Alterations in Rat Brain Proteins after Desflurane Anesthesia

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Background: Volatile anesthetics disappear from an organism after the end of anesthesia. Whether changes of protein expression persist in the brain for a longer period is not known. This study investigates the question of whether the expression of proteins is altered in the rat brain after the end of desflurane anesthesia.

Methods: Three groups (n = 12 each) of rats were anesthetized with 5.7% desflurane in air for 3 h. Brains were removed directly after anesthesia, 24 h after anesthesia, or 72 h after anesthesia. Two additional groups (n = 12 each) served as naive controls, in which the brains were removed without previous anesthesia 3 or 72 h after the start of the experiment. Cytosolic proteins were isolated. A proteome-wide study was performed, based on two-dimensional gel electrophoresis and mass spectrometry.

Results: Compared with conscious controls, significant (P < 0.05) increase/decrease was found: 3 h of anesthesia, 5/2 proteins; 24 h after anesthesia, 13/1 proteins; 72 h after anesthesia, 6/4 proteins. The overall changes in protein expression as quantified by the induction factor ranged from −1.67 (decrease to 60%) to 1.79 (increase by 79%) compared with the controls (100%). Some of these regulated proteins play a role in vesicle transport and metabolism.

Conclusion: Desflurane anesthesia produces changes in cytosolic protein expression up to 72 h after anesthesia in the rat brain, indicating yet unknown persisting effects.

VOLATILE anesthetics are administered to patients and animals with the aim of minimizing the effect of external stimuli, such as surgical incisions. How volatile anesthetics act is not known. In recent studies, it has been shown that volatile anesthetics can induce changes in gene expression in the lung1–3 and in the liver4 during anesthesia. In contrast to this, little is known about the action of volatile anesthetics referring to gene or protein expression in the brain. Two studies indicate that anesthetics can alter cerebral gene and protein expression either during or after application of a volatile anesthetic. One study showed an increase in c-fos RNA during isoflurane anesthesia.5 The other6 showed an increased concentration of the protein of the inducible nitric oxide synthase (NOS) up to 24 h after isoflurane anesthesia. Because changes in the messenger RNA (mRNA) level do not necessarily implicate an alteration in the amount of protein,7 we focused on proteins and conducted a proteome-wide study based on two-dimensional gel electrophoresis and mass spectrometry. Proteome analysis is a mass-screening approach to molecular biology. It mainly serves three goals: (1) to document the overall distribution of proteins in cells, (2) to identify and characterize individual proteins of interest, and (3) to elucidate the relations and functional roles of proteins.

The aim of the current study was to investigate whether the inhalational anesthetic desflurane can induce persisting changes in the expression level of intracellular proteins in the brain. The whole brain was chosen for a first approach to this question. To reach this goal, experimental times of 3, 24, and 72 h after the start of a 3-h anesthetic procedure were selected for brain analysis. It seemed mandatory to avoid any interference with the rats during the whole experiment, e.g., by leg catheterization, except for their exposure to desflurane in air for 3 h. By avoiding any surgical manipulation of the animals, a possible influence on protein expression in the brain caused by anything other than the desflurane anesthesia could be excluded. Desflurane was selected for three reasons: (1) Desflurane is commonly used in surgery. (2) Desflurane can be administered by simple inhalation without any further interference with the animal. (3) An interspecies-independent amount of desflurane can be applied using the minimum alveolar concentration (MAC). The used MAC of 1 corresponds to 5.7% of the inspired gas.8 The use of this dose was chosen because 5.7% desflurane is a common dose for clinical anesthesia. A lower dose was not chosen because, e.g., 2.75% desflurane does not result in anesthesia in rats and a higher dose, e.g., 8.45% desflurane, has major detrimental effects on the cardiopulmonary system, such as hypotension and hypoxia. Such changes could per se induce alterations in the protein distribution in the brain. A time period of 3 h was chosen for anesthesia because this represents a common duration of anesthesia in the

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clinical setting. The first time point for the proteome analysis was set immediately after the 3-h anesthesia to detect changes in the brain that might be induced during anesthesia. As second time point, 24 h after anesthesia was chosen because a recent study had shown that a change in the protein expression could persist for this time period at least for one protein, the inducible NOS. Finally, the third time point, 72 h after anesthesia, is beyond any reported changes of a protein in the brain after anesthesia.

