Effect of anaesthesia and cardiopulmonary bypass on blood endocannabinoid concentrations during cardiac surgery

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The human endocannabinoid system (ECS) is a complex and pleiotropic system that was discovered in the late 1980s. Major features of this system are: (i) at least two G-protein-coupled receptors, known as the cannabinoid receptors type 1 and 2 (CB1 and CB2), and (ii) endogenous ligands which are derivatives of arachidonic acid, the best known of which are anandamide (AEA) and 2-arachidonoylglycerol (2-AG); it has potentially important roles in cardiovascular regulation, cardiovascular diseases, inflammation, and pain pathways. While CB1 is expressed mainly on neuronal tissue, but also peripherally on endothelial cells, immune cells, and on solid organs, CB2 is present mainly on immune cells, but also on neuronal and other tissues. The ECS is involved in the regulation of a number of physiological and pathophysiological processes including inflammation, arterial and pulmonary arterial pressure regulation, coronary and cerebral vasodilation, and various types of shock. Furthermore, the ECS is thought to contribute to human atherosclerosis by activating pro-inflammatory activity in macrophages within atherosclerotic lesions. Only recently, endocannabinoid receptors have been identified on human myocardium and heart failure has been shown to substantially alter their expression pattern.

Background. The endocannabinoid system (ECS) is an endogenous signalling system which includes the endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG) and specific G-protein-coupled endocannabinoid receptors (CB1 and CB2). Recent studies have described important roles of the peripheral ECS in human atherosclerosis, cardiometabolic disorders, heart failure, and systemic inflammation. We sought to study changes in plasma endocannabinoid concentrations during cardiac surgery (CS) under general anaesthesia with isoflurane/sufentanil, and during cardiopulmonary bypass (CPB).

Methods. We studied 30 patients undergoing CS with CPB. All patients received midazolam and sufentanil for induction and isoflurane and sufentanil for maintenance of general anaesthesia. Blood samples were drawn before and after induction of general anaesthesia, after the beginning of surgery, during and after weaning from CPB, and after admission to intensive care unit (ICU) after surgery. Endocannabinoid measurements were performed by HPLC-tandem mass spectrometry.

Results. Induction of general anaesthesia led to a significant decline in plasma AEA concentrations [from mean (ss) 0.39 (0.03) to 0.27 (0.03) ng ml⁻¹, P<0.01]. CPB induced a pronounced increase in 2-AG concentrations [from 112.5 (163.5) to 321.0 (120.4) ng ml⁻¹, P<0.01], whereas AEA concentrations remained persistently low until admission to the ICU. 2-AG concentrations returned to preoperative values after surgery.

Conclusions. General anaesthesia with isoflurane significantly reduces plasma AEA concentrations. This could be a consequence of stress reduction after loss of consciousness. The significant increase in 2-AG after initiation of CPB may be part of an inflammatory response. These findings suggest that anaesthesia and surgery have differential effects on the ECS which could have substantial clinical consequences.

Key points
- The ECS includes the AEA and 2-AG; it has potentially important roles in cardiovascular regulation, cardiovascular diseases, inflammation, and pain pathways.
- In 30 adult patients undergoing cardiac surgery, AEA concentrations decreased after induction of anaesthesia and remained below baseline.
- 2-AG concentrations increased significantly after the onset of both surgery and CPB, but returned towards baseline after CPB.
- This may reflect different responses of the ECS to sympathetic nervous system activation, inflammatory pathways, anaesthetic drugs, and surgery.

Keywords: anaesthesia, general; cardiac surgical procedures; cardiopulmonary bypass; endocannabinoids, anandamide, 2-arachidonoylglycerol

Accepted for publication: 26 March 2010
Understanding these processes may help the anaesthetist to provide optimal perioperative care, particularly for patients suffering from cardiovascular diseases and undergoing major operative procedures, for example, cardiac surgery (CS) using cardiopulmonary bypass (CPB). In the field of pain medicine, recent research has discovered CB receptors to be involved in the pathogenesis of neuropathic pain, neuro-inflammation, and cannabinoids may exert analgesic effects. Cannabinoids acting via neuronal pre-synaptic CB1 receptors modulate neurotransmitter release. Exogenous cannabinoids reveal analgesic effects in animal models, whereas endocannabinoids may mediate a physiological anti-nociceptive ‘tone’. Synergism between CB receptor activation and opioid therapy may reduce opioid requirements.

