Effects of General Anesthesia on Anandamide Blood Levels in Humans

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Background: The endocannabinoid system includes G-protein-coupled cannabinoid receptors, the endocannabinoids N-arachidonylethanolamine (anandamide) and 2-arachidonoylglycerol, and multiple enzymes involved in the biosynthesis and degradation of endocannabinoids, including the anandamide metabolizing enzyme fatty acid amide hydrolase. Endocannabinoids play an important role in the physiologic control of sleep, pain processing, and emesis. The authors therefore investigated the effects of general anesthesia on the endocannabinoid system in humans.

Methods: The authors measured whole blood levels of anandamide in 12 patients after induction of general anesthesia with etomidate (an agent shown to have no effect on anandamide levels) and maintenance of anesthesia with the volatile agent sevoflurane as well as in 12 patients undergoing total intravenous anesthesia with propofol, a known inhibitor of fatty acid amide hydrolase in the mouse brain. Anandamide levels were measured using high-performance liquid chromatography–tandem mass spectrometry at four time points (before and at 10, 20, 30, and 40 min after induction of anesthesia).

Results: Patients of the sevoflurane group showed a significant decline in anandamide levels from induction of anesthesia to 40 min after induction, whereas anandamide levels in patients of the propofol group remained unchanged (type III sum of squares = 1725.66, F = 162.60, P < 0.001, repeated-measures analysis of variance).

Conclusion: General anesthesia influences the endocannabinoid system in a drug-dependent way, which may explain side effects of general anesthetics such as psychomimetic and antiemetic properties of propofol and the high incidence of postoperative nausea and vomiting after volatile anesthetics. These findings suggest new targets for anesthetic drug development.

THE endogenous cannabinoid system includes a distinct class of lipid mediators, the so-called endocannabinoids and specific G-protein-coupled receptors, which are currently divided into two types, CB₁ and CB₂. CB₁ receptors are found in most brain areas, generally with a presynaptic localization and an important role in controlling neurotransmitter release. Central endocannabinoid effects mediated by CB₁ receptors include important roles in disorders involving movement, cognition, mood, dependence, memory, and the regulation of visceral function, or fertility and the regulation of appetite or sexual behavior. In addition, endocannabinoids control sleep, pain, and emesis. CB₂ receptors and their ligands are found primarily in the periphery, especially in immune and endothelial cells. Cannabinoids have been shown to modulate a variety of immune cell functions in humans and animals, which include T-helper cell development, chemotaxis, and tumor development. In addition, the endocannabinoid system seems to be involved in the regulation of peripheral vascular tone, with a possible protective effect during shock and myocardial infarction. Likewise, hypotension associated with sepsis, hemorrhage, and the use of bone cement during orthopedic surgery may be mediated by activation of endocannabinoid receptors.

N-Arachidonylethanolamine (anandamide) is an important endogenous cannabinoid that is synthesized by neurons and peripheral tissues including nucleated blood cells. The biologic activity of anandamide is terminated mainly through hydrolyzes, which primarily occurs by means of the enzyme fatty acid amide hydrolase (FAAH). Propofol has recently been shown to inhibit FAAH in mice after intraperitoneal administration, thereby increasing brain anandamide content. Whether the endocannabinoid system is involved in humans undergoing general anesthesia with propofol or a volatile agent has, to our knowledge, never been studied. We performed a pilot study to test the hypothesis that the use of propofol results in an increase of systemic anandamide levels and thus in an activation of the endocannabinoid system in humans.

Materials and Methods

The study was approved by the Institutional Review Board of the Ludwig-Maximilians University, Munich, Germany (protocol No. 368/04). Data protection met the standard set by German law, and all patients gave written, informed consent to participate in the investigation.

Patient Selection and Characterization

Anandamide levels were measured in 24 patients undergoing routine orthopedic surgery after induction of anesthesia with etomidate (which is known to have no effect on anandamide levels and no in vitro effect on FAAH) and maintenance of general anesthesia with sevoflurane (n = 12) as well as in patients undergoing...
total intravenous anesthesia (TIVA) with propofol (n = 12).

