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SHORT COMMUNICATION

Effects of exercise stress on the endocannabinoid system in humans under field conditions

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Abstract The effects of physical exercise stress on the endocannabinoid system in humans are almost unexplored. In this prospective study, we investigated in a crossover design and under field conditions at different altitudes the effects of physical exercise on the endocannabinoid system (ECS) in 12 trained healthy volunteers. For determination of alterations on the ECS three different protocols were analyzed: *Protocol A* (physical exercise at lower altitude) involved strenuous hiking below 2,100 m, whereas *Protocol B* (physical exercise by active ascent to high altitude) involved hiking up to 3,196 m, an accommodation at the cottage and a descent the next day. *Protocol C* (passive ascent) included a helicopter ascent to 3,196 m, an overnight stay at this altitude and a flight back to the base camp the following day. The cumulative hiked altitude in Protocol A and B was comparable ($\sim$1,650 m). The blood EC concentrations of anandamide increased significantly in Protocol A/B from baseline (T0) 0.12 $\pm$ 0.01/0.16 $\pm$ 0.02 (mean $\pm$ SEM) to 0.27 $\pm$ 0.02/0.42 $\pm$ 0.02 after exercise (T1) ($p < 0.05$). Anandamide levels in Protocol C remained stable at 0.20 $\pm$ 0.02. We conclude that the ECS is activated upon strenuous exercise whereas the combination with hypoxic stress further increases its activity. The reduced partial pressure of oxygen at high altitude alone did not affect this system. In summary, physical exercise activates the endocannabinoid system, whereas the combination with high altitude enhances this activation. This discloses new perspectives to adaptation mechanisms to physical exercise.

Keywords Endocannabinoids · Anandamide · 2-AG · Exercise · Sport

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Introduction

One of the most common conditions of challenging the humans' physiological systems is physical exercise. While numerous reports have been published on the role of exercise and its effects on the various stress response systems [e.g. activation of the catecholaminergic system (Zouhal et al. 2008) or the hypothalamo-pituitary-axis (HPA) (Flinn et al. 2011)], only very few reports have investigated the role of the endocannabinoid system (ECS) under conditions of exercise stress (Sparling et al. 2003).

The ECS is an evolutionary well-preserved neurobiologic system that controls key elements in the organ homeostasis (Hill and McEwen 2010). This system which

was—in contrast to its long phylogenetic existence—discovered late in the 1980s consists of the following elements: cannabinoid receptors, endogenous ligands, specific proteins involved in the endocannabinoid biosyntheses and degradation enzymes like fatty acid amide hydrolase (FAAH) (De Petrocellis and Di Marzo 2009). Two G-protein-coupled receptors—the cannabinoid receptors type 1 or 2 (CB1, CB2)—were identified so far. Endogenous ligands binding onto those receptors are the so-called endocannabinoids which are synthesized from arachidonic acid. The best described endocannabinoids so far are anandamide (*N*-arachidonylethanolamide, AEA) and 2-arachidonoylglycerol (2-AG). AEA seems to have hereby a lower intrinsic activity and binds to CB1 receptors as a partial agonist, whereas 2-AG is considered a full agonist on both receptor types (Mackie 2006; Sugiura et al. 2006). Endocannabinoids are synthesized "on demand" and released from cells immediately after their synthesis and can hereby quickly react to different stressful conditions. It has been shown that the ECS is an important mediator in the interaction of man to conditions of stress of either psychological/emotional as well as of physical nature responses to stress (Carrier et al. 2005; Hohmann et al. 2005). Recently, it has been shown that the ECS has a crucial role in the regulation of the interaction with the HPA-axis during behavioral stress (Steiner and Wotjak 2008).

It remains yet unclear if, and if yes to which degree, this system is affected after several hours lasting continuous exercise stress. We, therefore, selectively tested these effects in a crossover design by measuring the blood concentrations of the endocannabinoid AEA and 2-AG in healthy man under different conditions of strenuous exercise under field conditions.

Methods

Location

The study took place at the South Tyrolean Alps in Italy at the Becherhaus summit at an altitude of 3,196 m above sea level. The base camp was located at an altitude of 1,416 m in the Ridnaun Valley.

Subjects

Twelve young healthy males of the South Tyrolean Mountain Rescue Team were included in the study (average age 27.6 years; range 24–38 years). The approval for this investigation was obtained by the ethic committee of the University of Munich and informed consent was signed by every participant. Prior to study start every volunteer completed a medical health checkup. Self-prescribed drugs before and during the study were not allowed. None of the volunteers lived at an altitude higher than 1,200 m; all were trained mountaineers with normal body mass indices (BMI 21.8 ± 2.7, mean ± SD) and further quantifiable tests to evaluate their physical fitness (e.g. VO_{2max}) were not performed.