In the perioperative setting, the role of the ECS seems to be of special interest, as Schelling and colleagues recently demonstrated that different types of general anaesthesia regimens (i.e. propofol vs etomidate/sevoflurane) modulate plasma AEA concentrations in different ways: while etomidate/sevoflurane led to a significant reduction in plasma AEA concentrations, propofol, which is an inhibitor of the AEA metabolizing enzyme fatty acid amide hydrolase (FAAH), resulted in a moderate increase in plasma AEA concentrations after induction of anaesthesia and for a further 40 min (including the initial steps of the respective operative procedures). However, 2-AG concentrations were not determined in that study, probably because correct measurement of 2-AG is difficult and has only recently been well established. Another important issue not addressed by that study is whether or not the deactivating effects of inhalation anaesthesia on the ECS are still present during major surgical procedures. Furthermore, it is yet unclear if the deactivating effect of sevoflurane is specific to the drug itself or to the substance group of volatile anaesthetics.

Therefore, we performed a prospective, observational study to determine the impact of CS with CPB under volatile anaesthesia using isoflurane on perioperative plasma AEA concentrations and 2-AG concentrations.

Methods

The study was approved by the Institutional Review Board of the Ludwig-Maximilians University of Munich (protocol number 089/04) and every patient gave informed consent before enrolment. Data protection met the standard set by German law.

Patients

Thirty patients >18 yr old undergoing coronary artery bypass grafting (CABG), valve surgery, and combined procedures using CPB were enrolled in this prospective-observational study from January to June 2007. Patients were excluded from the study if they were undergoing cardiac transplantation, minimally invasive or emergency procedures, if they had preoperative chronic renal dysfunction, as indicated by plasma creatinine concentrations >2.0 mg dl\(^{-1}\), or impaired liver function, as indicated by a plasma bilirubin concentration >1.2 mg dl\(^{-1}\).

Intraoperative anaesthetic management

Preoperative medication was maintained until surgery except for oral antidiabetic drugs and monoamine oxidase inhibitors. Monitoring included electrocardiography with ST-segment analysis of leads II and V5 and pulse oximetry. Anaesthesia was induced with midazolam 0.15–0.25 mg kg\(^{-1}\) body weight (midazolam has no known interference with the metabolism of endocannabinoids), sufentanil 1–3 \(\mu\)g kg\(^{-1}\), and pancuronium 0.1 mg kg\(^{-1}\), and maintained with sufentanil 1–2 mg kg\(^{-1}\) h\(^{-1}\) and isoflurane 0.3–1.0%. After oro-tracheal intubation, a double-lumen central venous catheter and an 8.5 G introducer for a pulmonary artery catheter were inserted into the right internal jugular vein. In cases of difficult weaning from bypass, a pulmonary artery catheter was inserted. If a mean arterial pressure of 70 mm Hg could not be maintained despite normovolaemia as indicated by a central venous pressure of 8–12 mm Hg or a pulmonary capillary wedge pressure of 12–15 mm Hg at zero PEEP, cardiac function was assessed by transoesophageal echocardiography (TOE). If impaired cardiac function could be ruled out, norepinephrine was used as a first-line vasopressor drug. If necessary, inotropic support, using epinephrine as the first-line drug, was guided by TOE examination.

CS with CPB was performed with mild hypothermia [mean (so) 31 (2) \(^\circ\)C]. During bypass, a mean arterial pressure of 60 mm Hg was maintained by a minimal flow rate of 2.4 litre m\(^{-2}\) min\(^{-1}\). Myocardial protection was performed with cold hyperkalaemic cardioplegic solution (HTK Bretschneider solution; Dr F. Köhler Chemie, Alsbach, Germany) and additional topical ice slush.

Perioperative measurements

We recorded patients’ baseline and clinical variables including age, sex, BMI, preoperative ejection fraction, and intraoperative details including the duration of CPB and the aortic cross-clamping time.

