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Research Article

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Effects of parabolic flight and spaceflight on the endocannabinoid system in humans

Abstract: The endocannabinoid system (ECS) plays an important role in the regulation of physiological functions, from stress and memory regulation to vegetative control and immunity. The ECS is considered a central and peripheral stress response system to emotional or physical challenges and acts through endocannabinoids (ECs), which bind to their receptors inducing subsequent effecting mechanisms. In our studies, the ECS responses have been assessed through blood concentrations of the ECs anandamide and 2-arachidonoylglycerol. In parallel, saliva cortisol was determined and the degree of perceived stress was quantified by questionnaires. This report summarizes the reactivity of the ECS in humans subjected to brief periods of kinetic stress and weightlessness during parabolic flights and to prolonged stress exposure during life onboard the International Space Station (ISS). Both conditions resulted in a significant increase in circulating ECs. Under the acute stress during parabolic flights, individuals who showed no evidence of motion sickness were in low-stress conditions and had a significant increase of plasma ECs. In contrast, highly stressed individuals with severe motion sickness had an absent EC response and a massive increase in hypothalamic-pituitary-adrenal axis activity. Likewise, chronic but well-tolerated exposure to weightlessness and emotional and environmental stressors on the ISS for 6 months resulted in a sustained increase in EC blood concentrations, which returned to baseline values after the cosmonauts' return. These preliminary results suggest that complex environmental stressors result in an increase of circulating ECs and that enhanced EC signaling is probably required for adaptation and tolerance under stressful conditions.

Keywords: 2-arachidonoylglycerol; anandamide; International Space Station; spaceflight; stress.

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Introduction

Spaceflight conditions reflect a complex environmental challenge with multiple aversive consequences for human health. In the light of manned orbital, lunar, and interplanetary mission, the assessment, monitoring, and maintenance of human health is a prerequisite for a successful mission accomplishment. Spaceflight, even when short in duration, can induce several adverse effects due to adaptation to the physical stressors of gravitational changes, radiation, malnutrition, and psychological stress (Choukèr, 2012). These complex environmental challenges are most pronounced for extended periods during interplanetary missions (Choukèr, 2012). Although stressful conditions of psychological or physical nature are known to activate multiple stress response systems (Selye, 1998), such as the hypothalamic-pituitary-adrenal (HPA) axis, it remains to be determined to which degree the phylogenetically old and evolutionary well-preserved endocannabinoid system (ECS) responds to stress and to spaceflight conditions in particular.

The ECS consists of central and peripheral G-protein-coupled receptors (CB1 and CB2; Pertwee et al., 2010) with their corresponding endogenous ligands, the endocannabinoids (ECs) anandamide and 2-arachidonoylglycerol (2-AG; Pertwee, 2008). Other structurally related fatty acids, such as palmitoylethanolamide and oleoylethanolamide, are part of the same chemical group, the N-acylethanolamines, but bind as well to other receptors, such as the transient

receptor potential vanilloid type 1 receptor system or the G-protein-coupled receptor GPR55, and have potentiated effects (entourage effects) on anandamide (Ryberg et al., 2007; Ho et al., 2008; Kallendrusch et al., 2012).

The ECs are known to play an important role in the modulation and regulation of various kinds of central and peripheral physiological responses to stress (Gorzalka et al., 2008). Neuroendocrine and neurochemical processes with an influence on behavior, mood, and memory (Campolongo et al., 2009; Atsak et al., 2012) are affected as well as immune (Tanasescu and Constantinescu, 2010) or vegetative functions such as gastrointestinal motility or myocardial contractility (Weis et al., 2010b). Those processes and control functions are transmitted by signaling pathways such as the HPA axis and the sympathetic nervous system. As they are stress modulated, the ECS influences their activity (Hill et al., 2010).