Materials and Methods

Animals and Treatment

After approval by the institutional animal care committee (Regierungspräsidium Karlsruhe, Germany), the experiments were performed in 70 male Wistar rats (Charles River Deutschland, Sulzfeld, Germany) weighing 310 ± 14 g, according to the regulations of the National Institutes of Health Guide for the Use of Laboratory Animals. The animals were kept under temperature-controlled environmental conditions on a 14 h:10 h light:dark cycle and were fed a standard diet (Altromin C1000; Altromin, Lage, Germany), with free access to food and water.

Experimental Protocol

Proteome Analysis. Analysis was performed in the brains of noncatheterized rats. The rats were divided into five groups, each group consisting of 12 rats (fig. 1). Of these, two control groups (control 1 and control 2) received no treatment, and three desflurane groups were exposed to anesthesia for 3 h (group 1, group 2, and group 3). The two control groups were chosen to investigate whether any changes in protein expression occurred during the observation period. The rats of the desflurane groups were anesthetized in a box with inhalation of 5.7% desflurane (Suprane; Pharmacia & Upjohn, Erlangen, Germany; Devapor; Dräger, Lübeck, Germany) in air using a precalibrated vaporizer. After induction of anesthesia in the box, the rats were taken out of the box, and anesthesia was maintained for 3 h via a nose cone. The experiments were performed in room light, and efforts were made to minimize ambient noise. During anesthesia, oxygen saturation was monitored using pulse oximetry at the hind paw (Nonin 8600V Pulse Oximeter; Nonin Medical, Plymouth, MN), and body temperature was kept constant at 37°C with a temperature-controlled heating pad. While anesthesia was performed, the animals were motionless and had no visible muscle tone. The animals of group 1 were killed immediately after anesthesia. The two other desflurane groups were allowed to wake up and were kept in their cages until decapitation (observation period), i.e., 24 h (group 2) and 72 h (group 3) after anesthesia. The two control groups were treated identically to the desflurane groups, but no anesthesia was given. We killed the animals after the respective time, i.e., 3 h (control 1) or 72 h (control 2), after the start of handling. For decapitation, all rats were anesthetized briefly with desflurane and then decapitated. The brains were removed immediately and stored at −80°C until further processing.

Physiologic Parameters. In two separate groups (n = 5 each), one control group (control–physiologic) and one desflurane group (desflurane–physiologic), physiologic variables were measured (fig. 1). These two groups were catheterized and therefore not used for proteome analysis. These rats were anesthetized by spontaneous inhalation of 8.6% desflurane in air via a nose cone. The right femoral artery and vein were cannulated using polyethylene catheters for measurement of arterial blood pressure and saline infusion. Body temperature was held constant at 37°C, and oxygen saturation was monitored using pulse oximetry at the hind paw (Nonin 8600V Pulse Oximeter). The wound site was covered with 2% lidocaine. Catheters were inserted for continuous arterial blood pressure monitoring and intravenous fluid administration, and the wound was closed. In the desflurane–physiologic group, anesthesia with 5.7% desflurane in air was maintained for an additional 3 h. During this time, oxygen saturation at the hind paw was monitored, and body temperature was kept constant at 37°C. At the end of this 3-h period,
anesthesia was terminated, and the rats were placed in tunnels (Brantree Scientific, Boston, MA). For the measurement of acid–base parameters, small blood samples were taken hourly. Two hours after the termination of anesthesia, the rats were killed. This 2h period was chosen to measure differences between these two groups in physiologic variables during the postanesthetic period. Until the end of surgery, the rats of the control-physiologic group were treated as the animals of the desflurane-physiologic group. After surgery, the animals of the control-physiologic group were placed in rat tunnels and were allowed to recover from anesthesia. Measurement of physiologic variables and application of saline was performed as in the desflurane-physiologic group. The animals were killed after a total of 5 h.

Two-dimensional Gel Electrophoresis.