Plasma concentrations of AEA and 2-AG were measured before operation in the awake state before anaesthesia, after induction of anaesthesia, and tracheal intubation, intraoperatively during surgery but before CPB, during CPB, after termination of CPB, and after operation at admission to the intensive care unit (ICU).

Measurement of endocannabinoid concentrations

We used a method to determine plasma concentrations of the endocannabinoids AEA and 2-AG in humans based on high performance liquid chromatography-tandem mass spectrometry (HPLC/MS-MS) which has been described previously. The method is linear within a range of 0.1–2 ng ml\(^{-1}\) for AEA and 0.5–10 ng ml\(^{-1}\) for 2-AG. The inter-assay coefficient of variation is 34% for a mean AEA concentration of 0.2 ng ml\(^{-1}\). The lower limit of detection of the method...
(defined as a signal/noise ratio $>4:1$) is 0.025 ng ml$^{-1}$ for AEA and 0.33 ng ml$^{-1}$ for 2-AG.

For endocannabinoid measurements, blood samples were drawn into EDTA-containing tubes (S-Monovette®, Sarstedt, Numbrecht, Germany) and immediately (within 5 min) centrifuged. The time interval between blood sampling and centrifugation was minimized because previous experiments have shown that endocannabinoid generation in blood samples is continued ex vivo.$^{17}$ Thus, delays in blood processing could result in false positive increases in plasma endocannabinoid concentrations. In biological matrices, 2-AG (including its deuterated analogue) is rapidly isomerized to 1-AG.$^{15}$ We therefore quantified 2-AG as the sum of 1- and 2-esters of arachidonic acid.

In healthy volunteers [$n$=20, 10 female, mean (so) age 36 (8) yr, BMI=22.0 (2.5)], our method for endocannabinoid measurements resulted in mean (so) plasma AEA concentrations of 0.25 (0.23) and 6.70 (4.10) ng ml$^{-1}$ for 2-AG.$^{17}$

Statistical analyses

Power analysis

This study intended to test the hypothesis that general anaesthesia with isoflurane results in a significant reduction in endocannabinoid plasma concentrations. The estimation of the required sample size for the study was based on our previous investigation which found a mean (so) decline of 2.1 (1.9) ng ml$^{-1}$ of AEA at 20 min after induction of anaesthesia.$^{14}$ On the basis of using a paired t-test and after correction for multiple comparisons ($\alpha=0.008$), a sample size of 10 was estimated to be sufficient to demonstrate a statistically significant difference in AEA concentrations. The final sample size in our study was considerably higher, particularly because 2-AG is known to have a larger inter-individual range.

Statistical procedures

All variables were tested for normal distribution using the Lilliefors modification of the Kolmogorov–Smirnov test. Changes in endocannabinoid plasma concentrations across the six time points of measurements were compared using one-way repeated-measures analyses of variance (one-way RM-ANOVA) with the Holm–Sidak post hoc test to control for multiple comparisons. The Pearson correlation coefficient was calculated as a measure of linear association between parametric variables, Spearman’s $\rho$ was used for non-normally distributed data. A P-value of $<0.05$ was regarded as statistically significant. Data are presented as mean (so) with exception of figures, where mean (SEM) is used to increase clarity. Statistical calculations were performed using PASW Statistics 17.0 and SigmaPlot 11.0, Chicago, IL, USA.

Results

Patient data

Thirty patients (24 males and six females) were recruited for this observational trial and all were included in the analysis (Table 1). CABG was performed in 73.3% of the patients, 26.6% underwent aortic valve replacement, and 10% mitral valve surgery. Intraoperative clinical data are shown in Table 2.

Perioperative plasma endocannabinoid concentrations

Induction of general anaesthesia led to a significant decline in AEA plasma concentrations [from 0.41 (0.15) at the awake state to 0.28 (0.13) ng ml$^{-1}$ after loss of consciousness ($P<0.01$, Fig. 1)]. Plasma concentrations of 2-AG were also lower after onset of anaesthesia and declined from 41.4 (57.2) to 30.4 (78.1) ng ml$^{-1}$, but this reduction was not statistically significant ($P=0.77$). While AEA concentrations remained significantly lower concentrations when compared with baseline awake values throughout the whole observation period, 2-AG concentrations increased significantly after the start of surgery and reached maximal concentrations during CPB [from 112.5 (163.5) to 321.0 (120.4) ng ml$^{-1}$, $P<0.01$]. After termination of CPB, 2-AG concentrations decreased significantly and were close to preoperative values at admission to the cardiovascular ICU (Fig. 1).

