DOI 10.1515/revneuro-2012-0058 — Rev. Neurosci. 2012; 23(5-6): 681–690

Research Article

Daniela Hauer*, Florian Weis, Patrizia Campolongo, Mirjam Schopp, Andres Beiras-Fernandez, Claudia Strewe, Markus Giehl, Roland Toth, Erich Kilger and Gustav Schelling

Glucocorticoid-endocannabinoid interaction in cardiac surgical patients: relationship to early cognitive dysfunction and late depression

Abstract

Background: Endocannabinoids (ECs) are rapidly acting immune-modulatory lipid-signaling molecules that are important for adaptation to stressful and aversive situations. They are known to interact with glucocorticoids and other stress-responsive systems. Maladaptation to acute or chronic stress represents a major risk factor for the development of psychiatric disorders. In the present study, we administered stress doses of hydrocortisone in a prospective, randomized, placebo-controlled double-blind study in patients undergoing cardiac surgery (CS) to examine the relationship between the use of glucocorticoids, plasma EC levels, and the occurrence of early postoperative cognitive dysfunction (delirium) and of later development of depression.

Methods: We determined plasma levels of the ECs anandamide and 2-arachidonoylglycerol (2-AG) in CS patients of the hydrocortisone (n=56) and the placebo group (n=55) preoperatively, at postoperative day (POD) 1, at intensive care unit discharge, and at 6 months after CS (n=68). Postoperative delirium was diagnosed according to *Diagnostic and Statistical Manual of the American Psychiatric Association IVth Edition* (DSM-IV) criteria, and depression was determined by validated questionnaires and a standardized psychological interview (Structured Clinical Interview for *DSM-IV*).

Results: Stress doses of hydrocortisone did not affect plasma EC levels and the occurrence of delirium or depression. However, patients who developed delirium on POD 1 had significantly lower preoperative 2-AG levels of the neuroprotective EC 2-AG (median values, 3.8 vs. 11.3 ng/ml; p=0.03). Preoperative 2-AG concentrations were predictive of postoperative delirium (sensitivity=0.70; specificity=0.69; cutoff value=4.9 ng/ml; receiver operating characteristic curve area=0.70; 95% confidence interval=0.54–0.85). Patients with depression at 6 months after CS (n=16) had significantly lower anandamide and 2-AG levels during the perioperative period.

Conclusions: A low perioperative EC response may indicate an increased risk for early cognitive dysfunction and long-term depression in patients after CS. Glucocorticoids do not seem to influence this relationship.

Keywords: cardiac surgery; delirium; depression; endocannabinoids; glucocorticoids.

***Corresponding author: Daniela Hauer,** Department of Anaesthesiology, Ludwig Maximilians University, D-81377 Munich, Germany, e-mail: daniela.hauer@med.uni-muenchen.de
Florian Weis: Department of Anaesthesiology, Ludwig Maximilians University, D-81377 Munich, Germany
Patrizia Campolongo: Department of Physiology and Pharmacology, La Sapienza University of Rome, 00185 Rome, Italy
Mirjam Schopp: Department of Anaesthesiology, Ludwig Maximilians University, D-81377 Munich, Germany
Andres Beiras-Fernandez: Department of Cardiac Surgery, Ludwig Maximilians University, D-81377 Munich, Germany; and Department of Cardiac Surgery, Johann Wolfgang Goethe-University, Frankfurt/Main, Germany
Claudia Strewe: Department of Anaesthesiology, Ludwig Maximilians University, D-81377 Munich, Germany
Markus Giehl: Department of Anaesthesiology, Ludwig Maximilians University, D-81377 Munich, Germany
Roland Toth: Department of Anaesthesiology, Ludwig Maximilians University, D-81377 Munich, Germany; and School of PhD Studies, Semmelweis University, Budapest, Hungary
Erich Kilger: Department of Anaesthesiology, Ludwig Maximilians University, D-81377 Munich, Germany
Gustav Schelling: Department of Anaesthesiology, Ludwig Maximilians University, D-81377 Munich, Germany

Introduction

Dysregulation of the endocannabinoid (EC) system is thought to contribute to a diverse number of human dis-

eases, including atherosclerosis (Mach and Steffens, 2008), systemic inflammation, obesity and the metabolic syndrome (Cote et al., 2007), arterial hypertension (Batkai et al., 2004), heart failure (Fajardo and Bernstein, 2007; Weis et al., 2009b), psychiatric disorders such as anxiety and depression (Gobbi et al., 2005; Hill and Gorzalka, 2009), and stress-related chronic pain syndromes (Kaufmann et al., 2008). The EC system is a ubiquitous neurobiological and immune-modulatory system, mainly consisting of two endogenous ligands – anandamide and 2-arachidonoylglycerol (2-AG) – and two types of receptors, that is, the cannabinoid 1 receptor (CB1) and the cannabinoid 2 receptor (CB2) (Pertwee, 2008). The ECs are not circulating hormones and therefore not bound to proteins but synthesized on demand and rapidly degraded. CB1 receptors are mainly expressed in the brain (Pertwee, 2008) but also in peripheral tissues such as heart (Weis et al., 2009b), muscle, liver, and adipose tissue (Begg et al., 2001). CB2 receptors are primarily found on immune cells (Eisenstein et al., 2007), but also within the central nervous system (Onaivi et al., 2006) and the heart (Weis et al., 2009b). Because of the ubiquitous distribution of CB receptors throughout the brain, animal experiments have shown a multitude of central physiological functions of ECs, for example, memory processing (Marsicano et al., 2002; Campolongo et al., 2009; Hauer et al., 2011; Atsak et al., 2012), neuroprotection and neuroinflammation (Wolf et al., 2008), anxiety (Bortolato et al., 2006; Domschke and Zwanzger, 2008), reward (Maldonado et al., 2006), and feeding behavior (Vickers and Kennett, 2005). Despite this diversity of central function modulation, recent studies in animals point to a major role of EC signaling in controlling adaptive processes to aversive situations and in regulating and limiting stress reactions (Patel and Hillard, 2008). Maladaptation to acute or chronic stress represents a major risk factor for the development of stress-related disorders such as depression, anxiety, and even posttraumatic stress disorder (Schelling et al., 2003).