The investigation was designed as a pilot study to generate data on possible effects of general anesthetics on anandamide levels. The allocation of patients to the treatment groups was therefore not randomized, and the choice of anesthetic technique was left to the discretion of the team of anesthesiologists responsible for perioperative care. Patient selection was performed in a balanced way, however, to result in an equal size of both groups and in an approximately equal distribution of surgical procedures between the propofol–TIVA and the etomidate–sevoflurane group (see Table 1 for a comparison of patient and treatment data between the two study samples). The choice of opioids (sufentanil) and muscle relaxants (atracurium) during the procedures was standardized between groups. All patients were premedicated with 7.5 mg oral midazolam approximately 30 min before induction of anesthesia. Anandamide levels were measured at four time points: before induction, 10 min after intubation, 20 min after intubation, and 40 min after intubation. This time interval did not include the administration of bone cement in patients with total hip or knee arthroplasty, which was used later during surgery. Bone cement has been shown to increase anandamide levels, and blood sampling was therefore terminated before bone cement was administered.

Measurement of Anandamide
We developed and validated a method for measurement of anandamide based on high-performance liquid chromatography–tandem mass spectrometry (HPLC/MS-MS). We applied automated on-line solid phase extraction using column switching with subsequent direct transfer to HPLC and a tandem mass spectrometry system (Waters Quattro Ultima Pt; Waters Corporation, Milford, MA). Stable isotope labeled anandamide (fourfold deuterated) was used as the internal standard for HPLC/MS-MS measurements. Electrospray ionization in the positive mode was applied; the following mass transitions are monitored: native anandamide, 348 \( \rightarrow \) 62; 4d3-anandamide (internal standard), 352 \( \rightarrow \) 66. We saw a linear response from 100 to 1 g/l anandamide and a total coefficient of variation of 10.6% at a mean concentration of 8.5 g/l. Using plasma from healthy volunteers (n = 12), we found plasma concentrations of 1.3 ± 0.3 g/l.

During the validation process of the HPLC/MS-MS technique, we also studied possible effects of variations in preanalytic handling conditions of blood samples on anandamide concentrations. For this purpose, we measured anandamide in whole venous blood. For complete cell lysis and protein precipitation, a methanol–zinc sulfate solution was used in these experiments. After centrifugation, the supernatant was submitted to automated on-line solid phase extraction with subsequent direct transfer to HPLC/MS-MS. Electrospray ionization in the positive mode was applied; the following mass transitions are monitored: native anandamide, 348 > 62; 4d3-anandamide (internal standard), 352 > 66. We saw a linear response from 100 to 1 µg/l anandamide and a total coefficient of variation of 10.6% at a mean concentration of 8.5 µg/l. Using plasma from healthy volunteers (n = 12), we found plasma concentrations of 1.3 ± 0.3 µg/l.

**To convert µg/l to pmol/ml, multiply by 2.9.**
of related arachidonic acids to anandamide. 23 Because every delay in centrifugation and freezing of a blood sample apparently resulted in an increase in anandamide concentrations, we decided to use anandamide measurements in whole blood, which was sampled in EDTA vials, frozen immediately, and stored at −20°C until analyses. All anandamide measurements were performed in a blinded fashion regarding group allocation.

Statistical Analyses

The primary endpoints of this study were anandamide concentrations, and the primary hypothesis to be tested was that anandamide concentrations were significantly higher in patients receiving propofol–TIVA as compared with etomidate–sevoflurane. Because a conservative estimate regarding the number of patients required demonstration of a significant difference between both groups, we assumed a difference of 3.0 ± 2.0 µg/l (SD) at 10 min after induction in favor of the propofol group. This resulted in a required sample size of 10 patients per group for α = 0.05 at a power of 0.8 (unpaired t test, two sided).

Continuous variables between groups were compared using the t test. Discrete variables were compared with the chi-square or Fisher exact test, when appropriate. The Pearson correlation coefficient was calculated as a measure of linear association between variables. Comparisons between treatment groups at the different time points of measurement were made using a general linear model with repeated measurement (repeated-measures analysis of variance). In the model, time points of measurements were defined as within-subject variables, and the administered treatment was regarded as a between-subject variable. Data are presented as mean ± SD except in the figure where mean ± SEM is used to increase clarity. All statistical calculations were performed using SPSS 13.0 (SPSS, Chicago, IL). A P value less than 0.05 was regarded as statistically significant.