Study protocols

In total, three different study protocols were performed, whereas a time interval of more than 6 weeks was maintained between implementation of every study arm. Temperature variations throughout the day were ±5°C in all three study protocols (Choukèr et al. 2005).

Sample collections were performed in all protocols around the same time of day before breakfast and before start (T0, early morning ~7 a.m.), at the summit cottage 60–90 min upon arrival (T1), in the morning after the overnight stay (T2) and 60–90 min after returning to base camp the next day (T3) (see also Fig. 1). Venous blood drawing from the antecubital vein for measuring plasma endocannabinoids was done in up-right sitting participants. Food intake was scheduled during the regular meals and was not different in the three study protocols also in respect to the amount and to the composition of the food consumed. The degree of social interactions between the subjects in the different protocols was comparable.

Protocol A: physical exercise by hiking at lower altitude

Volunteers were subjected to physical exercise by hiking for a total time of 4–4.5 h below an altitude of 2,100 m. This protocol included a fourfold ascent achieving a cumulative altitude of ~1,650 m. Hereby a comparable degree of climbed altitude was achieved as under Protocol B. The overnight stay was at the base camp.

Protocol B: physical exercise by active ascent to high altitude

This study arm combined the effects of strenuous physical exercise and high altitude. Mountaineers had to climb from the base camp to the Becherhaus summit. The difference in altitude was 1,780 m and the total hiking time was between 3.5 and 5.5 h, respectively. Volunteers spent the night at the Becherhaus hut and descended the following day—24 h after their ascent.

Protocol C: passive ascent to high altitude

This protocol involved the passive ascent to high altitude by helicopter, an accommodation at the Becherhaus summit and a flight back to the base camp 24 h after the ascent. The flight duration from the base camp to the summit hut

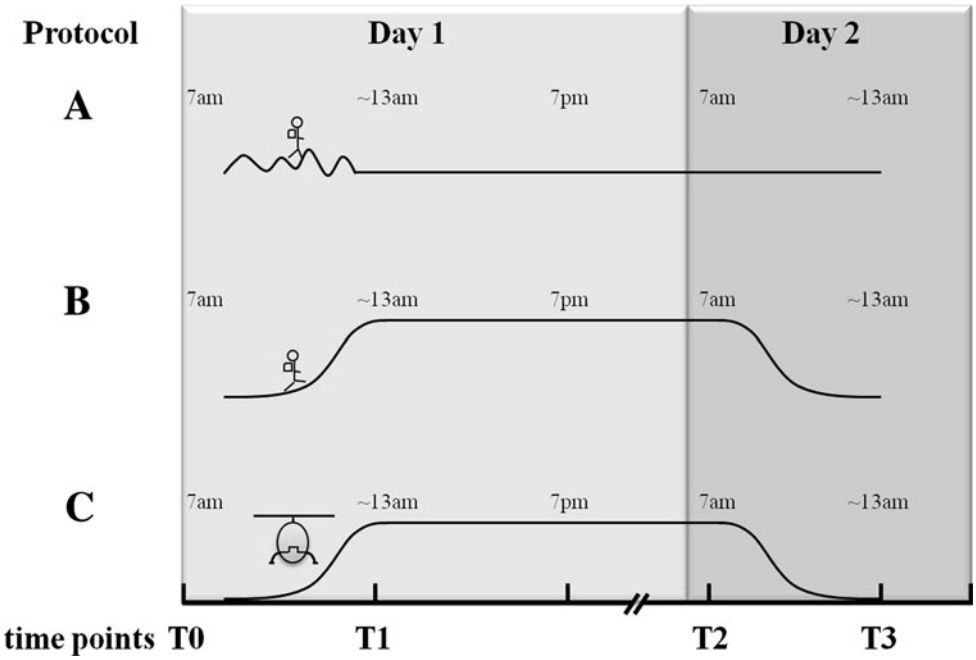

Fig. 1 Blood collection time points in the three study protocols: *A* physical exercise by hiking at lower altitude (<2,100 m), *B* physical exercise by active ascent to high altitude (3,196 m) and *C* passive ascent to high altitude (3,196 m)

was 8 min. Volunteers rested at the cottage and were not allowed to do any exercises during this observation period.

Endocannabinoids

Blood samples were collected into EDTA containing tubes (S-Monovette®, Sarstedt, Nümbrecht, Germany) and then immediately centrifuged. Plasma was transferred into Eppendorf tubes before final sample storage at −80°C occurred. These conditions allow storage of endocannabinoid samples for at least 6 months (Di Marzo et al. 2009), or to more than 3 years (unpublished data).