The acute stress response and its effects constitute an important survival reaction of the human body, whereas the chronic stress response or a persistent activation of the stress response system can lead to maladaptation syndromes causing disorders characterized by a chronically increased allostatic load (McEwen, 2002), such as depression, chronic pain, posttraumatic stress disorder (Yehuda, 2002), or autoimmune disease (McEwen and Dhabhar, 2002). The question whether there is a positive or negative relationship between EC activity and the level of stress response is not yet understood (Di Marzo, 2008), and no data in humans subjected to stress in space are available. In this context, we performed two different studies addressing stressful conditions that are important during spaceflight, either during training or the mission to space, respectively. We asked (i) for the role of the peripheral ECS as a modulator of stress and stress responses, (ii) if the changes in peripheral EC signaling are associated with HPA axis activity (e.g., the cortisol response), and (iii) to which degree acute or chronic environmental changes affect this system.

Acute vs. chronic stress

We tested the responsiveness of the ECS under two conditions representing acute and chronic space-related stress exposures. Healthy subjects were investigated during spaceflight-associated conditions such as parabolic flights (http://www.novespace.com) as an acute stress model.

During the flights, the subjects are exposed to several abrupt changes due to gravitational acceleration/deceleration causing short violent kinetic stress on the human body. The brevity of the different acceleration phases prevents any possible physiological adaptation mechanisms. Additionally, the hypergravity phases are interrupted by phases of microgravity evoking weightlessness. Those severe, fast-changing conditions heavily impact on the human sense of balance, leaving the subjects with only little control of their well-being and no possibility to escape the situation and therefore causing acute stress (Schneider et al., 2007, 2009; Chouker et al., 2010).

In contrast, long-duration space mission to the International Space Station (ISS) offers a unique, complex model of chronic stress as a result of permanent microgravity, high radiation doses, and confinement in a purely technical, hazardous, and potentially deadly condition.

EC and glucocorticoid measurements and stress quantification

During both studies, we measured the concentrations of the ECs anandamide and 2-AG from venous blood samples. ECs were determined in whole blood collected into EDTA-containing tubes (S-Monovette, Sarstedt, Nümbrecht, Germany). During the parabolic flight campaign (PFC), the samples were immediately frozen in-flight on dry ice and stored at -80°C. The time delay between sampling and freezing was kept at a minimum, as previous experiments have shown that EC generation in native blood samples continues *ex vivo* (Vogeser et al., 2006). On the ISS, blood vials were stored at -80°C until downloaded to Earth and analyzed (Figure 5). The details of the high-performance liquid chromatography tandem mass spectrometry technique used for EC quantification are described elsewhere (Vogeser et al., 2006; Chouker et al., 2010).

Cortisol levels were analyzed from salivary samples taken in the morning before breakfast at approximately 8:00 a.m. Saliva for cortisol measurements was collected by wetting a cotton swab for 60–90 s (Salivette, Sarstedt, Nümbrecht, Germany) and kept at room temperature (18–25°C). Free cortisol was quantified by an automated immunoassay system based on the principle of electrochemiluminescence (Elecsys Cortisol, Roche Diagnostics, Mannheim, Germany; Chouker et al., 2010). During the PFC, salivary and blood samples were taken at the time points T0–T5 [in-flight before the start of the parabolic maneuvers (T0); after 10 (T1), 20 (T2), and 30 (T3) parabolas; in-flight after the end of the parabolic maneuvers (T4); and 24 h later (T5)].

For the long-duration missions on the ISS, the preflight measurements were taken during a period of 30 to 7 days before the launch of the mission. In-flight samples were collected after 3–4 months and after almost 6 months before return to Earth. Postflight measurements

were carried out on days 1 and 7. These time points were chosen with regard to the changes following adaptation to microgravity and reexposure to gravitation.

The intensity of self-perceived stress was quantified using a short and well-validated instrument, the KAB stress symptom questionnaire (German: Kurzfragebogen zur akutellen Beanspruchung; Mueller and Basler, 1993), which was completed at each indicated time point in combination with the saliva and blood collections. The KAB is able to repeatedly determine an individuals' psychological state under the conditions of acute or chronic stress and is highly sensitive to short-term or situational changes during a stressful experience (Chouker et al., 2010). It consists of six matched bipolar adjectives describing the states of stress or relaxation (e.g., 'worried-happy' and 'comfortable-uneasy'). The rating is done on a 6-point Lickert scale, which ranges between 1 and 6 for all six matched adjectives. Higher KAB values indicate increased perceived stress levels.