Whereas early studies mainly pointed out the central effects of EC signaling, more recent investigations have also demonstrated important peripheral functions of ECs. The EC system is activated in obesity and associated with cardiometabolic disorders in humans (Sugamura et al., 2009). The CB1 receptor blocker rimonabant in obese humans has resulted in weight loss and multiple beneficial effects on plasma triglycerides, high-density lipoprotein levels, glucose tolerance, and systemic inflammation (Pacher, 2009). Impairment of EC signaling by CB1 receptor blockade, however, led to a significant increase in the incidence of depression and anxiety, particularly in patients exposed to stressful situations (Nissen et al., 2008). As a consequence, rimonabant never received approval by the US Federal Drug Administration and was withdrawn from the European market in 2008. Thus, activation of the EC system appears to play an important role in the adaptation to stressful situations while simultaneously resulting in an increased risk for the development of obesity and cardiometabolic disorders associated with systemic inflammation.

Cardiac surgery (CS) is frequently performed in patients with multiple cardiometabolic risk factors and induces a profound proinflammatory response related to surgery and cardiopulmonary bypass. CS represents a considerable stress exposure (Schelling et al., 2003) and carries a substantial risk of psychiatric sequelae such as chronic stress reactions (Stoll et al., 2000) or anxiety and depression (Burker et al., 1995).

Patients with depression show significantly lower plasma EC concentration (Hill and Gorzalka, 2009) as well as changes in glucocorticoid signaling (Treggiari et al., 2009). These findings suggest that alteration of circulating levels of glucocorticoids and ECs might influence stress-related outcome parameters. We therefore evaluated whether alterations of EC signaling could be predictive of early cognitive dysfunction and later development of depression in CS patients treated with placebo or stress doses of hydrocortisone (cortisol).

Patients and methods

Participating patients

We studied a cohort of CS patients in a prospective, randomized, double-blinded trial that compared the effects of stress doses of hydrocortisone to placebo on several short- and long-term outcome parameters. The patients were randomized to either hydrocortisone or placebo treatment, and then followed until 6 months after CS. We included patients undergoing CS for coronary artery disease or cardiac valve replacement. We excluded individuals if they met any of the following criteria: combined surgical procedures, emergency surgery, pregnancy, plasma interleukin-6 (IL-6) levels higher than 10 pg/ml preoperatively, hepatic dysfunction (bilirubin >3 mg/dl), renal dysfunction (plasma creatinine>2 mg/dl), a positive serological test result for HIV or hepatitis, manifest insulin-dependent diabetes mellitus, use of steroidal or nonsteroidal antiphlogistics (except low-dose aspirin) during the last 7 days before surgery, an extracardial septic focus, chronic or acute inflammatory disease, and an inability to give informed consent. In addition, patients with previous intensive care unit (ICU) treatment (with the exception of brief stays in coronary care units) and those who required glucocorticoids for medical reasons (e.g., asthma or rheumatism) were excluded.

We screened 535 patients of whom 424 were not eligible for randomization on the basis of the above-mentioned exclusion and inclusion criteria. Of the 111 patients randomized, three died perioperatively in the ICU and 40 were lost to follow-up because of early

postoperative transfer to other ICUs away from the study center (n=11) or because they could not be contacted after discharge from the hospital (n=29). The final study population evaluated at the first time point (ICU discharge) consisted of 97 patients (87.4% of those originally randomized). Of these 97 patients, 68 (70.1%) were available for assessment at 6 months after CS. Of these 68 patients, 32 had received placebo and 36 had received hydrocortisone.

All patients received a detailed explanation of the purpose of the study and were able to give informed consent. The study was approved by the Institutional Review Board of the Ludwig-Maximilians University of Munich (protocol number 149/00, Amendment) and the relevant government and regulatory agencies. Data protection met the standard set by German law.

Sequence of actions during the perioperative period and follow-up

Patients were evaluated at five time points [1 day before CS, postoperatively on admittance to the ICU, on postoperative day 1 (POD 1), at discharge from the ICU, and at 6 months after surgery]. The sequence of actions during the study is depicted in Figure 1.

Study intervention

The study drug hydrocortisone (Hydrocortisone-21-hydrogensuccinate, Pharmacia, Berlin, Germany) was administered by using a loading dose (100 mg over 10 min, intravenous [IV]) before induction of anesthesia, followed by a continuous infusion of 10 mg/h for 24 h (POD 1), which was reduced to 5 mg/h on POD 2, and then tapered to 3×20 mg IV on POD 3, and 3×10 mg IV on POD 4 (Kilger et al., 2003; Weis et al., 2009a).

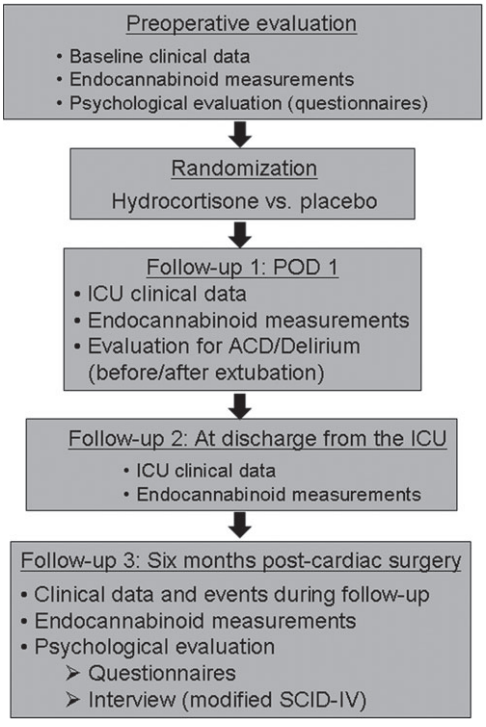

Figure 1 Flow chart depicting the sequence of actions during the study. POD 1, postoperative day 1; SCID-IV, Structured Clinical Interview for *Diagnostic and Statistical Manual of the American Psychiatric Association IVth Edition*.

Primary and secondary outcome measures

The primary outcome parameters of this part of the study were the occurrence of delirium and the incidence and intensity of depression at 6 months after CS in relation to EC plasma concentrations and the use of hydrocortisone.