We suspended each brain separately in 2 ml sample buffer consisting of 40 mM tris, 7 mM urea, 4% 3-[3-cholamidopropyl]dimethylamino]-1-propanesulphonate (CHAPS), 10 mM 1,4-dithiothreitol, and 1 mM EDTA. The suspension was homogenized in a Potter-Elvehjem glass tissue grinder (Wheaton Science Products, Millville, NJ) and centrifuged for 60 min at 100,000g. We determined the protein content in the supernatant by the Bradford method. Aliquots containing 500 μg protein were stored at –80°C.

The protocol for two-dimensional electrophoresis was followed as previously described by Fountoulakis et al. For isoelectric focusing, we applied 500 μg of each sample on nonlinear gradient gel strips (Immobiline DryStrips, pH 3–10 NL, 18 cm; Amersham Biosciences, Uppsala, Sweden). After 12 h of reswelling time at 30 V, voltages of 200, 500, and 1,000 V were applied for 1 h each. Then voltage was increased to 8,000 V within 30 min and kept constant at 8,000 V for 12 h, resulting in a total of 100,513 Vh. For the second dimension, we used 12.5% polyacrylamide gels in the presence of 10% sodium dodecyl sulfate. The gels were run at 30 mA for 30 min and 100 mA for approximately 4 h in a water-cooled electrophoresis apparatus. Analytical gels for image analysis were stained with silver nitrate, and preparative gels for spot identification were stained with colloidal Coomassie blue. The preparative gels were performed from one brain, with the aim to excise the spots that we found significantly altered in the analytical gels. The location of the spots is defined by their isoelectric point and their molecular mass. Therefore, it was possible to locate each spot on the preparative gel for identification. Then, those spots were excised. For identification, the Center for Molecular Medicine (University of Cologne, Cologne, Germany) performed a matrix-assisted laser desorption/ionization mass spectrometry and time of flight (MALDI-TOF) and a database search.

Results

Approximately 1,200 different spots of defined volumes (defined by spot area and optical density) could be discriminated in each two-dimensional gel. Corresponding spots of one of the five groups were matched by their position in the gel to create a mean volume for each spot within each experimental group. To minimize relative errors, a spot was considered as relevant for data analysis when an accurate matching was possible in at least 10 of the 12 gels within all experimental groups. This procedure resulted in 263 spots that met the criteria. The changes in protein expression acquired by measuring the mean spot volumes are presented by their induction factors. The induction factor is formed by comparing the value of the spot volume in the experimental group with that of the control group. If the value of the spot volume in the experimental group is larger than that in the control group, the induction factor is formed by the ratio of the mean value in the experimental group to the mean value in the control group. If the mean value of the spot volume in the experimental group is lower than in the control group, the induction factor is defined as the reciprocal of the mean value in the control group. Induction factors that are lower in the experimental than in the control group are assigned a negative value, e.g., an induction factor of 1.5 corresponds to an increase in a spot volume by 50%. Conversely, a decrease in spot volume by 50% yields an induction factor of –2.0. P < 0.05 was considered statistically significant for testing changes in mean spot volumes between the groups.
at the level of significance ($P < 0.01$) did not show differences in the spots between the two control groups (control 1 and control 2).

In contrast, in the three desflurane groups, 22 spots differed significantly from controls (fig. 2). Of these 22 spots (fig. 2), all could be identified, representing 20 different proteins (table 1). This difference might be explained by the fact that several spots can represent the same protein, because most proteins are composed of several isoforms. In addition, a protein may also appear as several spots when the spots differ by their molecular mass. In such a case, use of MALDI-TOF would result in the identification of a special protein sequence (motif) that can be found in various proteins of different molecular masses. In group 1, 2 proteins were decreased and 5 proteins were increased with regard to their mean spot volume. In group 2, 1 protein was decreased and 12 proteins were increased. In group 3, 4 proteins were decreased and 5 proteins were increased. Ten percent of the affected proteins were changed at all three time points, whereas 24% were altered at two time points, and 66% were changed at only one of the time points. The changes in protein expression expressed by their induction factors ranged from a factor of $1.67$ for the decrease to a factor of $1.79$ for the increase. The changes were not consistent for all spots at all three time points.