For determination of plasma concentrations of the endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG), high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) was applied which has been previously described (Schmidt et al. 2006; Vogeser et al. 2006). This method is linear within a range of 0.1–2 ng/ml for anandamide and 0.5–10 ng/ml for 2-AG. The lower detection limit of this method (defined as a signal/noise ration >4:1) is 0.025 ng/ml for anandamide and 0.33 ng/ml for 2-AG. In biological matrices, 2-AG (including its deuterated analog) rapidly isomerizes to 1-AG (Vogeser and Schelling 2007). For this reason, we quantified 2-AG as the sum of 1- and 2- esters of arachidonic acid.

Epinephrine

Epinephrine blood concentrations were determined in plasma samples (EDTA, S-Monovette®, Sarstedt, Nümbrecht, Germany) by HPLC (Chromosystems, Martinsried, Germany) and are expressed in mg/l as previously shown (Choukèr et al. 2005).

Statistical analyses

Deviation from normal distribution of sample data was tested using the Kolmogorov–Smirnov test. Changes in plasma endocannabinoid concentrations across the 4 time points of measurement were analyzed with a repeated measurement general linear model (RM-GLM) with time point as a within-subject variable and group assignment as a between-subject variable. Fisher's LSD test was used to define which time points differed significantly from each other. Possible correlation between anandamide and epinephrine was tested by Pearson correlation analyses. For all testing, a p value <0.05 was regarded as statistically significant. Data are presented as mean ± SEM. Statistical calculations were performed using SPSS 15.0, PASW Statistics 17.0 and Sigma Plot 11.0, Chicago, Illinois, USA.

Results

Anandamide and 2-Arachidonoylglycerol (2-AG)

All twelve subjects completed the three study protocols. The RM-GLM demonstrated a significant within—subject change in endocannabinoid plasma concentrations over time (type III sum of square = 0.38, $F = 24.5$, $p < 0.01$ for AEA and type III sum of square = 51.3, $F = 4.2$, $p < 0.01$ for 2-AG) with a significant time point by group interaction for AEA (type III sum of square = 0.20,

$F = 6.6$, $p < 0.01$ for AEA and type III sum of square = 42.4, $F = 1.7$, $p = 0.12$ for 2-AG).

In detail, AEA levels measured during the physical exercise below 2,100 m were significantly increased directly and after the exercise (Fig. 2) and additionally, an increase of the 2-AG concentrations could be quantified (T0: 3.70 ± 0.72; T1: 5.91 ± 1.47; T2: 2.72 ± 0.49; T3: 4.86 ± 0.99). Hiking to 3,196 m caused also a significant, triple fold increase of AEA with no remarkable changes in 2-AG levels. In contrast, passive ascent by helicopter had no effect on the endocannabinoid blood concentrations of AEA and 2-AG.

With the exception of anandamide in the active ascent group B—which remained significantly elevated compared to baseline—all levels returned to baseline values (Fig. 2).

Anandamide and epinephrine

An increase of the catecholamine epinephrine was measured after physical activity to high altitude (T1, Protocol B) (Choukèr et al. 2005) and showed a positive and significant correlation with anandamide: AEA (ng/ml)/epinephrine (mg/l): T1: 0.42 ± 0.06/86.4 ± 11.5; T1: $p = 0.026$, $R^2 = 0.73$. No significant correlations were observed for other time points or in study protocols A and C.

Discussion

This investigation aimed to analyze the responses of the endocannabinoid system in healthy mountaineers experiencing different exercise stress conditions. Little is known about the interaction of the endocannabinoid system and exercise in humans. Sparling provided first evidence about an increase of anandamide as well as 2-AG levels in blood after 45 min of either cycling or running (Sparling et al. 2003). Our results confirm in humans for the first time Sparling's findings of an increase of anandamide in relation to exercise. Furthermore, we extended these observations by quantification of anandamide and 2-AG blood concentrations in volunteers who were physically challenged for a longer-duration lasting up to 5 h. The major finding is that exercise for several hours leads itself to an increase of anandamide blood levels. Interestingly, the effect of exercise on the blood anandamide concentrations was enhanced when exercise was combined with moderate hypobaric hypoxic conditions at high altitude (Protocol B). This additive effect of altitude was not due to the alone standing effect of hypobaric hypoxia as high altitude alone (Protocol C) had no effect on the endocannabinoid blood concentration.