Randomization generation and allocation concealment

Randomization to hydrocortisone or placebo treatment was performed in blocks of four by using a computer-generated randomization list. Patients, investigator staff, persons performing the assessments, and data analysts remained blinded to the identity of the treatment from the time of randomization until database lock when data entry was finished.

Biological and clinical measurements

EC levels in human blood

Plasma levels of anandamide and 2-AG were determined with high-performance liquid chromatography-tandem mass spectrometry as described elsewhere (Vogeser et al., 2006; Weis et al., 2010).

For EC measurements, 4.5 ml of arterial blood was withdrawn from a cannula placed into the femoral artery. Blood was sampled into ethylenediaminetetraacetic acid-containing tubes (S-Monovette, Sarstedt, Numbrecht, Germany) and immediately (within 5 min) centrifuged at 5000 *g* over 5 min at 4°C and stored. The time interval between blood sampling and centrifugation was minimized because previous experiments have shown that EC generation in blood samples is continued *ex vivo* (Schmidt et al., 2006; Vogeser et al., 2006). Thus, delays in blood processing could result in false-positive increases in plasma EC concentrations. After centrifugation, the supernatant was immediately aspirated, transferred into 1-ml plastic vials, and stored at -80°C until measurement.

In biological matrices, 2-AG (including its deuterated analog) is rapidly isomerized (Vogeser and Schelling, 2007; Di Marzo et al., 2009). We therefore quantified 2-AG as the sum of 2-AG and 1-AG and refer to the sum of both compounds as 2-AG throughout the paper although this basically represents a chemical misnomer.

Clinical measurements

The clinical and demographic variables measured are depicted in Tables 1 and 2.

Baseline variables	Hydrocortisone (n=56)	Placebo (n=55)	*p*-Value
Age (year)[a,b]	69.3±8.9	68.0±8.3	0.94
Body mass index (BMI)[a]	28.0±6.4	26.9±5.3	0.37
Sex (male/female)	45/11	47/9	1.00
Preoperative beta-blocker use (n)	34	35	0.65
Diabetes mellitus type II (n)[b]	11	12	0.57
Coronary artery disease (n)	25	35	0.21
ASA Score[a,b,c]	3.1±0.3	3.0±0.0	0.11
NYHA Score[a,d]	2.5±0.5	2.2±1.5	0.65
CCS Score[a,e]	2.8±0.4	2.3±1.5	0.42
Preoperative hsCRP[f] values (mg/dl)	0.76±1.41	0.48±0.56	0.20
Preoperative anandamide concentration (ng/ml)[a]	0.38± 0.11	0.36±0.15	0.51
Preoperative 2-AG concentration (ng/ml)[a]	27.9±46.7	27.3±57.8	0.96

Table 1 Comparison of preoperative baseline variables between the hydrocortisone and placebo groups.
[a]Values are mean±SD.
[b]Indicates significant differences ($p<0.05$) between males and females.
[c]American Society of Anesthesiologists classification of perioperative risk.
[d]New York Heart Association classification of heart failure.
[e]Canadian Cardiovascular Society classification of angina pectoris.
[f]Denotes highly sensitive C-reactive protein.

Psychometric measurements

Depression

The German version of the Hamilton Depression Rating Scale (HDRS) was used as a continuous outcome measure for depression (Hamilton, 1960) and was administered during the 6-month follow-up evaluation. As an additional measure of depression, we asked the patients to rate their answers to the question 'I feel depressed/downtrodden' on a rating scale ranging from 1 (never) to 7 (always). The depression rating scale was administered at the preoperative time points and at 6 months after surgery (Figure 1).

Diagnosis of early, acute cognitive dysfunction and delirium

Acute postoperative cognitive dysfunction (ACD) was defined as an inadequate neurological response in an awake patient at the first attempt of postoperative extubation requiring resedation and prolonged ventilation. In extubated patients, delirium was diagnosed according to the *Diagnostic and Statistical Manual of the American Psychiatric Association IVth Edition* (*DSM-IV*) criteria. Patients were evaluated for ACD/delirium on POD 1 (Figure 1).

Psychiatric interview

At approximately 6 months after CS, the patients received a follow-up phone call from study personnel and, after repeated oral consent to participate in the investigations, were invited to our study center for an evaluation of their general health and a psychological interview. During the interview, a psychologist (M.S.) performed a thorough psychological assessment by using a modified Structured Clinical Interview for *DSM-IV* (SCID-IV) technique focusing on anxiety disorders and depression (Figure 1).

Statistics

All variables were tested for normal distribution using the Lilieforts Modification of the Kolmogorov-Smirnov test. Normally distributed

	Hydrocortisone (n=56)	Placebo (n=55)	*p*-Value
Duration of extracorporeal circulation[a] (min)	114.0±58	107.3±57	0.54
Length of postoperative stay in the ICU[a,b] (h)	38.3±31.7	68.4±49.9	<0.01[c]
Postoperative hsCRP[d] values (mg/dl)	4.8±0.3	6.0±0.4	<0.01[c]
Duration of postoperative mechanical ventilation[a] (h)	17.1±12.0	21.0±17.0	0.42
Duration of epinephrine therapy[a] (h)	14.1±22.1	16.6±28.8	0.64

Table 2 Perioperative variables in the placebo group and the hydrocortisone group.
[a]Values are mean±SD.
[b]Indicates significant differences ($p<0.05$) between males and females.
[c]Significantly lower values in patients from the hydrocortisone group (*t*-test).
[d]Highly sensitive c-reactive protein.

continuous variables between the two groups were analyzed with *t*-tests; nonparametric continuous data were analyzed with Mann-Whitney *U*-tests. For discrete variables, the χ^2-test or Fisher's exact test, when appropriate, was applied. Changes in parameters (e.g., EC concentrations) across the five time points of measurement were analyzed with a repeated-measurement general linear model (RM-analysis of variance) with time point as a within-subject variable and group assignment as a between-subject variable.

The predictive value and optimal cutoff threshold of preoperative EC plasma concentrations for the postoperative development of ACD/delirium was quantified by receiver operating characteristic (ROC) curve analyses.

A *p*-value of <0.05 was considered statistically significant. Statistical analyses were by intention-to-treat by using the 'as randomized as analyzed principle'. Data are presented as mean±SD when normally distributed with the exception of figures, where mean±SEM is used to increase clarity. Nonparametric data are presented as median and range. All statistical calculations were performed by using PASW 18.0 (IBM SPSS Statistics, Chicago, IL, USA).

