of the monitored elite athletes were also determined for the data interpretation. We hypothesized that the acute *vita maxima* load by treadmill tests is suitable for the determination of the relationship between the physical performance and physiological processes induced by stress in athletes involved in different sport activities.

Our aim was also to identify which from the aforementioned neurotransmitter and vasoconstrictor peptides would be suitable for the differentiation of the selected sport activities. We also hypothesized that the response of the organism of athletes to the disturbed homeostasis can be characterized with the concentration change of catecholamines – especially with that of *NA* - upon acute stress. According to literature data, the role of *C* in training is decisive. However, due to the contradictory reports on the *C* levels upon stress in athletes, our further aim was to clarify the relationship between cathecolamines and *C*. According to our hypothesis, the changes in the cathecolamine and *C* levels can be in close connection between them. To complete this task our aim was to relate the cathecolamine concentration data to those of *C* ones before and after the workload test between the different investigated groups.

Materials and methods

Selection of volunteers for our study

Forty-four non-smoker, healthy Caucasian elite athlete took part in the present study. We selected two team sports (soccer, *n* = 8 and handball, *n* = 12) and two individual sports (triathlon, *n* = 9 and kayaking, *n* = 9). The first group consisted of ball game players (*n = 20*), the second one of cycling physical activity athletes (*n = 18*). Kayakers and triathletes were classified as cycling sport activity athletes. The elite athletes took part on a voluntary basis in the study. The control group consisted of medical students (*n* = 6) of the Semmelweis University of Budapest. The monitored athletes were in their preparatory phase of the forthcoming competitions.

Anthropometric studies

The anthropometric measurements of the present study were conducted according to the recommendations of the International Biology Program. The calibrated utensils were in accordance to international standards and they were as follows: stadiometer, anthropometeter, digital scale, compass caliper compass, flexible metallic tape and caliper.

For the anthropometric measurements, 24 variables were included as follows: body weight (BW), height, seven skinfolds (biceps, triceps, subscapular, suprailiac, abdominal, thigh and medial calf), five widths (shoulder, elbow, thorax, iliac spine and knee), thorax depth and nine girths (thorax, upper arm relaxed, upper arm flexed, upper arm tensed, lower arm, ankle, hand, thigh and maximum calf).

Based on the data obtained, the nutrition status (body mass index, BMI) expressed as kg m^{-2}, the body constitution and subsequently, the physical constitution could be estimated. For the assessment of the four-component body composition, the recommendations of Drinkwater and Ross (1980) were used, while for the two-component body constitution those of Pařižková (1961) were applied.

Workload protocol

An Ergosana ERG 911 treadmill equipped with a Cardiovit AT-104 ECG recorder, in conjunction with O_2 and carbon-dioxide gas analysis unit, was used. The volunteers performed a maximal workload experiment to determine the maximal oxygen uptake (VO_{2max}) and the maximal performance. The cardiorespiratory observation of the volunteers was achieved by placing an electrocardiogram (ECG) on their chest that was removed after having reached the total rest condition. The measurement of heart rate was achieved with a pulse measuring watch.

The experiment ended when the participants reached the above-defined VO_{2max} or when they reported subjective fatigue. The anaerobic threshold was determined based on a modified Bruce protocol through a maximal exercise type of spiroergometric test performed on the aforementioned treadmill. Briefly, the modified Bruce protocol was of ascending grade type starting at 0% for all volunteers. Athletes ran from an initial speed of 9 km h^{-1} to 12 km h^{-1} applying increments of 1.5% every minute. The control group ran from 6 km h^{-1} to 9 km h^{-1} applying the same increments as for the athletes.

Quantitative determination of lactate

Lactate (*LAC*) concentration was determined in a blood drop taken from the ear lobe of each volunteer by using a blood *LAC* measuring meter supplied by Nova Biomedical. The collected blood drop was placed onto a single use analyzing stripe. The range of quantitative determination was between 0.3 mM and 25 mM. Samples were collected before the test and after the maximal workload had been achieved.