Interestingly and despite the limited number of participants and variability of biological systems, this study demonstrates that the endocannabinoid system is one of the stress response systems which is modulated by strenuous physical exercise. Among the well-investigated stress response systems it is known that exercise activates the catecholaminergic system (Zouhal et al. 2008). Also in this study the catecholamine epinephrine increased upon physical activity (Choukèr et al. 2005). Taken once again into account the limited number of participants in this study a positive relationship between anandamide and epinephrine after physical activity (Protocol B) to high altitude was

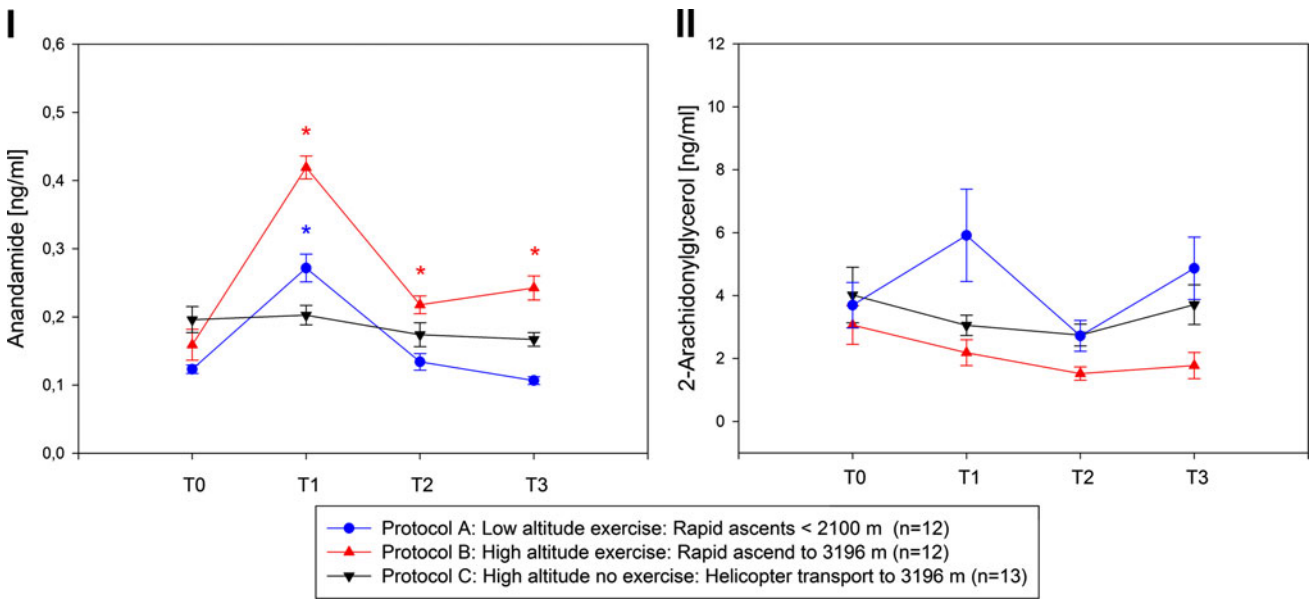

Fig. 2 Endocannabinoid plasma levels of **(I)** Anandamide and **(II)** 2-Arachidonoylglycerol at different time points during the study protocol *A* (physical exercise by hiking at lower altitude), *B* (physical exercise by active ascent to high altitude), and *C* (passive ascent to high altitude); Data are Mean ± SEM, *$p < 0.05$ versus T0, one-way RM ANOVA with Fisher's LSD post hoc test

seen. The complexity of the interaction of these two G-protein receptor coupled systems (Chen-Izu et al. 2000; Mackie 2008)—the ECS and the catecholaminergic system—can therefore just be assumed, especially when looking at the regulation of the nutritional blood flow. It is known that physical exercise affects blood circulation and is able to increase the blood flow to strained tissues (muscles). Recent reports in experimental settings showed an increase in nutritional blood flow under the influence of anandamide (Movahed et al. 2005). Not only the catecholaminergic system is activated under exercise stress but also the HPA-axis is affected by exercise and also subject to modulation by the endocannabinoid system. It is reported that the ECS seems also to have a major role in the regulation of the activation of the HPA-axis during and after stress (Steiner and Wotjak 2008). Furthermore, the endocannabinoid system is considered to be a modulator not only of stress-related endocrine but also of behavioral responses (Tasker 2004). One might therefore carefully speculate, as the ECS has also an impact on mood and motivation (Carrier et al. 2005), that endocannabinoids could contribute to the well-known "runner's high" experienced after strenuous physical exercise.

In conclusion, the activation of the ECS through physical exercise indicates an important systemic response which is probably involved in maintaining human organ homeostasis. Further studies are warranted to confirm these findings and to investigate the impact on different physiological mechanisms and affected organ systems, respectively.

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Conflict of interest The authors have no conflicts of interest or financial ties to disclose.

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