Results of stress and EC measurements

Parabolic flight experiments

One of the aims of the PFC experiments was to characterize the effects of kinetic stress on the response of the peripheral ECS and to relate the responsiveness of the system to stress-related outcomes of the experiment. Twenty-one volunteers participated in the PFC. Seven of these individuals reacted with a severe stress reaction accompanied by pronounced symptoms of motion sickness, whereas the remaining 14 participants tolerated the experiment well and were in a low-stress condition without evidence of kinetosis (see Table 1 for a comparison of the KAB scores). Interestingly, stress-intolerant individuals with acute motion sickness showed an absent EC response to kinetic stress and a massive activation of the HPA axis, whereas stress-tolerant participants showed the opposite pattern with a significant increase in plasma ECs and nearly unchanged plasma cortisol concentrations (Chouker et al., 2010; Figure 1).

Furthermore, both groups showed different baseline levels of the ECs and nearly equivalent levels of cortisol before the parabolas. The group identified further as 'highly stressed' showed already low activation of the ECS before the kinetic stress in contrast to the 'low stressed' subjects who had higher initial concentrations of ECs.

Long-term space mission

During the long-term ISS missions, cosmonauts reported increased KAB stress levels during their stay on the ISS with maximal scores at the 6-month time point. The increase of stress scores was, at that number of investigated subjects, however, not yet statistically significant (Figure 2). KAB scores paralleled the upsurge in EC blood concentration seen at the 6-month time point (Figure 3) and the increase of free cortisol in saliva (Table 2). In contrast to the acute stress model of PFC, the chronic stress exposure during long-duration missions demonstrates a positive relationship between stress, the ECS response, and the HPA axis activation. With the exception of the increase in whole-blood anandamide concentrations, the observed changes in 2-AG, saliva cortisol, and KAB stress scores were not statistically significant, so these observations need to be interpreted with caution.

Discussion and conclusions

The responsiveness of the EC system in humans to acute stress has been demonstrated in several recent studies (Hill et al., 2009; Dlugos et al., 2012). The exposure

Score	In-flight before parabolas (T0)		After 10 parabolas (T1)		After 20 parabolas (T2)		After 30 parabolas (T3)		In-flight after parabolas (T4)	
	Motion sickness		Motion sickness		Motion sickness		Motion sickness		Motion sickness	
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
KAB stress score	2.4±1.2	2.3±0.9	3.4±1.4[a]	2.1±0.7	4.1±1.3[a]	1.9±0.6	3.7±1.2[a]	1.5±0.5	2.8±1.1[a]	1.7±0.7

Table 1 Comparison of KAB stress symptom scores between high and low stressed participants with and without motion sickness during the parabolic flight experiment.

Data are mean±SEM. Adapted from Chouker et al. (2010).

[a]$p<0.01$ compared with participants without motion sickness.

