Molecular targets of inhaled anesthetics must be represented in the group that specifically bind these drugs, but the paucity of direct binding data has limited the number of candidates for further evaluation. To find candidate targets, we used a combination of photolabeling, two-dimensional gel electrophoresis, and mass spectrometry to identify halothane-binding targets in rat neuronal membranes. Of the 265 spots detected on the two-dimensional gels, 90 were labeled by [14C]halothane, and 34 were identified. Mitochondrial proteins, especially respiratory complex and voltage-dependent anion channels, dominated the labeled group, and there were several examples of subunit- and state-dependent binding. A significant correlation was found between internal protein cavities and binding in a group of proteins with high resolution structures. Therefore, in addition to identifying novel neuronal targets, these data suggest a general molecular feature, the buried cavity, as a dominant attribute of volatile anesthetic-binding sites found in a limited number of neuronal membrane proteins.

Molecular targets of inhaled anesthetics remain ambiguous, despite electrophysiological evidence for potentially relevant effects at ion channels (1–3). Unfortunately, few direct binding data exist for these small hydrophobic compounds because of the experimental difficulties associated with low affinity, volatile ligands (4). These difficulties have hindered verification that direct binding of anesthetics to sites in the protein matrix mediate the well-documented changes in ion channel activity. The use of photoaffinity labeling with halothane has overcome some of these difficulties (5) and has allowed demonstration of specific and selective binding sites in a wide variety of both membrane (6) and soluble (7, 8) protein models. Extending the results of these studies to the mammalian central nervous system, we have also demonstrated widespread binding (9) and multiple specific targets (10) for the anesthetic halothane. Interestingly, although similar haloalkanes (e.g. chloroform) could compete with halothane at these sites, the halothane isoflurane could not. The anesthetic affinity for many of these targets predicts significant occupancy at clinical concentrations of anesthetic, suggesting relevance to the principle anesthetic action. Therefore, inhaled anesthetics exhibit a degree of binding specificity and selectivity to justify further identification of the binding targets. Such an effort should produce novel targets for further study and for new hypotheses of anesthetic action.

Here, we use a combination of [14C]halothane photolabeling, two-dimensional gel electrophoresis, and matrix-assisted laser desorption ionization-mass spectrometry (MALDI-MS) to identify a group of membrane proteins that are specifically photolabeled by halothane. Although there are clearly multiple labeled targets, we found a greater degree of selectivity than predicted, a surprising number of mitochondrial proteins, and unanticipated evidence for an important protein structural feature.

EXPERIMENTAL PROCEDURES

Halothane was obtained from Halocarbon Laboratories (River Edge, NJ). The [14C]halothane (2-bromo-2-chloro-1,1,1-[1-14C]trifluoroethane; specific activity, 55.4 mCi/mmol) was from PerkinElmer Life Sciences. Quartz cuvettes were purchased from Markson LabSales, Inc. (Wayne, NJ), and a 254-nm UV low pressure mercury (argon) pencil calibration lamp was obtained from Thermo Orion (Stratford, CT). Prism software (version 4.0) from GraphPad was used for graphing, and Z3 software (version 2.1) from Compugen was used for two-dimensional gel and autoradiogram spot quantitation. Hyperfilm-MP was obtained from Amersham Biosciences. Coomassie Blue, human serum albumin, hemoglobin, myoglobin, EDTA, urea, and thiourea were obtained from either Sigma or Fisher. Immobilized pH gradient (IPG) strips, carrier ampholytes (Resolyte pH 3–10), SDS-PAGE standards, CHAPS, and tributylphosphine were purchased from Bio-Rad.

