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Does exercise training affect NO/GC/cGMP pathway in the brain?

by

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The mechanism(s) behind brain adaptation to physical training has not been fully investigated. The exercise-induced rise in energy turnover results in increased metabolic activity both in skeletal muscle, liver and fat tissue and some metabolic products may cross the blood brain barrier. In turn, this may influence brain metabolism and neuronal signaling pathways. In this paper we provide a concise overview of the field. Additionally, preliminary indications are given that NO/GC/cGMP may play a significant role in the signaling pathway involved in the control of movements (coordination).

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Introduction

The decrease in skeletal muscle performance that occurs with different types of exercise is termed as muscle fatigue (Enoka ans Stuard 1992). For many years the limit to performed exercise was explained in terms of metabolic and cardio-respiratory capacity and, more accurately, was attributed to factors, such as maximal oxygen uptake (VO_2max), aerobic enzyme capacity, cardiac output, muscle glycogen and phosphocreatine stores, perturbations in electrochemical coupling, calcium regulation, excess protons and inorganic phosphate accumulation inside muscle cells, etc. However, this paradigm does not explain the limitations to exercise performed with large muscle groups at altitude, when exhaustion occurs without limb locomotor fatigue and during submaximal cardiac output (Dill et al. 1932, Noakes et al. 2001). Thus, an alternative reason(s) and mechanism(s) explaining the limitation to perform exercise in such circumstances must exist. The obvious candidate is the central nervous system (CNS), which integrates input from various sources, all related to the exercise and limits the intensity and duration of recruitment of limb skeletal muscle to prevent jeopardizing of the disintegrity of the exercising organism. Thus, it seems that the cardio-respiratory and muscle metabolism capacities act as prime factors influencing performance, while crediting the CNS for its pivotal role as the ultimate site where exercise starts and ends (Kayser 2003).

Central fatigue

It is well known that prolonged, exhausting exercise inevitably leads to fatigue. Factors of peripheral fatigue as depletion of energy stores, accumulation of metabolic by-products or impairment of muscle contractile mechanism have been well documented but they cannot be exclusively accounted for fatigue symptoms appearing during long bouts of endurance exercise (Bigland-Richte et al 1986, Cooper et al. 1988). Since the stimulus for muscular contraction is initiated in the brain, the so called central fatigue may be generated if alternations within the central nervous system decrease the ability to voluntary send a signal to neuromuscular junction (Davis and Bailey 1997).

A possible role of serotonin in central fatigue – animals and human studies.

Serotonin neurons play an important role in various behavioral and autonomic functions such as arousal, feeling, temperature regulation, activation of hypothalamic–pituitary-adrenal axis and locomotion (Wilckens et al. 1992, Di-

nan 1996). Romanowski and Grabiec (1974) suggested for the first time that an increase in the brain concentration of 5-hydroxytryptamine (5-HT) might be involved in fatigue. They observed higher 5-HT in whole brain homogenates after endurance exercise performed to exhaustion in comparison to values obtained from brain homogenates of sedentary rats. A hypothesis of possible role of 5-HT in central fatigue was than developed by Newsholme et al. (1987). According to their argumentation, a marked increase in the plasma FFA level (over 1 mM) observed during prolonged exercise, especially when liver glycogen store is depleted, can release from plasma albumin tryptophan, as FFA displace some of albumin-bound tryptophan (TRY) (Fernstrom 1990, McMenamy 1965). During prolong exercise FFA released from adipose tissue increase the plasma concentration of FFA and thereby also free thryptophan (f-TRY). A higher plasma concentration of f-TRY can lead to its elevated transport across blood brain barrier by specific transporters that TRY shares with other large neutral amino acids (BCAA). f-TRY is the precursor for synthesis of 5-HT and increased f-TRY availability is expected to elevate the cerebral 5-HT level (Newsholme et al. 1987). The fact that TRY can not be synthesized in the brain and the rate-limiting enzyme in the serotogenic pathway (tryptophan mono-oxygenaze) is not saturated with the substrate implies that the rate of 5-HT formation in the brain is strongly dependent on the supply of its precursor f-TRY (Olendorf and Szabo, 1976). There is much convincing data obtained from animal studies to support the hypothesis that premature fatigue during prolonged exercise is caused by higher synthesis of 5-HT in rat. Chaouloff at al. (1985) demonstrated that plasma f-TRY was elevated during low intensity treadmill running and it was accompanied by an increase in TRY and 5-HIAA concentration in brain but no effect of exercise on 5-HT was found (Chaouloff et al. 1985). Similar changes were found in cerebrospinal fluid (Chaouloff et al. 1985a). Blomstrand et al. (1989) found elevated regional rat brain concentrations in 5-HT and 5-HIAA after a treadmill run to exhaustion. The association between enhanced 5-HT level and fatigue was also indicated by the observations that pharmacological manipulation of the serotoninergic activity affected endurance exercise in rats (Davis and Baily, 1997). This approach was mainly based on neuropharmacological studies with agonists and antagonists of serotoninergic receptors (Bailey at al. 1992, Bailey et al. 1993, Bailey et al. 1993a). The release of 5-HT is inhibited by the stimulation of presynaptic 5-HT receptors located on serotoninergic terminal neurons and controlling the release of serotonin via a negative feed back mechanism (Engel et al 1986, Maura et al. 1986). Moreover, Chennaoui et al. (2001) was the first to demonstrate a total 5-HT_{1B} receptors desintization in rat substantia nigra after intensive training. Later studies indi-