Results

Clinical parameters

Preoperative baseline values, including highly sensitive C-reactive protein (hsCRP) values, did not differ significantly between patients from the hydrocortisone group and the patients from the placebo group (Tables 1 and 2). Patients from the hydrocortisone group had a significantly shorter postoperative stay in the ICU and significantly lower postoperative hsCRP values than did patients receiving placebo (Table 2), without any other significant differences in early clinical outcome parameters.

Hydrocortisone and EC plasma concentrations

The use of hydrocortisone had no significant effect on EC plasma concentrations throughout the perioperative period (Figure 2A and B).

EC plasma concentrations changed, however, significantly during the perioperative period. Immediately after CS, when the patients were deeply sedated and admitted to the ICU for postoperative care, anandamide plasma levels were significantly lower than preoperatively (0.22±0.13 vs. 0.38±0.13 ng/ml; $p<0.01$).[a] 2-AG concentrations at this time point were significantly higher than before surgery (46.7±44.4 vs. 32.3±60.7 ng/ml; $p<0.01$)[b] and also

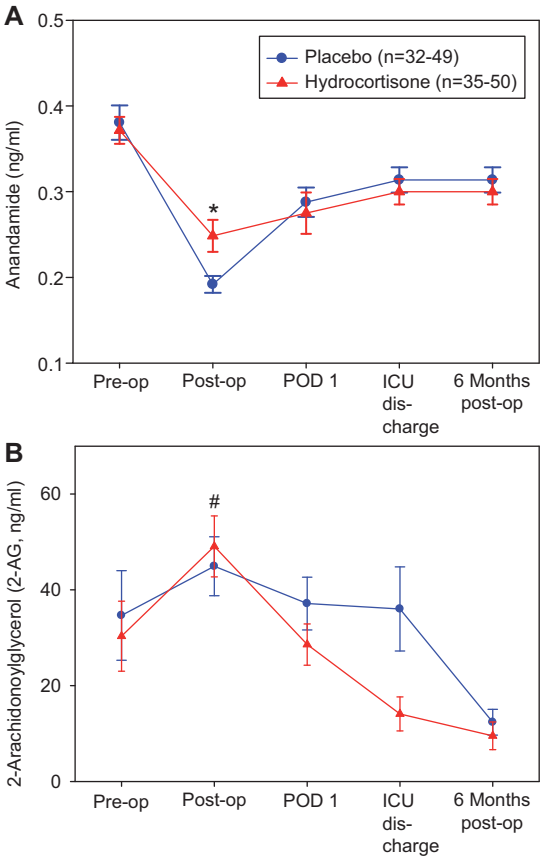

Figure 2 Stress doses of hydrocortisone and plasma EC levels during the perioperative period and 6 months thereafter in patients undergoing cardiac surgery.
There was a significant within-subject effect of time point on anandamide (Figure 2A, type III sum of squares=0.267; $F=4.04$; $p<0.01$) and 2-AG plasma concentrations (Figure 2B, type III sum of squares=11978.90; $F=4.56$; $p<0.01$), no interaction between group assignment and anandamide (type III sum of squares=0.058; $F=0.851$; $p=0.496$) and 2-AG concentrations (type III sum of squares=5572.2; $F=2.12$; $p=0.083$), and no between-group effect of hydrocortisone (type III sum of squares=0.064; $F=2.853$; $p=0.102$ for anandamide, and type III sum of squares=131.06; $F=0.080$; $p=0.779$ for 2-AG). Data are mean±SEM. EC, endocannabinoid; RM-ANOVA, repeated-measurement analysis of variance; 2-AG, 2-arachidonoylglycerol. *Indicates significantly lower postoperative anandamide plasma concentrations compared with preoperative baseline values (RM-ANOVA with Holm-Sidak post hoc test; $p<0.01$). #Significantly higher postoperative 2-AG concentrations compared with baseline values and with plasma concentrations 6 months after surgery ($p<0.01$).

significantly higher than at 6 months after surgery (11.0±12.4 ng/ml; $p<0.01$). At POD1 and at discharge from the ICU, anandamide levels were still lower than preoperatively but the difference from the baseline values was not statistically significant. At these two time points, 2-AG levels in patients from the placebo group declined to baseline (preoperative) values. In the hydrocortisone group,

[a] To convert ng/ml of anandamide into pmol/ml, multiply by 2.88.
[b] To convert ng/ml of 2-AG into pmol/ml, multiply by 2.64.

2-AG plasma concentrations showed a more pronounced decline but the difference from the placebo group was not statistically significant (Figure 2A and B).

Acute cognitive dysfunction and delirium

Thirteen patients developed ACD/delirium during ICU treatment. The occurrence of delirium was not significantly associated with any of the major study end points, including postoperative EC levels or the occurrence of depression. Patients with ACD/delirium had higher postoperative proinflammatory IL-6 levels on POD 1 than did patients without acute brain dysfunction (median values, 2378 vs. 81 pg/ml; p=0.002). Patients who developed postoperative ACD/delirium had significantly lower preoperative 2-AG levels (median values, 3.8 vs. 11.3, ng/ml; p=0.032) and significantly higher hsCRP values (1.5 vs. 0.53 µg/ml; p=0.004). Low preoperative 2-AG levels were predictive for postoperative ACD/delirium (ROC curve area=0.70; 95% confidence interval [CI]=0.54–0.85; Figure 3), whereas hsCRP was not (ROC curve area=0.34; 95% CI=0.14–0.54). Patients with delirium showed a trend toward a longer stay in the ICU (p=0.070). The use of hydrocortisone had no effect on the incidence of ACD/delirium (six patients from the placebo group developed ACD/delirium vs. seven patients from the hydrocortisone group; p=0.771).