Possible effects of desflurane on physiologic parameters of the animals were investigated in another two groups of rats (control–physiologic and desflurane–physiologic; $n = 5$ each). These values were compared with those of the control–physiologic group in which no anesthesia was administered after termination of anes-

**Fig. 2. A representative silver-stained gel.** Spots marked by their numbers show the location of the proteins, which were altered in their expression (table 1). The horizontal arrow shows the pH gradient, which increases from 3 to 11 in a nonlinear fashion. The vertical arrow shows the direction of a decrease in molecular weight (MW) of the separated spots starting at 120 kd (top) to 14 kd down (bottom) in a nonlinear setting.

The proteins that were changed at all time points were the histidine triad nucleotide-binding protein and the ribosomal protein L6. The enzyme fructose-bisphosphate aldolase C was decreased at 3 and 24 h after anesthesia. The mitochondrial aconitase precursor protein was increased only at 3 h after anesthesia. Three proteins were changed in their abundance at 3 and 72 h after anesthesia: NG,NG-dimethylarginine dimethylaminohydrolase (DDAH), T-complex protein 1, and dynamin-1. The protein α enolase was increased at 24 and 72 h after anesthesia. The following proteins were changed at 24 h after anesthesia: a protein similar to phosphoglycerate mutase, a malate dehydrogenase-like enzyme, a protein belonging to the aldehyde dehydrogenase family 1, the stress-induced protein 1, the dihydropyrimidinase-related protein 2, the serum albumin precursor, albumin, and the tubulin α chain protein. Two of the proteins were changed only in group 3: nucleoside diphosphate kinase B and brain-enriched WD-repeat protein. Table 1 lists the identified proteins according to their appearance early or late after treatment. Also listed are the accession numbers of the respective spots, which can be accessed via the Internet at SwissProt® and GenBank®. In addition, the Mascot score is shown, which quantifies the probability of identification of the fragment match of the peptide mass fingerprint obtained by MALDI-TOF. A definition and interpretation of the meaning of the Mascot score can be found on the Internet at Matrix Science.††
Table 1. Identified Proteins of the Rat Brain, which Were Altered in Their Expression after Desflurane Anesthesia