cated desensitization of 5-HT 1B was also accompanied after chronic physical exercise (intense training) by changes in the level of mRNA $5HT_{1B}$ receptors in brain cortex and cerebellum. A study exists that shows no significant change in $5HT_{1B}$ receptor mRNA levels in striatum and hippocampus after a training session (Chennaoui et al 2001).

Evidence for a serotonin role in human central fatigue during exercise remains indirect. Pharmacological blocking of reuptake of released 5-HT before exercise decreased endurance time and increased perceived effort (Wilson and Maughan, 1992, Struder et al. 1998). There is also some evidence that elevated level of plasma f-TRY in humans is associated with fatigue (Davis et al., 1992, Wilson and Maughan, 1992). However, human studies show contradictory results: no effect of nutritional manipulation on mental and endurance performance has been seen (Galiano et al. 1991, Varnier et al. 1994, Van Hall et all. 1995).

Taken together, majority of presented above observations suggest that changes in brain serotonin level may be affected by exercise due to elevated lipolysis in adipose tissue and in turn brain f-TRY metabolism. There are also evidences suggesting that exercise affects serotonin receptor sites via transcriptional or post-transcriptional mechanisms. Both phenomenons are dependent on exercise type and within the brain are located in its exercise-stimulated area.

Central fatigue - a possible role of ammonia

Ammonia accumulation in the blood can occur during very intense short-term exercise or also during moderately intense but prolonged exercise. This occurs in parallel to deamination of adenine nucleotides in working muscles (Broberg et al. 1988, Broberg and Sahlin 1988). It is known that high plasma ammonia level may affect the function of the CNS and cause neurological disturbances (Mutch and Banister 1983, Guezennec et al. 1998). An exercise-induced elevation in plasma ammonia may be a contributing factor in fatigue through an effect on the CNS causing convulsions and a loss of coordination (Banister and Cameron 1990, Mutch and Banister 1983). An exercise induced hiperammonemia may also lead to muscle cramps through an effect on the peripheral nervous system (Banister and Cameron 1990, Mutch and Banister 1983). Plasma ammonium (NH_4^+) was assessed as it stimulates glycogen metabolism in actrocytes (Tsacopoulos et al. 1997). It was proved during liver failure that hyperammonemia lowered the cerebral concentrations of both glutamate and GABA which may strongly influence cerebral circulation, osmotic regulation, and neuronal metabolism (Bachman 2002, Jones and Weissenborn 1997, Larsen and Wenden 2002). Again, the results of such experiments re-

vealed that metabolites released into blood from exercising skeletal muscle may affect brain metabolism. This seems to be the case during prolonged or very intense exercise when systemic hyperammonemia reaches above 250 μmol x l^{-1} (Banister and Cameron 1990, Brouns et al. 1990).

Brain metabolic responses to exercise

During past decade several imaging methods for evaluation of cerebral metabolism have been developed (Abdelmalki et al 1997, Guezennec et al. 1998). Because of methodological limitations all of them can be applied from low to moderate exercise intensity (Christensen et al. 2000). To overcome accompanied methodological difficulties with using the imaging techniques during exercise that involve large muscle groups and/or intense exercise, Dalsgaard et al. (2002) developed a human exercise model for the measurement metabolism within the brain based on determination of a-v substrate differences across the brain combined with the assessment of global CBF (cerebral metabolic ratio defined as 0_2/glucose + 1/2lactate). They were able to show a marked lactate uptake by the brain with exhaustive exercise which is probably metabolized by this tissue (Ide et al. 1999, Dalsgaard et al. 2004). The same effect in humans was seen after recovery from maximal exercise (Ide et al. 2000). Again this notion suggests that exercise induced production of some muscle metabolites may influence brain metabolism. Muscle metabolic receptors and mechanoreceptors may also be involved n this phenomenon (Dalsgaard et al. 2002a).