Results

There were no significant differences regarding patient or clinical data (including duration of surgery, type of surgical procedure, or dosage and type of other drugs administered during anesthesia and surgery or the use of additional regional anesthesia) between the two groups (table 1). Baseline whole blood anandamide levels before induction of anesthesia were nearly identical between the groups (5.0 ± 2.6 µg/l in the etomidate–sevoflurane group vs. 5.0 ± 1.6 µg/l in the propofol–TIVA group; P = 0.95). At 10 min after induction, anandamide levels declined significantly in the etomidate–sevoflurane group to 2.9 ± 0.9 µg/l but increased to 5.3 ± 2.4 µg/l in the propofol–TIVA group. The difference in anandamide levels between both groups at this time point was statistically significant (P = 0.004). Anandamide levels remained close to this level in the propofol–TIVA group throughout the observation period but showed a further decline in the etomidate–sevoflurane group (fig. 1). Repeated-measures analysis of variance demonstrated a significant within-subject effect (type III sum of squares = 18.41, F = 4.25, P = 0.008), a significant interaction of time point of measurement by group (type III sum of squares = 20.48, F = 4.73, P = 0.005), and a significant between-groups effect (type III sum of squares = 1725.66, F = 162.60, P < 0.001).

There was no significant correlation between the administered dosage of propofol and anandamide concentrations at any time point (P ≤ 0.720). Likewise, we saw no relation between anandamide levels at baseline or during the study period and patient sex or age (data not shown).

Discussion

In this study, general anesthesia with sevoflurane resulted in a significant decline in whole blood anandam-
ide levels, whereas anandamide concentrations were slightly increased in patients receiving TIVA with propofol. The decrease in anandamide concentrations after induction of anesthesia in the etomidate–sevoflurane group could be the result of nonspecific stress reduction associated with the induction of general anesthesia. This hypothesis is supported by data showing that stress and physical exercise are known to activate the endocannabinoid system. In addition, there is a close link between the endocannabinoid system and other important stress response systems. In contrast, the higher cell lysis resulted in comparable levels of anandamide. Presumably, an inhibitory effect of propofol on anandamide activity seen in the propofol–TIVA group is, to our knowledge, unreported, none of the other important endocannabinoids (e.g., 2-arachidonoylglycerol). The design of our study was primarily based on experimental findings from Patel et al., who described an increase in brain anandamide content after administration of propofol in mice. In this study, 2-arachidonoylglycerol also increased, but the increase was not statistically significant. In addition, the presumed mechanism for anandamide increase was propofol-sensitive FAAH inhibition, whereas for 2-arachidonoylglycerol, a different means of degradation exists (monoglyceride lipase). Whether monoglyceride lipase is also inhibited by propofol is, to our knowledge, unknown. We therefore focused this pilot study on anandamide whole blood levels.

Previous studies in humans have reported blood levels of anandamide ranging between 2.6 and 15 pmol/ml, whereas our study found higher levels, in the 30-pmol/ml range. A possible explanation for this difference could be that previous investigations measured blood levels of anandamide in serum or plasma where blood was centrifuged after sampling and anandamide was determined in the supernatant, whereas in our study, anandamide was measured in whole blood after cell lysis and protein precipitation. Using our method of anandamide determination in plasma of healthy volunteers after blood centrifugation without cell lysis resulted in comparable levels of anandamide.

Our findings regarding the effects of general anesthesia on anandamide carry several possible clinical implications. On a central level, propofol reduces postoperative nausea and vomiting, alters postoperative mood, and is associated with a higher incidence of dreaming compared with other general anesthetics. The data from this and another study in mice suggest that sustained anandamide signaling may contribute to these unique properties of propofol. Alternatively, the decline in anandamide levels seen in the sevoflurane group could also be a mechanism leading to postoperative nausea and vomiting in patients undergoing general anesthesia with a volatile agent.

In summary, this pilot study shows that two common general anesthetic techniques have differential effects on the endocannabinoid system and that these effects have possible clinical and scientific implications. Nonetheless, the exact mechanisms underlying these effects must be elucidated in further studies.

References

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