<table>
<thead>
<tr>
<th>Spot</th>
<th>Accession No. (NCBI GenBank, SwissProt)</th>
<th>Full Name</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Mascot</th>
</tr>
</thead>
<tbody>
<tr>
<td>3558</td>
<td>XP_126166</td>
<td>Histidine triad nucleotide-binding protein</td>
<td>1.70</td>
<td>1.66</td>
<td>1.43</td>
<td>2.33</td>
</tr>
<tr>
<td>3552</td>
<td>S55922, P47911</td>
<td>Ribosomal protein L6</td>
<td>1.79</td>
<td>1.97</td>
<td>1.73</td>
<td>3.64</td>
</tr>
<tr>
<td>825</td>
<td>ALF_RAT, P09117</td>
<td>Fructose-bisphosphate aldolase C (brain specific)</td>
<td>−1.34</td>
<td>−1.32</td>
<td>−1.14</td>
<td>2.52</td>
</tr>
<tr>
<td>588</td>
<td>NP_071633, O08557</td>
<td>NG,NG-dimethylarginine dimethylaminohydrolase</td>
<td>1.30</td>
<td>1.15</td>
<td>1.30</td>
<td>2.90</td>
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<tr>
<td>277</td>
<td>S42723, P80318</td>
<td>T-complex protein 1, γ subunit [Mus musculus]</td>
<td>1.39</td>
<td>1.45</td>
<td>1.34</td>
<td>2.82</td>
</tr>
<tr>
<td>3507</td>
<td>S11508, P21575</td>
<td>Dynamin-1 (D100 protein)</td>
<td>−1.43</td>
<td>1.01</td>
<td>−1.28</td>
<td>2.49</td>
</tr>
<tr>
<td>154</td>
<td>Q9ER34</td>
<td>Mitochondrial aconitase precursor</td>
<td>1.28</td>
<td>1.13</td>
<td>1.09</td>
<td>2.10</td>
</tr>
<tr>
<td>435</td>
<td>NP_036666, P04764</td>
<td>α Enolase</td>
<td>1.37</td>
<td>1.52</td>
<td>1.41</td>
<td>2.13</td>
</tr>
<tr>
<td>3790</td>
<td>XP_126166</td>
<td>Histidine triad nucleotide-binding protein [Mus musculus]</td>
<td>2.47</td>
<td>1.87</td>
<td>1.73</td>
<td>2.32</td>
</tr>
<tr>
<td>349</td>
<td>NP_647549, Q9JM4</td>
<td>Neuronal differentiation related</td>
<td>1.07</td>
<td>1.38</td>
<td>−1.20</td>
<td>1.95</td>
</tr>
<tr>
<td>678</td>
<td>XP_124890, P25113</td>
<td>Similar to phosphoglycerate mutase B chain—rat [Mus musculus]</td>
<td>2.15</td>
<td>1.38</td>
<td>1.01</td>
<td>4.01</td>
</tr>
<tr>
<td>621</td>
<td>NP_150238</td>
<td>Malate dehydrogenase-like enzyme</td>
<td>2.00</td>
<td>1.48</td>
<td>1.03</td>
<td>3.94</td>
</tr>
<tr>
<td>370</td>
<td>XP_131377, Q9CZS1</td>
<td>Aldehyde dehydrogenase 1 family, member B1 [Mus musculus]</td>
<td>1.32</td>
<td>1.51</td>
<td>1.16</td>
<td>1.27</td>
</tr>
<tr>
<td>286</td>
<td>NP_620266, P31948</td>
<td>Stress-induced phosphoprotein 1</td>
<td>1.22</td>
<td>1.53</td>
<td>1.34</td>
<td>2.69</td>
</tr>
<tr>
<td>3437</td>
<td>NP_034085, P47942</td>
<td>Dihydropyrimidinase related protein-2</td>
<td>1.07</td>
<td>1.58</td>
<td>1.09</td>
<td>5.26</td>
</tr>
<tr>
<td>3431</td>
<td>ABRTS, P02770</td>
<td>Serum albumin precursor</td>
<td>1.19</td>
<td>1.68</td>
<td>1.22</td>
<td>6.03</td>
</tr>
<tr>
<td>3533</td>
<td>NP_599153, Q903036</td>
<td>Albumin</td>
<td>1.22</td>
<td>1.92</td>
<td>1.36</td>
<td>4.07</td>
</tr>
<tr>
<td>425</td>
<td>UBRTA, P02551</td>
<td>Tubulin α chain</td>
<td>1.18</td>
<td>2.41</td>
<td>−1.02</td>
<td>5.13</td>
</tr>
<tr>
<td>770</td>
<td>A38369, P19804</td>
<td>Nucleoside diphosphate kinase B</td>
<td>−1.19</td>
<td>1.06</td>
<td>−1.67</td>
<td>2.59</td>
</tr>
<tr>
<td>267</td>
<td>NP_476543, Q99M63</td>
<td>Brain-enriched WD-repeat protein</td>
<td>−1.41</td>
<td>−1.13</td>
<td>−1.49</td>
<td>3.07</td>
</tr>
<tr>
<td>123</td>
<td>S11508, P21575</td>
<td>Dynamin-1 (D100 protein)</td>
<td>−1.25</td>
<td>1.25</td>
<td>−1.38</td>
<td>4.71</td>
</tr>
<tr>
<td>426</td>
<td>NP_060713, Q8BV3</td>
<td>Hypothetical protein FLJ10849 [Homo sapiens]</td>
<td>1.11</td>
<td>−1.03</td>
<td>−1.28</td>
<td>1.65</td>
</tr>
</tbody>
</table>

Differentially expressed proteins in groups 1, 2, and 3 vs. control. Proteins are listed according to their appearance early or late after treatment. The Mascot score quantifies the probability of identification of the fragment match of the peptide mass fingerprint obtained by matrix-assisted laser desorption/ionization mass spectrometry and time of flight (MALDI-TOF). The induction factor (IF) reflects the change in spot volume, whereas the IF of the control group is set to 1 ± 1.0.

* P < 0.05.

NCBI = National Center for Biotechnology Information.

Discussion

The current study shows for the first time that the volatile anesthetic desflurane induces changes in protein expression in the brain that persist up to 72 h after anesthesia. Previous findings of persisting effects of another volatile anesthetic, i.e., isoflurane, in the brain for the cannulation of the femoral vessels. Desflurane produced a decrease in arterial partial pressure of oxygen and in mean arterial pressure during the time of anesthesia but not in the postanesthetic period (table 2). To exclude any influence of the surgical procedure on cerebral protein expression, these two groups were not used for proteome analysis.