Brain Membrane Protein Preparation—Animal protocols were approved as required by the University of Pennsylvania Institutional Animal Care and Use Committee, and rats were treated in accordance with American Pain Society-National Institutes of Health guidelines. Four white Wistar male adult rats were used in this study. Each animal was deeply anesthetized with halothane, and the brain was perfused through the left ventricle of the heart with ice-cold saline. The whole brain was quickly removed, rapidly frozen in liquid nitrogen, and stored at −80 °C. The brain tissue was thawed, homogenized in 0.32 M sucrose, 20 mM Tris-HCl (pH 7.5), 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, and 1 μM protease inhibitor mixture (P-8340, Sigma) and centrifuged at 100,000 × g for 60 min. The pellet was washed and resuspended in lysis buffer (20 mM Tris-HCl (pH 7.5), 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, and 1 μM protease inhibitor mixture). This was centrifuged at 15,000 × g for 15 min, and the final crude synaptosomal pellet was collected and stored at −80 °C.

Halothane Photoaffinity Labeling—Photolabeling was conducted as described previously (5). Briefly, the pellets were resuspended in 5 mM

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‡ The abbreviations used are: MALDI-MS, matrix-assisted laser desorption ionization-mass spectrometry; IPG, immobilized pH gradient; CHAPS, 3-[[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonic acid; PVDF, polyvinylidene difluoride; CAF, chemically assisted fragmentation; PSD, post-source decay; HSP, heat shock protein; VDAC, voltage-dependent anion channel; PDB, protein data bank.
Tris-HCl (pH 7.5) buffer with 0.5 mM [14C]halothane, plus or minus 5 mM non-radioactive halothane (higher competing concentrations of halothane were not used because of limited solubility and UV absorption), in 2-mL quartz cuvettes (5-mm path length) at 25 °C. The samples were exposed to 254-nm light at a distance of ~5 mm for 60 s with continuous stirring from enclosed micro stir bars. The photolabeled membrane pellet was centrifuged and washed with 10 mM Tris-HCl (pH 7.5).

Two-dimensional Gel Electrophoresis—The membrane pellet was mixed with sample buffer consisting of 7 M urea, 2 M thiourea, 4% cationic ampholytes, 0.2 mM tributylphosphine, and 0.2% carrier ampholytes. Protein concentrations were determined using a modified Bio-Rad protein assay (11). Approximately 150 µg of total protein was applied for the isoelectric focusing on immobilized pH 3–10 nonlinear gradient strips (7 cm). The IPG strips were rehydrated with samples for 16 h at 20 °C. Focusing proceeded from 0 to 350 V over 20 min, and then the voltage was gradually increased to 4000 V over 2 h and kept constant until 20,000 V-h was reached. Focused strips were either stored at ~80 °C or used directly for the second dimension. The IPG strips were then equilibrated, first in a solution containing 6 m urea, 2% dithiothreitol, 2% SDS, 0.05 M Tris-HCl (pH 8.8), and 20% glycerol for 10 min and then in 6 M urea, 0.25% iodoacetamide, 2% SDS, 0.05 M Tris-HCl (pH 8.8), and 20% glycerol for 10 min. The equilibrated strips were loaded onto a 12% polyacrylamide gel for further separation in the second dimension.

After running SDS-PAGE, gels were electroblotted onto PVDF membranes with mini-Trans-Blot Cell (Bio-Rad) overnight at 4 °C and stained with Coomassie Brilliant Blue R-250 for protein visualization. The PVDF membranes were placed on x-ray film at 0 °C for 12 days. The PVDF membrane and autoradiogram film were scanned (GS-710 Scanner, Bio-Rad), processed, and quantified with Z3 software to calculate the area and density of each spot as well as the molecular weight and isoelectric point. Four full sets of data were collected and averaged.

Protein Identification and Labeling Quantitation—Some gels were stained with Coomassie Blue and saved for spot excision and identification. Based on the autoradiograms, the most prominently labeled (23 spots) and nine unlabelled spots were selected. Unlabeled spots were defined as those not detected by the Z3 software on the autoradiogram but clearly detected on the stained blot. The spots were digested using a standard trypsin in-gel protocol (12) and analyzed via peptide mass fingerprinting and CAF/PSD (MALDI-time of flight Pro mass spectrometry).