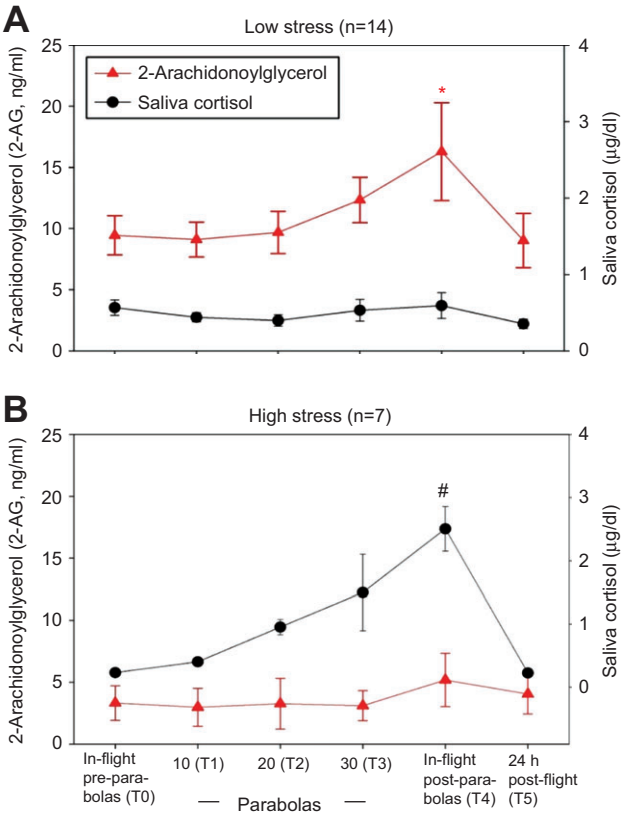

Figure 1 Comparison of salivary cortisol levels and blood concentrations of the EC 2-AG in volunteers with and without motion sickness undergoing a parabolic flight experiment.
(A) Inverse relationship between blood concentrations of the EC 2-AG and saliva cortisol in participants reporting low-stress conditions without evidence of motion sickness. The increase in 2-AG during the parabolic maneuvers was associated with low saliva cortisol concentrations. *Significantly higher 2-AG concentrations in volunteers experiencing low-stress conditions. Data are expressed as mean±SEM. (B) Opposite association between 2-AG concentrations and saliva cortisol in highly stressed volunteers with motion sickness. These individuals had low blood 2-AG levels and very high saliva cortisol values. #Significantly higher saliva cortisol concentrations in highly stressed participants ($p<0.01$, *t*-test). Data are expressed as mean±SEM. Adapted from Chouker et al. (2010).

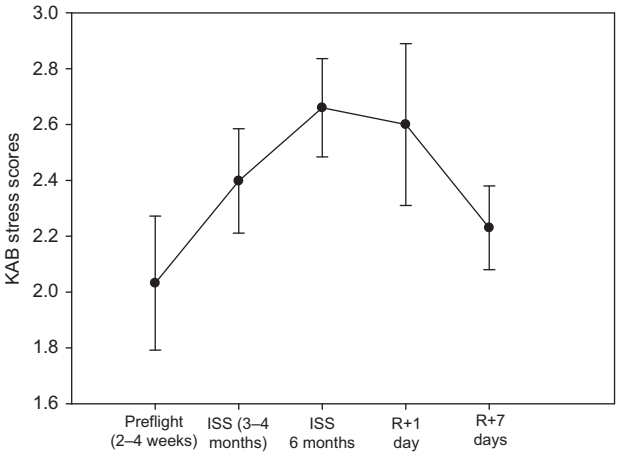

Figure 2 Changes in KAB stress symptom scores during long-duration missions on the ISS.
The increase in stress score is an indicator for psychological stress during the in-flight period in space (ISS). Preflight samples were taken in the first month before launch to the ISS, whereas the following time points were collected on the ISS after 3–4 and 6 months of stay in space. Upon return, collections were performed after the first and seventh days, respectively (R+1 and R+7). Data are expressed as mean±SEM.

of healthy volunteers to kinetic stress during our parabolic flight experiments resulted in a significant increase in plasma EC concentrations in stress-tolerant participants, whereas highly stressed individuals with acute motion sickness showed an absent EC response and a massive activation of the HPA axis (Chouker et al., 2010). Recent animal experiments have confirmed the antinausea and antiemetic effects of cannabinoids and their receptors (Van Sickle et al., 2003; Parker et al., 2009). Cannabinoids are also used to treat chemotherapy-induced emesis in cancer patients (Machado Rocha et al., 2008).

Physical stress in trained and physically fit individuals induced by hard exercise during mountaineering or cycling also resulted in elevated EC concentrations, which returned to baseline after the termination of the stressful activity (Feuerecker et al., 2012; Heyman et al., 2012). Likewise, stress induced by the Trier Social Stress Test, a type of pure emotional stress without any physical challenge, is associated with a significant increase in 2-AG plasma levels with a baseline return during the recovery phase (Hill et al., 2009). These findings suggest that an increased EC signaling during acute stress is a protective response to maintain homeostasis and to enhance the recovery process. EC signaling is also known to maintain HPA axis homeostasis by buffering basal activity as well as by mediating glucocorticoid-induced fast feedback mechanisms, which represents the most important mechanism of the organism for stress adaptation and termination of the stress response (Evanson et al., 2010; Riebe and Wotjak, 2011).