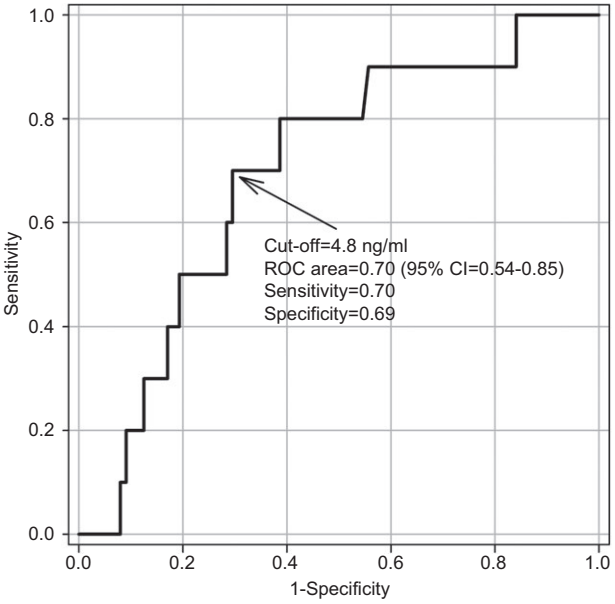

Figure 3 ROC curve analysis showing sensitivity and specificity of preoperative plasma 2-AG concentrations as a predictor of postoperative ACD/delirium on POD 1. ACD, acute postoperative cognitive dysfunction; ROC, receiver operating characteristic; POD1, postoperative day 1; 2-AG, 2-arachidonoylglycerol.

Glucocorticoids and depression

At 6 months after CS, patients from the hydrocortisone group had significantly lower HDRS depression scores than did those from the placebo group [median values, 2 (0–5) vs. 6 (1–9); p=0.03]. This finding could, however, not been confirmed by the psychological interview. According to SCID-IV, 5 out of 36 patients (13.8%) from the hydrocortisone group and 11 out of 32 patients (34.4%) from the placebo group had evidence of depression, but this difference did not reach significance (p=0.160).

The 16 patients diagnosed with depression had significantly higher scores on the depression rating scale at 6 months after CS (2.8±3.0 vs. 1.1±0.25; p=0.003), without significant differences at the preoperative time point (2.0±1.1 vs. 1.8±1.2; p=0.550), indicating new onset of depression after CS.

EC plasma concentrations and emotional outcomes

When compared with patients without depression, patients with evidence of depression at 6 months after CS had significantly lower anandamide plasma levels during the immediate postoperative period (p=0.002) (Figure 4A). The perioperative 2-AG response in patients with depression was significantly lower than in mentally healthy individuals who showed significantly increased 2-AG levels throughout the entire postoperative period (Figure 4B).

Discussion

This study investigated the relationship between changes in the peripheral EC system and emotional as well as early neurocognitive outcomes in patients treated with placebo or stress doses of hydrocorticosterone and undergoing highly stressful CS. Whereas hydrocortisone had no direct effects on plasma ECs, early cognitive dysfunction, or depression, patients with a lower perioperative EC response were at an increased risk for both types of adverse outcomes.

It has been suggested that EC signaling is required for stress adaptation and that a dysfunction of the EC system or an inability to upregulate its activity during stressful conditions could predispose individuals to the development of depression or other stress-related disorders (Gorzalka et al., 2008; Patel and Hillard, 2008; Hill et al., 2009, 2010; Moreira et al., 2009; Atsak et al., 2012).

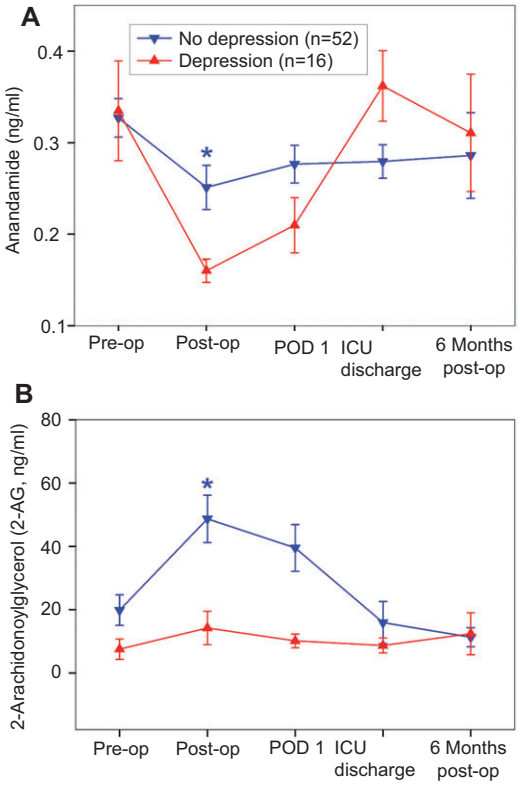

Figure 4 Changes in anandamide and 2-AG plasma concentrations across the study period in patients with depression and in patients without symptoms of depression at 6 months after CS.
Data are mean±SEM. Panel (A) shows anandamide plasma concentrations. There was a strong trend toward a between-group effect for depression on anandamide levels (type III sum of squares=0.27; F=4.1; RM-ANOVA; p=0.05). CS, cardiac surgery; RM-ANOVA, repeated-measurement analysis of variance; 2-AG, 2-arachidonoylglycerol. *Indicates significantly higher postoperative anandamide concentrations in patients who did not show evidence of later depression (p<0.01). Panel (B) shows 2-AG plasma concentrations. RM-ANOVA revealed a significant between-group effect of depression (type III sum of squares=8967.8; F=6.9; p=0.02). *Indicates significantly higher postoperative 2-AG levels in patients without depression at 6 months after CS (p<0.01).

At least two recent studies reported reduced plasma levels of anandamide or 2-AG in patients with depression (Hill et al., 2008, 2009). Moreover, patients from four large studies who received the CB1 receptor blocker rimonabant for treatment of obesity and cardiometabolic disorders were 2.5 times more likely to drop out from the study because of depression and depressive symptoms than those who took placebo, and 3 times more likely to discontinue treatment because of anxiety (Mitchell and Morris, 2007). Consistent with these findings, patients from our study who developed new-onset depression or depressive-like symptoms after CS had significantly lower plasma anandamide and 2-AG concentrations during the highly stressful perioperative period. Interestingly, a similar EC response pattern was found in severely stressed participants of a recent parabolic flight experiment who developed acute motion sickness. In contrast, volunteers who tolerated the experiment well and were only minimally stressed showed a significant increase in plasma ECs throughout the parabolic flight maneuvers (Chouker et al., 2010).