Correlations between plasma concentrations of endocannabinoids and clinical parameters

Preoperative AEA concentrations correlated positively with the patients’ BMI ($r=0.43$; $P=0.02$) (Fig. 2). AEA concentrations during CPB correlated positively with the maximum intraoperative dose of norepinephrine ($r=0.39$; $P=0.04$), whereas concentrations after CPB correlated negatively with the highest dose of insulin ($r=-0.56$; $P<0.01$) and length of stay (LOS) in the ICU ($r=-0.39$; $P=0.03$). Concentrations of AEA after admission to the ICU were negatively correlated with the time of weaning the patient from vasopressors ($r=-0.39$; $P=0.03$).

Table 1 Patient data expressed as mean (range), mean (so) or number. NYHA, New York Heart Association class; CCS, Canadian Cardiovascular Society class; ASA, American Society of Anesthesiology

<table>
<thead>
<tr>
<th>Variable</th>
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<tbody>
<tr>
<td>Age (yr)</td>
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<tr>
<td>Height (m)</td>
<td>1.71 (0.09)</td>
</tr>
<tr>
<td>Weight (kg)</td>
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<tr>
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<tr>
<td>CCS</td>
<td>III (0)</td>
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<td>ASA</td>
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<tr>
<td>Mitral regurgitation (n)</td>
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</tr>
<tr>
<td>Beta-blockers (n)</td>
<td>21</td>
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<td>ACE inhibitors (n)</td>
<td>16</td>
</tr>
<tr>
<td>Statins (n)</td>
<td>21</td>
</tr>
<tr>
<td>Ejection fraction (%)</td>
<td>57 (16)</td>
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in plasma concentrations of AEA and a non-significant decline in 2-AG concentration. The surgical procedure and CPB, however, appeared to have differential effects on both endocannabinoids. Whereas CPB was associated with an extreme elevation of plasma 2-AG concentrations, AEA concentrations were not significantly influenced by extracorporeal circulation. Plasma concentrations of AEA were related to the patients’ BMI, their preoperative ejection fraction, intraoperative need for vasopressors, the amount of insulin administered, and the LOS in the ICU. On the other hand, 2-AG concentrations were related to postoperative plasma concentrations of IL-6 and pulmonary dysfunction.

The effects of general anaesthesia based on propofol with that of sevoflurane. Comparable with the present findings, the use of sevoflurane resulted in a significant decline in blood AEA concentrations. In contrast, during propofol anaesthesia, plasma AEA concentrations showed a moderate increase and were significantly higher than in patients anaesthetized with sevoflurane. The latter effect of propofol can probably be explained by an inhibitory effect of propofol on FAAH, the main degradation enzyme of AEA.13 In both studies, propofol was omitted for induction and for maintenance of anaesthesia, which should preclude a significant interaction with the endocannabinoid metabolism.18

The mechanisms by which the onset of general anaesthesia results in a decline of plasma endocannabinoid concentrations are unknown. There is evidence that endocannabinoid signalling in the brain19 and in the periphery20 increases in response to stress. Physiological stress is associated with increased concentrations of circulating catecholamines which bind among other targets to

### Discussion

The present study shows that general anaesthesia using midazolam/sufentanil/isoflurane leads to a significant reduction related to the preoperative ejection fraction \(r = -0.468\); \(P = 0.03\) and the highest intraoperative dose of insulin \(r = -0.51; P < 0.01\).