Sampling protocol and quantitative determination for the investigated hormones

From each volunteer, 12 mL of blood was taken at the basal and after the maximal workload levels into Vacuette tubes coated with K3 EDTA. Samples were centrifuged at 3,000 g and 4 ºC for 10 minutes and then, further divided into six aliquots (2 mL each) for the determination of *A*, *NA*, *DA*, *AGT*, *ET* and *C*. In the case of *A*, *NA* and *DA*, 200 µL of aprotinin was added prior to centrifugation.

After centrifugation, the supernatant was collected and frozen in liquid nitrogen. Levels of *A*, *NA*, *DA*, *AGT*, *ET* and *C* from plasma were determined by solid phase enzyme-linked immunosorbent assay.

Statistical analysis

The mean and standard deviation and, in some cases, he standard error were calculated for each sport activity and group. Because of the limited sample size, the Shapiro-Wilkes normality test was performed to investigate normality. As all data were found to be normal, parametric statistical method was used afterwards. Student's (one sample) paired *t*-test for dependent samples was the adequate statistical method to describe differences gathered after and before the exercise test. ANOVA was used for comparing differences in the subgrops. As *post hoc* test, we applied the Tukey *honest* significant difference method for different sample sizes (Statistica 11.0 software, StatSoft, Tulsa, Oklahoma, USA). The significance level was set at $p < 0.05$ or $p < 0.01$ for all variables.

The measured parameters of the present study are summarized in Figure 1.

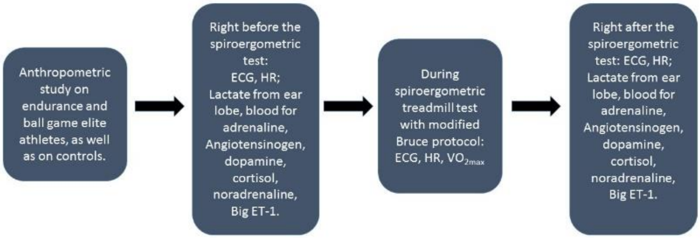

Figure 1. Summary of the parameters determined in our study (own figure). Abbreviations: ECG = electrocardiogram; HR = heart rate; big ET-1 = big endothelin-1; VO_{2max} = maximal oxygen uptake.

Results

During our work, the complex hormonal response of elite athletes of ball games was compared with those of cyclic sport activity ones upon gradually increasing workload experiment performed on a treadmill, since the procedures applied up to now have not allowed comparison of data obtained for athletes of different sport activities.

Results of anthropometric and physiological performance measurements

We did not find any significant difference in the fractionated mass data of all volunteers except for the body fat percentage between handball and soccer volunteers (p = 0.0124) as well as control group and soccer volunteers (p = 0.0326).

There was not either any significant difference in the performance data registered with the *vita maxima* workload protocol data of the volunteers involved in the present study, not even when the registered performance data were related to BW or lean body mass (LBM). At the same time, by summing the performance data obtained by the workload protocol minute-by-minute, the so-called cumulative workload was obtained. By relating these latter data to LBM, the cumulative workload of handball volunteers (p = 0.0249) and that of triathletes (p = 0.000194) were significantly higher than that of the control group. The cumulative workload of triathletes was significantly higher than that of soccer players (p = 0.00572) and kayakers (p = 0.0183).

The VO_{2max} values were significantly higher for handball volunteers (p = 0.00874) and triathletes (p =0.0496) compared to the control group. The relative aerobic capacity values expressed as VO_{2max} BW^{-1} were significantly higher for triathletes compared to control (p = 0.00249) and handball volunteers (p = 0.00628), whereas the mean VO_{2max} and cumulative workload of triathletes were also significantly higher compared to the control group and soccer and kayak volunteers.

_Intra_group hormonal change

By looking at the *intra*group hormonal variability, significant differences between the mean values of *A* for the control (p = 0.0261), ball game (p = 0.0015 and p = 0.0148 for handball and soccer athletes, respectively) were observed.

For *NA* mean values before and after executing the exercise protocol, there was a significant difference for each group (p = 0.0074, p = 0.0001, p = 0.0001 and p = 0.0062 for control, handball, soccer and kayaking, respectively) except for triathletes, where the *NA* concentration determined after the test was not significantly different from the one obtained before executing the exercise protocol (p = 0.0523).