Our small study in cosmonauts during a long-term mission onboard the ISS resulted in a comparable observation by demonstrating a more chronic activation of the ECS, probably the result of persistent and highly complex stressors acting on the crew. Although increased EC signaling under acute or chronic conditions of stress might be beneficial for the organism in terms of stress tolerance, there is evidence of elevated plasma levels of anandamide and 2-AG in patients with atherosclerosis and coronary

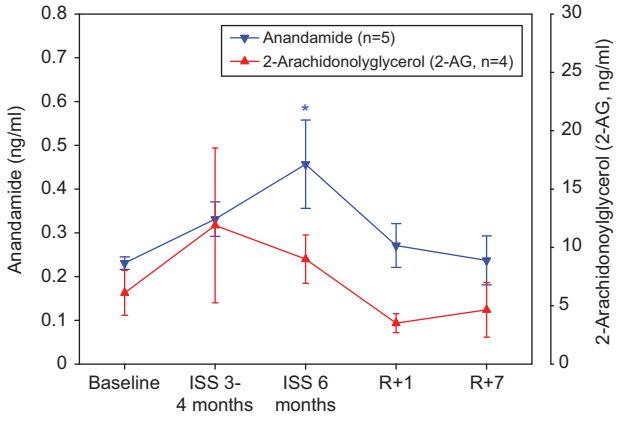

Figure 3 Whole-blood EC concentrations during long-duration space missions on the ISS.
Blood anandamide levels increased significantly under in-flight conditions after 6 months on the ISS (n=4–5; *$p<0.05$ vs. baseline for the EC anandamide). The increase in 2-AG blood concentrations appeared earlier but was not statistically significant. For the definition of time points, see Figure 2. Data are expressed as mean±SEM.

artery disease (Sugamura et al., 2009; Weis et al., 2010a), whereas an inhibition in EC signaling by CB1 receptor blockade in circulating macrophages is associated with a significant reduction in proinflammatory mediators (Sugamura et al., 2009) as activated macrophages, and other nuclear blood cells are believed to be the major source of circulating ECs in the blood (Vogeser et al., 2006; Centonze et al., 2008). Therefore, an activated peripheral EC system might play a causal role in the reported increased risk for coronary artery disease and the associated enhanced mortality in chronically stressed individuals, such as patients with posttraumatic stress disorder (Boscarino, 2008). These observations in humans illustrate the complex immunoregulatory functions of the ECS as mediated through the binding to the CB1 but also to the CB2 receptors as expressed on immune cells. The EC ligands are involved not only in the suppression of immune cell activation but also in the inhibition of proinflammatory cytokine production and the modulation of T-cell functions. CB2 receptors, for instance, modulate the antigen presentation properties of macrophages as well as their migration, phagocytosis, and adhesion characteristics (Pandey et al., 2009). Conversely, the EC receptors are themselves also regulated through cell activation by humoral factors, which modulate their expression. Furthermore, the lymphocyte TH1/TH2 ratio is influenced by ECs through CB2 receptors (Klein et al., 2003).

With regard to those multiple interactions of the ECS with variable immune functions, one could hypothesize that the increased activity of the EC system in chronically stressed individuals as observed in space might have long-term adverse consequences. In acute stress situations, however, an appropriate ECS response seems to help maintaining organ homeostasis. One might further conclude that EC baseline concentrations measured before defined stress situations could give a hint of the individuals' stress resistance during spaceflight conditions and thus might represent an indicator to detect stress-sensitive individuals for which special countermeasures could then be provided.

These assumptions need, however, experimental and clinical confirmation and may also be of great importance during long-term space missions. However, despite all efforts taken, challenged by methodological problems, low case numbers, and download limitations, the cause-effect relations (e.g., of the ECS activation) are far from being understood, as their attribution to one or more defined stressors during space voyage, such as confinement and weightlessness, can barely be separated during a mission. In this respect, however, a full and differential view on the causes and effects of the ECS activation can only be systematically achieved in Earth-bound studies, where individuals are subjected not to the entire range of stressors as on the ISS but separately to each of them or to their combinations. For instance, (i) confinement conditions on Earth as mimicked (e.g., in the MARS500 isolation study or during overwintering in Antarctica) allow to investigate the consequences of isolation and confinement without weightlessness. In contrast, (ii) continuous bed rest under conditions of a 6° head down tilt for up to 120 days are imitating microgravity effects (Chouker et al., 2001).