Interestingly, we have previously shown that loss of consciousness in our CS patients as a result of general anesthesia with a benzodiazepine and a volatile agent (isoflurane) led to a significant decline in plasma anandamide concentrations (Weis et al., 2010). Anandamide levels remained low during postoperative sedation with midazolam in the ICU. When patients regained consciousness after surgery in the ICU, anandamide levels returned to baseline. Comparable effects were seen in patients anesthetized with the volatile agent sevoflurane undergoing orthopedic surgery. In contrast, in patients from the same study who were anesthetized with the intravenous agent propofol, a known inhibitor of the anandamide degradation enzyme fatty acid amide hydrolase, anandamide blood concentrations were maintained and no significant reduction was seen during general anesthesia and surgery (Schelling et al., 2006). We have recently shown that administration of anesthetic doses of propofol shortly after an aversive learning experience elevated brain anandamide content and strengthened memory formation of the learning experience in rats (Hauer et al., 2011). These effects were not seen with midazolam. As the development of traumatic memories after a stressful situation is an important risk factor for the occurrence of anxiety and other stress-related disorders (Schelling et al., 2003; Kapfhammer et al., 2004), these findings suggest that the choice of sedative agents in the ICU might contribute to the neuroemotional outcome of CS (Hemmings and Mackie, 2011).

An interesting finding of the present study is the relationship between low preoperative 2-AG levels and the later development of ACD/delirium. Although there is currently no unequivocal biological explanation for this observation, there is clear evidence that ECs are markedly increased in response to pathogenic events in the brain (Shohami et al., 2011). Numerous experimental studies on models of brain toxicity, neuroinflammation, and trauma support the notion that ECs are part of the brain's compensatory or repair mechanisms (Shohami et al., 2011). 2-AG is known to regulate neurotransmission and neuroinflammation by activating CB1 receptors on neurons and CB2 receptors and abnormal cannabidiol-sensitive receptors on microglia in the brain, suggesting a

possible neuroprotective effect of 2-AG (Kreutz et al., 2009; Lourbopoulos et al., 2011). Patients with an inadequately low perioperative 2-AG response might be at risk for early postoperative ACD/delirium and for the later development of depression. A significant association between depression and delirium has been demonstrated in CS patients (Ronksley et al., 2011).

The present study has some technical limitations. The clinical nature of our study limited our study to investigations of peripheral EC activity, which may not necessarily reflect central EC signaling. Preclinical findings show, however, that elements of the peripheral EC system may mirror central dysfunctions of EC signaling (Centonze et al., 2008). There is, indeed, evidence that EC activity in peripheral nucleated blood cells is related to changes in central EC signaling, which could theoretically allow the use of peripheral EC measurements as a diagnostic tool for a number of neuropsychiatric disorders (Centonze et al., 2008). In addition, the stress associated with footshock administration to rats resulted in an almost simultaneous increase in anandamide concentrations in blood and several memory-related brain areas including the amygdala and hippocampus (Hauer et al., in preparation).

A further limitation of our study results from the fact that the number of patients available for the 6-month follow-up was considerably smaller than the number of patients initially randomized. Despite our intention-to-treat approach for data analyses, this could have led to a substantial degree of selection bias (e.g., patients with a less favorable outcome or a higher degree of depression could have avoided traveling to the study center). Furthermore, psychological interviews could be performed only at the 6-month follow-up. Thus, some patients could have had preexisting depression at study inclusion, which is common in patients undergoing CS (Stroobant and Vingerhoets, 2008). However, as we found no significant difference in depression self-rating scores at the preoperative time point between patients who had evidence of depression at 6 months after CS and those who did not, the findings suggest new onset of depression after CS.

In summary, a more pronounced perioperative EC response, independently of glucocorticoid treatment, appears to be protective with regard to the development of ACD and depression in severely stressed patients undergoing CS.

Acknowledgments: Else Kröner-Fresenius-Stiftung supported this study.
Clinical Trial Registration Information: NCT00490828 (http://clinicaltrials.gov/).

Received April 28, 2012; accepted August 1, 2012; previously published online September 24, 2012

References

Atsak, P., Hauer, D., Campolongo, P., Schelling, G., McGaugh, J.L., and Roozendaal, B. (2012). Glucocorticoids interact with the hippocamapal endocannabinoid system in impairing retrieval of contextual fear memory. Proc. Natl. Acad. Sci. USA *109*, 3504–3509.

Batkai, S., Pacher, P., Osei-Hyiaman, D., Radaeva, S., Liu, J., Harvey-White, J., Offertaler, L., Mackie, K., Rudd, M.A., Bukoski, R.D., et al. (2004). Endocannabinoids acting at cannabinoid-1 receptors regulate cardiovascular function in hypertension. Circulation *110*, 1996–2002.

Begg, M., Baydoun, A., Parsons, M.E., and Molleman, A. (2001). Signal transduction of cannabinoid CB1 receptors in a smooth muscle cell line. J. Physiol. *531*, 95–104.

Bortolato, M., Campolongo, P., Mangieri, R.A., Scattoni, M.L., Frau, R., Trezza, V., La Rana, G., Russo, R., Calignano, A., Gessa, G.L., et al. (2006). Anxiolytic-like properties of the anandamide transport inhibitor AM404. Neuropsychopharmacology *31*, 2652–2659.

Burker, E.J., Blumenthal, J.A., Feldman, M., Burnett, R., White, W., Smith, L.R., Croughwell, N., Schell, R., Newman, M., and Reves, J.G. (1995). Depression in male and female patients undergoing cardiac surgery. Br. J. Clin. Psychol. *34*(Pt 1), 119–128.

Campolongo, P., Roozendaal, B., Trezza, V., Hauer, D., Schelling, G., McGaugh, J.L., and Cuomo, V. (2009). Endocannabinoids in the rat basolateral amygdala enhance memory consolidation and enable glucocorticoid modulation of memory. Proc. Natl. Acad. Sci. USA *106*, 4888–4893.

Centonze, D., Battistini, L., and Maccarrone, M. (2008). The endocannabinoid system in peripheral lymphocytes as a mirror of neuroinflammatory diseases. Curr. Pharm. Des. *14*, 2370–2342.

Chouker, A., Kaufmann, I., Kreth, S., Hauer, D., Feuerecker, M., Thieme, D., Vogeser, M., Thiel, M., and Schelling, G. (2010). Motion sickness, stress and the endocannabinoid system. PLoS One *5*, e10752.