For *big ET-1*, significant differences were observed between the maximal and basal values for control (p = 0.0291) and ball game volunteers (p = 0.0277 and p = 0.0001 for handball and soccer volunteers, respectively).

Generally, there were few significant changes in the case of *DA*, *C* and *AGT* levels in the case of comparing before and after the test values. By comparing mean concentration data determined before and after executing the test, small significant difference could be established for *DA*, *C* and *AGT*. Thus, the *DA* concentration data for soccer volunteers (p = 0.0414) and triathletes (p = 0.0075) were slightly significantly higher after executing the test. For *C*, the corresponding values of the handball (p =0.0308) and triathletes (p = 0.0044) changed significantly. In the case of *AGT*, the mean concentration data increased significantly after the execution of the test (p = 0.0040).

For each group of volunteers, the *NA* and *A* concentration ratios after and before the test were calculated. These ratios were always higher than one. The ratios of *LAC* concentration levels after reaching the maximal workload and at basal level were the highest for controls and the lowest for triathletes. The concentration ratios of *A* and *LAC* levels determined after reaching the maximal workload were similar for the control group, kayaking and triathlon volunteers but were considerably higher for ball game athletes by a factor of 4.7 and 2.5 for soccer and handball athletes, respectively.

Noradrenaline and cortisol concentration ratios for the investigated groups before and after the execution of the workload protocol

The cathecolamine levels obtained before and after executing the workload protocol were divided by the corresponding *C* ones for each investigated group. However, significant changes ratios were observed only for the *NA*/*C* ratios. Among the *intra*group *NA*/*C* before/after ratios, only those for control, handball and soccer were significantly higher.

By comparing the *NA*/*C* ratios calculated for the investigated groups, those for the kayakers and triathletes differed significantly from the control, handball and soccer especially after the workload had been applied.

Among all calculated ratios, significant differences ($p < 0.05$) were observed in more than 60% of the cases. Stronger statistically supported differences ($p < 0.01$) were observed in one third of the cases.

The highest *NA*/C ratios were observed for soccer players. This phenomenon can be related to their training characterized by short, intensive and fast loads. The higher *NA*/*C* values obtained for the control group can be the consequence of stress, because these volunteers could not be accustomed to acute workload.

Changes in the hormone levels upon physiological maximum performance

For *A*, significant increases of a factor of 2.9 and 3.9 in the case of soccer and cyclic sport volunteers upon physical exercise were observed by comparing the normalized means for soccer/kayaking and soccer/triathlon. For *NA*, we could establish an even stronger significant difference roughly by a factor of 3 between by comparing ball game volunteer mean data with those of cyclic sport ones.

The concentration change in the plasma serum for *A* and *NA* related to physical exercise was higher than for *DA*. By using these parameters, differentiation between cyclic sport activities and ball games, as well as between cyclic sport activities and controls could be made.

Our study proved that a systematic comparison of stress-related hormones (*A, NA, C*), neurotransmitter (*DA*) and vasoconstrictor peptides (*AGT* and *big ET-1*) levels are needed for cyclic sport activities and ball game individual and team players upon performing exercise tests applying Bruce protocol. Normalization of before and after test concentration levels to VO_{2max} data reflected better the changes in *A*, *NA*, *DA* and *C* upon physical exercise. During our investigation, ball games could be clearly distinguished from cyclic sport activities upon changes occurring in the *NA* concentration levels. The results obtained for *NA* showed that reaction of ball game players upon acute stress induced by workload exercise was the highest, then, the control group. In the case of soccer players, this phenomenon can be related to the sudden cardiac death. The reaction of cyclic sport activity athletes was the lowest. This trend reveals the topic of the sport adaption of different sport activities. Higher *NA* secretion capacity is more advantageous for training adaption in the case of ball game players. To the contrary, the opposite can be stated for cyclic sport activity athletes.