Endurance training as a possible factor modifying NO/GC/cGMP pathway in the brain

NO has been found to be a messenger molecule abundantly present in the nervous system (Snyder and Bredt 1991). As chemically instable, studies of its formative enzyme, nitric oxide synthase (NOS) has been served to establish NO's disposition. NO formation starts with the conversion of L-arginine to L-citrilline by NOS in the presence of several cofactors (Forestmann and Kleinert 1995, Marletta 1993). Typical classifications of NOS reveal only three isoforms which are all present in the CNS: inducible NOS (iNOS), endothelial NOS (eNOS) and neuronal NOS (nNOS). It must be pointed out that of these, nNOS is mainly expressed in neurons (Prast and Philippu 2001). A number of lines of indirect evidence may suggest an association between exercise and NO formation in the CNS. Thus, the link between NOS, nNOS and exercise was found by Kim et al (2003). In their study, food deprivation increased NOS and nNOS expressions in paraventricular nucleus (PVN) of the hypothalamus and intense

exercise suppressed food deprivation enhancing of NOS and nNOS expressions in the PVN (Kim et al. 2003). Furthermore, a close relationship was observed between NO production and exercise in various tissues, such as blood, skeletal muscles, kidney, lungs and also in the hypothalamus (Roberts et al. 1999, Bredt 1999, Kim et al. 2003). It is tempting to speculate that exercise may also affect CNS NO production through elevation of calcium level in the brain. This is because among known NOS isoforms, nNOS and eNOS are calcium dependent (Marletta, 1993). It is well established that blood calcium level significantly increases after exercise in blood (Ruben and Bennett 1981, Cordova et al 1990) and might be transported to the brain (Nielsen et al 1977). Lessons from animal model of parkinsonism are also in agreement with assumption that NO production within the brain may be affected by exercise. Thus, evidence obtained by Chalimoniuk et al. (2004) suggests that the meaningful mechanism through, which NO acts in striatum is an activation of NO-dependent guanylyl cyclase (soluble guanylyl cyclase, sGC) which in turn elevates cGMP level. Observed increased activity of sGC was due to the elevation of protein concentration of cytosolic fraction of CGβ1 subunit. Similar changes were seen in SN (Chalimoniuk M, Lukacova N, Strosznajder J, Marsala J and Langfort J – submitted). Some evidences revealed that cGMP may regulate dopamine level in the brain (Sutoo and Akiyama 1996). Since it is known that striatal dopamine deficiency results in complex changes in the brain's motor circuitry and causes motor deficit characteristic of PD, eg. hypokinesia (reduced movement), akinesia (absent movement), tremor, rigidity, postural instability and a slow short-stepped and shuffling gait pattern (Morris 2000, Hagell and Winder 1999), it may be assumed that the observed in mild to moderate PD patients improvement of motor performance by participation in various forms of exercises may be due to the activation of NOS/GC/cGMP in SN and striatum (Palmer et al. 1986, Sunvission et al. 1997, Reuther et al. 1997, Baatile et al. 2000, Lokk 2000, Miyai et al. 2002). Thus, to testify hypothesis if NO/CG/cGMP pathway may be affected by endurance training we measured mRNA nNOS, GCβ1 protein level and cGMP concentration in different parts of the brain 48 h after the last bout of exercise using analytical methods as described elsewhere in details (Chalimoniuk et al. 2004). To do this, female Wistar rats (250-290g) to be exercise-trained were running 40-60 min for 5 days x week^{-1} for 7 weeks. More detailed description of the above procedure was reported previously (Langfort et al. 1988, Langfort et al. 1996). Applied in the present study training regime was investigated for its effectiveness previously and resulted in an increase of 2-oxoglutarate dehydrogenase activity in the soleus muscle, in increase the ratio of the heart weight to body weight and also shifted the anaerobic threshold to higher exercise load