Table 2. Physiologic Parameters

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Control–Physiologic End of 3 h</th>
<th>Deflurane–Physiologic End of 3 h</th>
<th>Control–Physiologic End of 5 h</th>
<th>Deflurane–Physiologic End of 5 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pao2, mmHg</td>
<td>92.6 ± 2.8</td>
<td>84.1 ± 3.6*</td>
<td>91.0 ± 4.0</td>
<td>87.6 ± 3.7</td>
</tr>
<tr>
<td>Paco2, mmHg</td>
<td>42.2 ± 1.4</td>
<td>42.3 ± 1.4</td>
<td>40.0 ± 3.0</td>
<td>39.8 ± 1.3</td>
</tr>
<tr>
<td>pH</td>
<td>7.39 ± 0.02</td>
<td>7.39 ± 0.03</td>
<td>7.41 ± 0.01</td>
<td>7.42 ± 0.02</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>41.8 ± 2.8</td>
<td>42.2 ± 0.3</td>
<td>42.0 ± 2.0</td>
<td>42.4 ± 0.7</td>
</tr>
<tr>
<td>Plasma glucose concentration, mg/dl</td>
<td>154 ± 18.8</td>
<td>161 ± 23.9</td>
<td>154 ± 18.7</td>
<td>151 ± 12</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>430 ± 46</td>
<td>390 ± 30</td>
<td>430 ± 41.5</td>
<td>382 ± 21</td>
</tr>
<tr>
<td>Mean arterial blood pressure, mmHg</td>
<td>130 ± 12</td>
<td>87 ± 11*</td>
<td>125 ± 15</td>
<td>111 ± 7.5</td>
</tr>
</tbody>
</table>

Values are mean ± SD. Conscious controls (n = 5) breathed air. Animals in the desflurane group (n = 5) breathed 5.7% desflurane in air during the first 3 h. In the postanesthetic period, animals in the desflurane group breathed air.

* P < 0.05 vs. control.

Paco2 = arterial carbon dioxide tension; Pao2 = arterial oxygen tension.
were confined to a change of expression of one protein and one mRNA. The novel finding of the current study is a demonstration of a persisting change in protein expression of a large number of proteins in the brain, which lasts up to 3 days after the end of desflurane anesthesia. This finding of sustained changes in protein abundance is a challenge for the common opinion that effects of volatile anesthetics disappear from the brain within minutes or hours.

The method of proteome analysis used to investigate changes in the abundance of proteins has the advantage that mass screening of proteins is possible. Two dimensional-based proteome analyses allows visualization of up to 10,000 individual spots in a single gel. On the other hand, our approach using this method has some limitations. A 12.5% polyacrylamide gel was selected because it allows separation of spots of a molecular mass ranging from 9,000 to 120,000 kd. Most proteins of interest can be expected in this range. However, at this range of molecular masses, some proteins could not be detected, such as membranous proteins and part of the soluble cytosolic proteins. In addition, the method of proteome analysis is purely descriptive and does not allow a direct analysis of mechanisms of protein functions.

For the discussion of the changed abundance of several proteins after anesthesia, we want to focus on those proteins in which a change occurred directly after anesthesia. In this context and in relation to possible interactions with anesthesia, one of the found proteins participates in the nitric oxide (NO) pathway. DDHA increased directly and 72 h after anesthesia is an enzyme that hydrolyzes NG,NG-dimethyl-l-arginine (ADMA) and NG-monomethyl-l-arginine (MMA). ADMA and MMA act as inhibitors of NOS. By hydrolyzation of those inhibitors of NOS, DDHA seems to regulate NO production by increasing NOS activity. However, the role of the NO system has been investigated extensively during anesthesia, the influence of volatile anesthetics on NO synthesis is controversial. It has been shown that volatile anesthetics can decrease NOS activity. In addition, desflurane, like other volatile anesthetics, decreases the expression of NOS and NOS activity in cultured macrophages. However, increased synthesis of nitric oxide has been observed in the rat brain cortex during anesthesia by volatile anesthetics using paramagnetic resonance spectroscopy. In the same study, increased NO synthesis could be blocked by NOS inhibitors, which confirms that the increase in NO is due to enhanced NOS activity. Such enhanced NOS activity may even persist beyond the anesthetic period because an increase in inducible NOS has been shown after the end of isoflurane anesthesia up to 24 h after the end of anesthesia. Our finding of increased expression of DDHA after desflurane anesthesia is congruent with these latter findings. In the current study, increased expression of DDHA was found at the end of desflurane anesthesia and lasted for up to 72 h. These findings support the hypothesis of persisting increased NOS activity, mediated by decreased NOS inhibition.