2-AG during surgery correlated positively with interleukin-6 (IL-6) concentrations in the ICU \(r = 0.68; P < 0.01\), and the 2-AG concentrations after weaning from CPB correlated negatively with maximum IL-6 concentrations during the ICU stay \(r = -0.67; P = 0.04\). Preoperative 2-AG concentrations were positively related to the lowest \(P_{acp} / F_i_{CO}_2\) ratio \(r = 0.51; P = 0.01\) and negatively with the duration of mechanical ventilation \(r = -0.43; P = 0.03\).
α-adrenergic receptors on blood cells and in many peripheral organs. Stimulation of α-adrenergic receptors has been shown to induce the synthesis of endocannabinoids through activation of phospholipase C.21 22 Because CB1 receptors are present on sympathetic nerve terminals and their activation results in inhibition of norepinephrine release,23 it has been suggested that a major function of the ECS lies in buffering the sympatho-adrenergic response to stress.22 This view is corroborated by the fact that the ECS also has an inhibitory effect on stress-induced activation of the hypothalamic–pituitary–adrenal axes.23 The exact mechanism of interaction between the ECS and general anaesthesia has to be determined in animal studies.

It is, however, of interest to note that AEA concentrations stayed low throughout the study period despite the physical and inflammatory stress of the surgical procedure.26 In contrast, 2-AG concentrations were significantly elevated by surgery and in particular during CPB. This could be explained by the fact that 2-AG appears to be the immunologically more active endocannabinoid:25 26 2-AG concentrations at 30 min after skin incision correlated positively with the IL-6 concentrations on admission, whereas 2-AG concentrations after termination of the CPB were negatively correlated with the maximum plasma concentrations of IL-6 in the ICU. As 2-AG is currently regarded as an anti-inflammatory mediator,27 these findings may be conclusive.

The source of blood endocannabinoids has not been fully elucidated. Adipocytes, endothelial cells, visceral organs, and peripheral mononuclear cells are all able to synthesize endocannabinoids. In this regard, it is important to note that endocannabinoid synthesis by blood cells is not limited to the in vivo situation but has been shown to continue ex vivo in stored blood samples.16 17 Thus, valid endocannabinoid measurements require immediate centrifugation and freezing (within a few minutes) of blood samples. Whereas the measurement of AEA appears to be straightforward, the exact quantification of 2-AG may be more problematic. 2-AG undergoes rapid non-enzymatic isomerization into the biological inactive 1-AG in samples of whole blood and in plasma.18 This effect seems to be less pronounced if blood samples are processed immediately. Nevertheless, there is currently no practicable solution to this problem other than processing blood samples immediately and being aware of the fact that at least a part of the measured 2-AG is in fact biologically inactive 1-AG. Thus, most studies including our investigations report the sum of 1-AG and 2-AG sample concentrations. This should not play a major role when measurements across time are performed and all blood samples are processed in the same way, as it was the case in our study. Furthermore, the level of statistical significance of the comparison of the six time points was very high for 2-AG which increased more than 10-fold, thereby allowing the conclusion that the relative changes found are real and of clinical relevance.

The fact that plasma AEA concentrations showed a positive correlation with patients’ BMI (Fig. 2) is consistent with a number of studies demonstrating that the ECS is overactivated in human obesity, especially in visceral adipose tissue, which is closely related to a high risk of type-2 diabetes and cardiovascular disease. We found that patients with high intraoperative doses of insulin have low post-CPB values of AEA. This may be the consequence of an insulin-induced increase in the activity of the FAAH (i.e. the enzyme that degrades AEA) as described by Murdolo and colleagues.28

High concentrations of AEA were also associated with the administration of higher doses of norepinephrine. This is in line with the abovementioned ability of endocannabinoids to limit the endogenous sympatho-adrenergic stress response.19 AEA itself has been shown to induce hypotension via activation of CB1, but also of transient receptor potential vanilloid type 1 (TRPV1) receptors.29 This AEA-induced hypotension has already been demonstrated to be of relevance in septic, haemorrhagic, and cardiogenic shock.8 Thus, the potential role of AEA-induced hypotension as part of the post-pump syndrome is a very interesting finding, especially as CB1 antagonists have already been used successfully in various types of experimental shock.30

In conclusion, general anaesthesia using midazolam/sufentanil/isoflurane leads to a significant reduction in plasma concentrations of AEA. Although 2-AG concentrations are substantially elevated by the surgical procedure and especially CPB, AEA concentrations do not show significant changes during the operative procedure. Changes in plasma concentrations of endocannabinoids are related to a number of clinical and immunological parameters which may be of significant biological relevance in the perioperative period.

Conflict of interest

None declared.

Funding

This study was supported by the Else Kröner-Fresenius Foundation (Project number P50/06/A40/06).

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