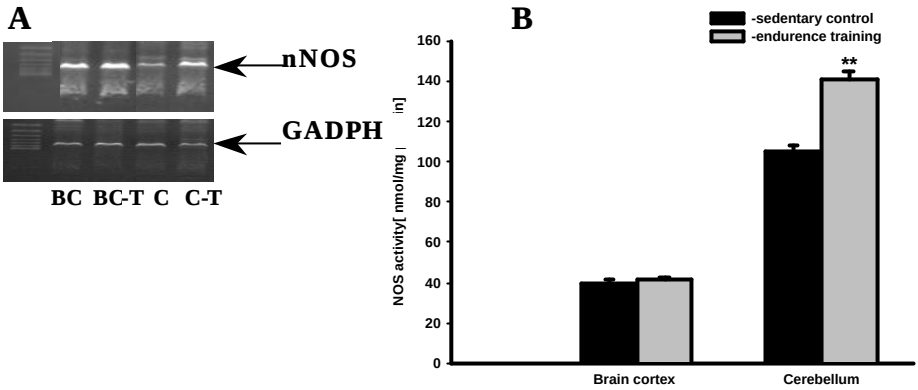

Fig. 1.

Effect of endurance training on nNOS expression and NOS activity in brain cortex and cerebellum

A. RT-PCR analysis of nNOS mRNA in brain cortex and cerebellum.

Reverse transcription of 5µg of total RNA was performed in final volume 20µl using 15 units of AMV reverse transcriptase, 0.5µg oligo(dT)$_{15}$ as a primer and 1mM each dNTP in one cycle: 42^{o}C for 1h and 99^{o}C for 5 min with subsequent cooling to 4^{o}C. Polymerase chain reaction of 5µl of (cDNA) RT product was carried out according to the manufacturer's manual using Taq PCR Master Mix and 20 pmol of each primer :

nNOS - sense: 5'-CCTTAGAGAATAAGGAAGGGGGCGGG-3', antisense: 5'-GGGCCGATCATTGACGCGAGAATGATG-3',

GAPDH sense: 5'-TGAAGGTCGGAGTCAACGGATTTGGT-3', antisense: 5'-CATGTGGGCCATGAGGTCCACCAC-3' in 30 cycles for CGβ1 and GAPDH: 1 min 94^{o}C, 2 min 60^{o}C, 2 min 72^{o}C, with pre-denaturation 94^{o}C for 5 min. and in 35 cycles for nNOS : 1min 94^{o}C, 1min 56^{o}C, 2min 72^{o}C with pre-denaturation 94^{o}C for 5 min. . A 7 min extension at 72^{o}C was carried out the end of the final cycle. Then the samples were cooled to 4^{o}C. 15 µl of PCR product was loaded onto one lane with 3 µl of sample buffer and electrophoresed at 100V through 2% agarose gel containing 200 µg/l ethidium bromide. nNOS mRNA PCR products were normalized by the intensity of GAPDH expression. A typical RT-PCR products is representative of 4 separate experiments

B. NOS activity in brain cortex and cerebellum.

NOS activity determined by conversion [^{14}C]L-arginine to [^{14}C]L-citrulline. Homogenate (300µg) was incubated with the mixture containing 50mM Tris-HCl pH 7.4, 100µM [U-^{14}C]L-Arginine, 0.2µCi, 2mM $CaCl_2$, 1µM calmodulin, 15µM FAD, 10µM tetrahydrobiopterin, 1mM NADPH, 1mM EDTA, 1mM dithiotreitol for 20 min at 37^{0}C. Reaction was stopped by addition of 1ml 100mM Tris-HCl buffer pH 5.5 containing 10mM EDTA. Protein removed by centrifugation at 3000xg for 10 min and supernatant was passed through 1 ml of Dowex™ 50WX-8 columns (Na^+ form) and [^{14}C]L-citrulline was eluted with 2x 1ml H_2O. Results are expressed as the means ± SEM of data from 5 separate experiments performed in triplicate. The statistical analysis performed by one-way ANOVA, followed by the Newman-Keuls post-hoc test. Statistical probability of $p<0.05$ was considered significant. *$p<0.05$ versus the control value.