Cote, M., Matias, I., Lemieux, I., Petrosino, S., Almeras, N., Despres, J.P., and Di Marzo, V. (2007). Circulating endocannabinoid levels, abdominal adiposity and related cardiometabolic risk factors in obese men. Int. J. Obes. (Lond.) *31*, 692–699.

Di Marzo, V., Verrijken, A., Hakkarainen, A., Petrosino, S., Mertens, I., Lundbom, N., Piscitelli, F., Westerbacka, J., Soro-Paavonen, A., Matias, I., et al. (2009). Role of insulin as a negative regulator of plasma endocannabinoid levels in obese and nonobese subjects. Eur. J. Endocrinol. *161*, 715–722.

Domschke, K. and Zwanzger, P. (2008). GABAergic and endocannabinoid dysfunction in anxiety – future therapeutic targets? Curr. Pharm. Des. *14*, 3508–3517.

Eisenstein, T.K., Meissler, J.J., Wilson, Q., Gaughan, J.P., and Adler, M.W. (2007). Anandamide and Delta9-tetrahydrocannabinol

directly inhibit cells of the immune system via CB2 receptors. J. Neuroimmunol. *189*, 17–22.

Fajardo, G. and Bernstein, D. (2007). Endocannabinoid inhibition: a new cardioprotective strategy against doxorubicin cardiotoxicity. J. Am. Coll. Cardiol. *50*, 537–539.

Gobbi, G., Bambico, F.R., Mangieri, R., Bortolato, M., Campolongo, P., Solinas, M., Cassano, T., Morgese, M.G., Debonnel, G., Duranti, A., et al. (2005). Antidepressant-like activity and modulation of brain monoaminergic transmission by blockade of anandamide hydrolysis. Proc. Natl. Acad. Sci. USA *102*, 18620–18625.

Gorzalka, B.B., Hill, M.N., and Hillard, C.J. (2008). Regulation of endocannabinoid signaling by stress: implications for stress-related affective disorders. Neurosci. Biobehav. Rev. *32*, 1152–1160.

Hamilton, M. (1960). A rating scale for depression. J. Neurol. Neurosurg. Psychiatry *23*, 56–62.

Hauer, D., Ratano, P., Morena, M., Scaccianoce, S., Briegel, I., Palmery, M., Cuomo, V., Roozendaal, B., Schelling, G., and Campolongo, P. (2011). Propofol enhances memory formation via an interaction with the endocannabinoid system. Anesthesiology *114*, 1380–1388.

Hemmings, H.C., Jr. and Mackie, K. (2011). The rivers of lethe and mnemosyne converge: propofol and memory consolidation. Anesthesiology *114*, 1277–1279.

Hill, M.N. and Gorzalka, B.B. (2009). Impairments in endocannabinoid signaling and depressive illness. J. Am. Med. Assoc. *301*, 1165–1166.

Hill, M.N., Miller, G.E., Ho, W.S., Gorzalka, B.B., and Hillard, C.J. (2008). Serum endocannabinoid content is altered in females with depressive disorders: a preliminary report. Pharmacopsychiatry *41*, 48–53.

Hill, M.N., Miller, G.E., Carrier, E.J., Gorzalka, B.B., and Hillard, C.J. (2009). Circulating endocannabinoids and N-acyl ethanolamines are differentially regulated in major depression and following exposure to social stress. Psychoneuroendocrinology *34*, 1257–1268.

Hill, M.N., Patel, S., Campolongo, P., Tasker, J.G., Wotjak, C.T., and Bains, J.S. (2010). Functional interactions between stress and the endocannabinoid system: from synaptic signaling to behavioral output. J. Neurosci. *30*, 14980–14986.

Kapfhammer, H.P., Rothenhausler, H.B., Krauseneck, T., Stoll, C., and Schelling, G. (2004). Posttraumatic stress disorder and health-related quality of life in long-term survivors of acute respiratory distress syndrome. Am. J. Psychiatry *161*, 45–52.

Kaufmann, I., Schelling, G., Eisner, C., Richter, H.P., Krauseneck, T., Vogeser, M., Hauer, D., Campolongo, P., Chouker, A., Beyer, A., et al. (2008). Anandamide and neutrophil function in patients with fibromyalgia. Psychoneuroendocrinology *33*, 676–685.

Kilger, E., Weis, F., Briegel, J., Frey, L., Goetz, A.E., Reuter, D., Nagy, A., Schuetz, A., Lamm, P., Knoll, A., et al. (2003). Stress doses of hydrocortisone reduce severe systemic inflammatory response syndrome and improve early outcome in a risk group of patients after cardiac surgery. Crit. Care Med. *31*, 1068–1074.

Kreutz, S., Koch, M., Bottger, C., Ghadban, C., Korf, H.W., and Dehghani, F. (2009). 2-Arachidonoylglycerol elicits neuroprotective effects on excitotoxically lesioned dentate gyrus granule cells via abnormal-cannabidiol-sensitive receptors on microglial cells. Glia *57*, 286–294.

Lourbopoulos, A., Grigoriadis, N., Lagoudaki, R., Touloumi, O., Polyzoidou, E., Mavromatis, I., Tascos, N., Breuer, A., Ovadia, H., Karussis, D., et al. (2011). Administration of 2-arachidonoylglycerol ameliorates both acute and chronic experimental autoimmune encephalomyelitis. Brain Res. *1390*, 126–141.

Mach, F. and Steffens, S. (2008). The role of the endocannabinoid system in atherosclerosis. J. Neuroendocrinol. *20*(Suppl 1), 53–57.

Maldonado, R., Valverde, O., and Berrendero, F. (2006). Involvement of the endocannabinoid system in drug addiction. Trends Neurosci. *29*, 225–232.

Marsicano, G., Wotjak, C.T., Azad, S.C., Bisogno, T., Rammes, G., Cascio, M.G., Hermann, H., Tang, J., Hofmann, C., Zieglgansberger, W., et al. (2002). The endogenous cannabinoid system controls extinction of aversive memories. Nature *418*, 530–534.

Mitchell, P.B. and Morris, M.J. (2007). Depression and anxiety with rimonabant. Lancet *370*, 1671–1672.