Normalization of the individual maximal workload related hormonal, neurotransmitter and vasoconstrictor peptide concentration differences before/after execution of the test to the corresponding basal levels (in %) was also done for each volunteer. For cathecolamines, higher changes could be observed for ball game volunteers compared to the athletes performing cyclic sport activities. For all investigated sport activities, these values were higher for at least three compounds than for control ones in the case of several athletes. Moreover, values higher than the mean for vasoconstrictor and neurotransmitter responses were observed for about 35% of the athletes. About two thirds of the group of handball athletes characterized by the highest age were affected by this aforementioned phenomenon. About one third of each kayakers and triathletes had higher than the average vasoconstrictor and neurotransmitter concentration difference response for at least three of the investigated compounds. For these latter sport activities the mean of the age of the athletes did not exceed 21 years. Although the number of volunteers for each sport activity of the present study cannot be considered as representative, our results suggest that the responses of the investigated neurotransmitter and vasoconstrictor peptides upon stress will be higher with ageing. Unequivocal trend as a function of the position hold by the athlete in the team could not be defined.

Taking into account the limiting factors of our studies it can be stated that further studies are needed concerning the investigation of the changes in the cathecolamine levels upon training in the case of different sport activities to draw more precise conclusions.

Conclusions

1. Up to our knowledge, during our research, we performed for the first time the simultaneous determination of the concentration change of several neuroendocrine compounds such adrenaline (*A*), noradrenaline (*NA*), cortisol (*C*), dopamine (*DA*) and vasoconstrictor peptides such as angiotensinogen and big endothelin-1 for athletes of different sports as a response upon stress through a treadmill experiment.

2. Same gender elite athletes with similar past experience and encountered in similar training phase of cyclic (kayaking and triathlon) as well as ball games (handball and soccer) were subjected simultaneously to harmonized workload test under controlled conditions by applying a modified *vita maxima* Bruce protocol.

3. For the comparability of the performed tests, workload was summed at each difficulty level to better follow the differences in the workload.

4. Among all studied compounds, *NA* proved to be the most reliable parameter for athletes of cyclic sports and ball games. For *NA*, a stronger difference of about factor of three has been observed between soccer players and athletes of cyclic games. For handball players and kayakers as well as handball players and triathletes, the significance level was $p = 0.0000587$ and $p = 0.0000567$, respectively. The significant difference between soccer players and kayakers as well as soccer players and triathletes were $p = 0.0028$ and $p = 0.0024$, respectively. Normalization of data with maximal

oxygen uptake values strengthened the *inter*group differences, since statistical data evaluation showed a stronger correlation for the concentration levels of *A*, *NA*, *DA* and *C*. This outcome represents a novelty for *DA* and *C* for athletes performing different type of physical activity, since there is scarce information on this topic, so far.

5. The change in the responses of each neuroendocrine and vasoconstrictor peptide compared to the basal levels has been individually determined and it can be stated that the aforementioned differences were higher than the mean values for at least three of the monitored compounds in about 20 out of the 44 athletes of the studied four sports. These results may indicate the need for a more thorough cardiac examination of the athletes.

Own Publication list

Publications related to the PhD thesis:

Szmodis M, Bosnyák E, **Protzner A**, Szőts G, Trájer E, Tóth M. (2016) Bone characteristics, anthropometry and lifestyle in late adolescents. Antr Anz. 73: 23-32.

Protzner A, Szmodis M, Udvardy A, Bosnyák E, Trájer E, Komka Zs, Györe I, Tóth M. (2015) Hormonal Neuroendocrine and Vasoconstrictor Peptide Responses of Ball Game and Cyclic Sport Elite Athletes by Treadmill Test. PLOS ONE 10: e0144691.

Publications not related to the PhD thesis:

Bosnyák E, Trájer E, **Protzner A**, Komka Zs, Györe I, Szmodis M, Tóth M. (2016) Osteocalcin gene polymorphism and bone density in Hungarian athletes. Anthr Anz. doi: 10.1127/anthranz/2016/0594.

Bosnyák E, Trájer E, Udvardy A, Komka Zs, **Protzner A**, Kováts T, Györe I, Tóth M, Pucsok J, Szmodis M. (2015) ACE and ACTN3 genes polymorphisms among female Hungarian athletes in the aspect of sport disciplines. Acta Physiol Hung. 4: 451–458.

Trájer E, Bosnyák E, Komka Zs, Kováts T, **Protzner A**, Szmodis M, Tóth Sz, Udvardy A, Tóth M. (2015) Retrospective Study of the Hungarian National Transplant Team's Cardiorespiratory Capacity. Transplant Proc. 47: 1600-1604.