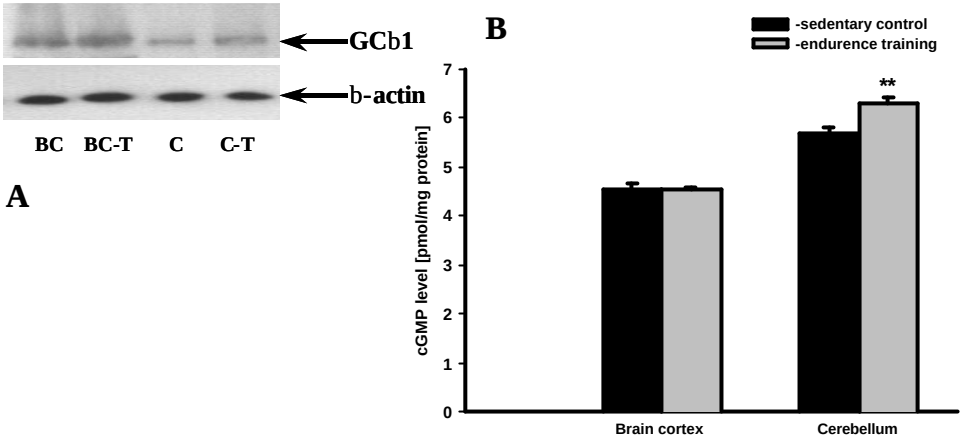

Fig.2.

Effect of endurance training on GC b1 expression and cGMP level in brain cortex and cerebellum

A. Western blot analysis for GC b1 subunit following endurance training in brain cortex and cerebellum.

40 μg of protein were subjected to 10% polyacrylamid gel SDS-PAGE and analyzed for GCβ1 and β-actin by immunobloting using polyclonal rabbit anti-GCβ1 antibody (Sigma, USA), anti-β-actin antibody (Cayman, USA) respectively. GCβ1 and β-actin antibodies complex was identified with the anti-rabbit IgG horsradish-peroxidase conjugate and visualized by using ECL kit (Amersham, UK). Results represent a typical immunoblot from 4 separate experiments.

B. Effect of endurance training on cGMP level in brain cortex and cerebellum.

The tissue was homogenized in 20% TCA, then cGMP level was determinated using immunoassay ELISA kit (Amersham, UK). Results are expressed as the means ± SEM of data from 5 separate experiments performed in triplicate. The statistical analysis performed by one-way ANOVA followed by the Newman-Keuls post-hoc test. Statistical probability of $p<0.05$ was considered significant. *$p<0.001$ versus the control value.

(Langfort et al. 1988, Langfort et al. 1996, Zarzeczny et al. 1996). Rats were killed after 48 hours after about of exercise and brain cortex and cerebellum were quickly isolated on ice-cold glass Petri dish. Samples were immediately frozen in liquid nitrogen and were stored at-80°C until analysis of mRNA nNOS, CGβ1 protein level and cGMP concentration. We observed that mRNA nNOS, CGβ1protein level, NOS activity and cGMP concentration were increased in cerebellum whereas no changes in all these variables were seen in brain cortex (Fig. 1 and Fig. 2). The well known fact that the brain cortex is known to be involved in planning and programming of movement and observed in our study the lack of influence of endurance training on NO/CG/cGMP in this part of brain indicate that activation of this pathway is not crucial for the processes by

which an abstract thought is converted into voluntary action. Opposite effect was seen in cerebellum which is permanently engaged in coordination control during exercise. Thus, it is temping to speculate that activation of NOS/CG/cGMP pathway in cerebellum may be involved in regulation of dopamine synthesis. This assumption bases on the immunohistochemistry study confirmed an increased dopamine levels in striatum and nucleus accumbens septi in exercised mice (Sutoo and Akiyama 1996). This finding are in agreement with successive report showing that dopamine and its metabolites enhanced following exercise (Chaouloff 1989, Freed and Yamamoto 1985, Heyes et al. 1988) and with the fact that the decrease in brain dopamine concentration and low dopamine to serotonin ratio may cause loss of motor coordination (Davis and Baily 1997). There are evidences that the brain dopamine synthesis is stimulated by elevated calcium transport into CNS which is elevated after exercise (Sutoo and Akiyama 1996, Cordova et al 1990). Studies with antagonists indicate that calmodulin-dependent pathway may be involved in brain dopamine production (Sutoo et al. 1989) but calcium can also act via NO/cGMP pathway (Bredt 1999).

Conclusions and perspective

Despite a huge scientific effort in exercise physiology during last decades, the marked systemic responses initiated by muscular exercise (for example: heart and respiration function, glucose production, etc) can not be fully explained by known endocrine and neuronal mechanisms (Galbo 1995). Preliminary results obtained in this research indicate that behind these regulatory mechanisms may lie changes in signaling pathways within neurons. Given the fact that no other physiological event taxes various system of body to the same extant as heavy muscular exercise, it seems to be clear that identification of new regulating factors secreted by exercising muscle potentially will have a high impact. On the other hand, it is necessary to fully explain a role of NO/GC/cGMP in control bodily movements map (coordination) and to investigate if exercise may modify functions of another signaling pathways in the brain.

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