Moreira, F.A., Grieb, M., and Lutz, B. (2009). Central side-effects of therapies based on CB1 cannabinoid receptor agonists and antagonists: focus on anxiety and depression. Best Pract. Res. Clin. Endocrinol. Metab. *23*, 133–144.

Nissen, S.E., Nicholls, S.J., Wolski, K., Rodes-Cabau, J., Cannon, C.P., Deanfield, J.E., Despres, J.P., Kastelein, J.J., Steinhubl, S.R., Kapadia, S., et al. (2008). Effect of rimonabant on progression of atherosclerosis in patients with abdominal obesity and coronary artery disease: the STRADIVARIUS randomized controlled trial. J. Am. Med. Assoc. *299*, 1547–1560.

Onaivi, E.S., Ishiguro, H., Gong, J.P., Patel, S., Perchuk, A., Meozzi, P.A., Myers, L., Mora, Z., Tagliaferro, P., Gardner, E., et al. (2006). Discovery of the presence and functional expression of cannabinoid CB2 receptors in brain. Ann. NY Acad. Sci. *1074*, 514–536.

Pacher, P. (2009). Cannabinoid CB1 receptor antagonists for atherosclerosis and cardiometabolic disorders: new hopes, old concerns? Arterioscler. Thromb. Vasc. Biol. *29*, 7–9.

Patel, S. and Hillard, C.J. (2008). Adaptations in endocannabinoid signaling in response to repeated homotypic stress: a novel mechanism for stress habituation. Eur. J. Neurosci. *27*, 2821–2829.

Pertwee, R.G. (2008). Ligands that target cannabinoid receptors in the brain: from THC to anandamide and beyond. Addict. Biol. *13*, 147–159.

Ronksley, P.E., Brien, S.E., Turner, B.J., Mukamal, K.J., and Ghali, W.A. (2011). Association of alcohol consumption with selected cardiovascular disease outcomes: a systematic review and meta-analysis. Br. Med. J. *342*, d671.

Schelling, G., Richter, M., Roozendaal, B., Rothenhausler, H.B., Krauseneck, T., Stoll, C., Nollert, G., Schmidt, M., and Kapfhammer, H.P. (2003). Exposure to high stress in the intensive care unit may have negative effects on health-related quality-of-life outcomes after cardiac surgery. Crit. Care Med. *31*, 1971–1980.

Schelling, G., Hauer, D., Azad, S.C., Schmoelz, M., Chouker, A., Schmidt, M., Hornuss, C., Rippberger, M., Briegel, J., Thiel, M., et al. (2006). Effects of general anesthesia on anandamide blood levels in humans. Anesthesiology *104*, 273–277.

Schmidt, A., Brune, K., and Hinz, B. (2006). Determination of the endocannabinoid anandamide in human plasma by high-performance liquid chromatography. Biomed. Chromatogr. *20*, 336–342.

Shohami, E., Cohen-Yeshurun, A., Magid, L., Elgali, M., and Mechoulam, R. (2011). Endocannabinoids and traumatic brain injury. Br. J. Pharmacol *163*, 1402–1410.

Stoll, C., Schelling, G., Goetz, A.E., Kilger, E., Bayer, A., Kapfhammer, H.P., Rothenhausler, H.B., Kreuzer, E., Reichart, B., and Peter, K. (2000). Health-related quality of life and post-traumatic stress disorder in patients after cardiac surgery and intensive care treatment. J. Thorac. Cardiovasc. Surg. *120*, 505–512.

Stroobant, N. and Vingerhoets, G. (2008). Depression, anxiety, and neuropsychological performance in coronary artery bypass graft patients: a follow-up study. Psychosomatics *49*, 326–331.

Sugamura, K., Sugiyama, S., Nozaki, T., Matsuzawa, Y., Izumiya, Y., Miyata, K., Nakayama, M., Kaikita, K., Obata, T., Takeya, M., et al. (2009). Activated endocannabinoid system in coronary artery disease and antiinflammatory effects of cannabinoid 1 receptor blockade on macrophages. Circulation *119*, 28–36.

Treggiari, M.M., Romand, J.A., Yanez, N.D., Deem, S.A., Goldberg, J., Hudson, L., Heidegger, C.P., and Weiss, N.S. (2009). Randomized trial of light versus deep sedation on mental health after critical illness. Crit. Care Med. *37*, 2527–2534.

Vickers, S.P. and Kennett, G.A. (2005). Cannabinoids and the regulation of ingestive behaviour. Curr. Drug Targets *6*, 215–223.

Vogeser, M. and Schelling, G. (2007). Pitfalls in measuring the endocannabinoid 2-arachidonoyl glycerol in biological samples. Clin. Chem. Lab. Med. *45*, 1023–1025.

Vogeser, M., Hauer, D., Christina Azad, S., Huber, E., Storr, M., and Schelling, G. (2006). Release of anandamide from blood cells. Clin. Chem. Lab. Med. *44*, 488–491.

Weis, F., Beiras-Fernandez, A., Schelling, G., Briegel, J., Lang, P., Hauer, D., Kreth, S., Kaufmann, I., Lamm, P., and Kilger, E. (2009a). Stress doses of hydrocortisone in high-risk patients undergoing cardiac surgery: effects on interleukin-6 to interleukin-10 ratio and early outcome. Crit. Care Med. *37*, 1685–1690.

Weis, F., Beiras-Fernandez, A., Sodian, R., Kaczmarek, I., Reichart, B., Beiras, A., Schelling, G., and Kreth, S. (2009b). Substantially altered expression pattern of cannabinoid receptor 2 and activated endocannabinoid system in patients with severe heart failure. J. Mol. Cell Cardiol. *48*, 1187–1193.

Weis, F., Beiras-Fernandez, A., Hauer, D., Hornuss, C., Sodian, R., Kreth, S., Briegel, J., and Schelling, G. (2010). Effect of anaesthesia and cardiopulmonary bypass on blood endocannabinoid concentrations during cardiac surgery. Br. J. Anaest. *105*, 139–144.

Wolf, S.A., Tauber, S., and Ullrich, O. (2008). CNS immune surveillance and neuroinflammation: endocannabinoids keep control. Curr. Pharm. Des. *14*, 2266–2278.