A second protein that was changed in the abundance in the brain and might be interesting for possible interactions with anesthesia is dynamin-1. This protein was decreased directly and 72 h after anesthesia. It is known that volatile anesthetics can decrease synaptic transmission. However, it is not known whether such changes in synaptic transmission (dynamic change) are paralleled by structural changes related to the functionality of synapses, e.g., the density of membrane proteins. One of those regulative pathways is the clathrin-dependent endocytosis of membrane proteins by which neurons regulate synaptic function and plasticity. This clathrin-dependent endocytosis is mediated by dynamin-1. By its function as a guanosine triphosphatase, dynamin-1 rate limits the cleavage and therefore the endocytosis of the formed vesicles from the cell membrane. Through the mechanism of clathrin-dependent endocytosis, several types of membrane proteins, such as ionotrophic membrane channels, and glutamate and γ-aminobutyric acid receptor proteins can be removed from the cell membrane. This clathrin-dependent reduction of membrane proteins may be one mechanism by which neurons regulate synaptic function and plasticity. In the context of the guanosine triphosphatase activity of dynamin-1, a second protein seems to have an important role. We found a decrease of the nucleoside diphosphate kinase B, a kinase that allows the phosphorylation of guanosine diphosphate to guanosine triphosphate. Guanosine triphosphate is needed by dynamin-1 to facilitate clathrin-dependent endocytosis. The observed decrease of these proteins could be an expression of an impairment of the clathrin-dependent endocytosis. By this potential restriction of the clathrin-dependent endocytosis, the ability of neurons to regulate their synaptic membrane proteins may be altered several days after the completion of anesthesia.

Some of the proteins that were altered in their abundance directly after anesthesia can be assigned to glycolysis and the tricarboxylic acid cycle. The mitochondrial aconitate precursor of the tricarboxylic acid cycle was found to be increased directly after anesthesia, whereas fructose bisphosphate aldolase C was decreased directly and 24 h after anesthesia. It is not clear whether these changes in expression induce an altered energy metabolism. It has been shown that desflurane decreases glucose metabolism during anesthesia. However, in a previous study, we showed that another volatile anesthetic, isoflurane, does not impair glucose metabolism 24 h after anesthesia. The two proteins that were altered at all three time points were the histidine triad-binding protein (HIT), and the ribosomal protein L6. HIT is a...
protein motif, which is a conserved protein sequence with an adenosine monophosphorimidase activity that can be found in several proteins. The ribosomal protein L6 belongs to the family of proteins that are included in the large subunit of ribosomes. Another protein that was increased directly after anesthesia is the T-complex protein 1 γ subunit (TCPG). TCPG belongs to a cytosolic chaperone that is involved in the folding of tubulins and actins.7,57

In conclusion, we have shown for the first time that changes in cerebral protein expression after desflurane anesthesia occur as rapidly as 3 h after the start of anesthesia and last up to 72 h. Generally, this is at variance with the clinical impression of a complete recovery from the effects of volatile anesthetics within a few hours after the end of anesthesia.58–60 It might be possible that the tests used in the above mentioned studies were not able to detect slight neurologic changes. The changes in protein abundance observed in the current study should trigger further, more sophisticated studies of postanesthetic effects of volatile anesthetics. As to the current situation, longer-lasting effects of volatile anesthetics may also be of importance for patients who undergo multiple surgical and anesthetic procedures within short time periods.

References


22. Nishikawa K, MacIver MB: Excitatory synaptic transmission mediated by NMDA receptors is more sensitive to isoflurane than are non-NMDA receptor-mediated responses. ANESTHESiology 2000; 92:228–36


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