	Preflight	SEM	ISS 3–4 M	SEM	ISS 6 M	SEM	R+1	SEM	R+7	SEM
Cortisol	0.53	0.3	1.01	0.73	0.74	0.23	0.77	0.41	0.57	0.2
2-AG	6.1	1.9	11.9	6.63	9	2.07	3.5	0.81	4.6	2.3

Table 2 Comparison of free cortisol concentrations in salivary samples and blood concentration of the EC 2-AG during long-duration missions on the ISS (see also Figure 3).
For the definition of time points, see Figure 2. Data are expressed as mean±SEM.
ISS 3–4 M, in-flight samples collected after 3–4 months; ISS 6 M, in-flight samples collected after 6 months before return to Earth; R+1, postflight measurements carried out on day 1; R+7, postflight measurements carried out on day 7.

In summary, the presented studies using spaceflight conditions provide a first and likely important piece to unveil the role of the ECS in humans under extreme environmental challenges. As the studies have been carried out under fully standardized conditions, maintaining the cooling chain for the frozen samples, the results demonstrate that stress and EC research in space is possible. Moreover, the differential changes can provide meaningful information for (planning) future space missions but also seem to be of benefit for humans on Earth (e.g., for kinetosis research). Further investigations on the role of the ECS and also the interactions with other organ functions are warranted.

Materials and methods

Participants and technical accomplishment

Parabolic flights were performed with a modified Airbus A300 that has been certified to fly specific flight maneuvers, producing 22 s periods of near weightlessness (close to '0 g'). For each parabola, an initial pullup occurred (~45° slope) inducing 1.8 g for about 20 s, before reaching '0 g' conditions and 1.8 g again in the pullout phase. A typical flight day included 30 subsequently flown parabolic maneuvers (http://www.novespace.com). After an approval by the local Ethics Committee, signed informed consent, flight medical approval, and on-site clinical check of health status, 21 males (41.0±1.5 years old) were included in the three PFCs accomplished between May 2006 and September 2007. Samples were taken before, during, and after the parabolic flight as indicated.

The long-duration missions were performed by five healthy male individuals (55.0±3.8 years old) who each stayed on the ISS for 6 months over a period of 2 years.

Figure 4 Expedition 13 crewmember with the ESA astronaut Thomas Reiter (left); Expedition 13 flight engineer; cosmonaut Pavel V. Vinogradov, ISS Commander representing Russia's Federal Space Agency; and Jeff Williams, NASA space station science officer and flight engineer, pose for a photo onboard of the ISS (credit to ESA).

Figure 5 Expedition 13 crewmember Jeff Williams, Expedition 13 NASA space station science officer and flight engineer, inserts a sample, such as the EC samples, in the -80°C laboratory freezer for ISS (MELFI) in the Destiny laboratory of the ISS.
MELFI is a low-temperature freezer facility with nominal operating temperatures of -80°C, -26°C, and +4°C that will preserve experiment materials over long periods (credit to ESA).

Blood sampling, sampling preparation in space, and download of samples to Earth

During the long-duration missions, the data collected in-flight and postflight were compared with the data gained at the baseline data collection time point approximately 1 month before flight. The ground-based work has mostly been conducted in Moscow and at the German Space Agency (DLR) site in Cologne. Additionally, return sample analysis was performed at the National Aeronautics and Space Administration (NASA)-Johnsons Space Center (Houston, TX). On-orbit work for this protocol included two in-flight sample collections by the crew (Figure 4): in the mid-period of flight as well as after the maximum stay (6 months) before return. Sample preparation on the ISS was necessary to ensure a reliable and adequate storage to best prepare for a further analysis on Earth. This included, after aseptic blood draw, centrifugation and storage procedures (Figure 5). The KAB questionnaire was performed on a hard copy and filled in with a pencil.

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