In all cases, 0.25 µL of sample was applied to the target, and a-cyano-4-hydroxycinnamic acid was used as the matrix. The CAF/PSD data were acquired after the samples were reacted with the Ettan CAF sequencing kit. After MALDI-MS identification of spots and calibration of gel positions, five additional spots were identified from previously published maps (13, 14). Spots on stained PVDF and autoradiograms were quantified by calculating the area × contrast (optical density) product. The ratio of this product in the autoradiogram (a measure of 14C incorporation) to that in the PVDF membrane (a measure of protein mass) provided a relative measure of labeling or binding. Multiplication by the molecular weight resulted in a relative binding stoichiometry (from isothermal titration calorimetry or crystallography) and incorporating the molecular weight, we were able to generate an estimate of absolute binding stoichiometry.

Statistical Analysis—Data are presented as the mean ± S.E. Multi-set data were compared by one-way analysis of variance using repeated measures followed by the Bonferroni test for multiple comparisons. Two-set data were compared with Student’s unpaired t test. A correlation between paired data (see Fig. 3) was tested with linear regression analysis and the nonparametric Spearman’s rank correlation test to reduce the impact of individual points. Significance was determined when p was less than 0.05.

RESULTS

Protein Separation and Visualization—Rat brain membrane proteins were separated by two-dimensional electrophoresis, and the spots were visualized with good resolution using Coomassie Blue (Fig. 1A, PVDF membrane). Treatment with UV plus halothane somewhat reduced gel resolution, but no significant difference in spot position was apparent. The associated 12-day autoradiogram (Fig. 1B) showed selective labeling of the resolved proteins. The Z3 software detected 265 spots on the PVDF membrane, but only 90 spots could be detected on the autoradiogram. Inclusion of 5 mM non-radioactive halothane eliminated detection of 47 proteins (100% reduced labeling) and reduced labeling of the remaining 43 proteins by an average of 58 ± 4%, ranging from 89 to 25%.

MALDI-MS Identification—Thirty-two spots were selected for MALDI-MS, but only 29 (21 labeled, 8 unlabeled) could be identified with confidence (Table I). The identification of these spots allowed gels to be calibrated so that five additional labeled spots (M1–M5) could be unambiguously identified based on published rat brain protein two-dimensional maps (13, 14);
this positive identification resulted in a total of 34 identified proteins (26 labeled, 8 unlabeled). Detailed information about the identified proteins is listed in Table I, along with the percentage of the label incorporation by 5 mM non-radioactive halothane (Disp.) were also presented. *, Represents no labeling by radioactive halothane.

TABLE I

Identified spot information

| Spot# | NCBI Protein ID | Protein Name | Mr kDa | pI | Cover. | Disp. |
|-------|----------------|--------------|--------|----|--------|-------|
| 1     | gi|242354|ref|NP_665726.1 | cytochrome C oxidase, subunit Va | 16.1 | 15 | 6.1 | 4.8 | 48.6 | 1OCC | 72 |
| 2     | gi|55992|emb|CA32424.1 | cytochrome C oxidase subunit Via | 12.7 | 15 | 6.5 | 5.2 | 62.4 | 1OCC | 24 |
| 3     | gi|95064|1|ref|NP_062256.1 | ATP synthase subunit d | 18.8 | 27 | 6.2 | 6.9 | 46.6 | 86 |
| 4     | gi|95064|1|ref|NP_062256.1 | ATP synthase subunit d | 18.8 | 27 | 6.2 | 6.9 | 46.6 | 86 |
| 5     | gi|95064|1|ref|NP_062256.1 | ATP synthase subunit d | 18.8 | 27 | 6.2 | 6.9 | 46.6 | 86 |
| 6     | gi|13747|1|gb|AAB02288.1 | ATP synthase beta subunit | 51.2 | 54 | 4.9 | 4.6 | 62.9 | 1MAB | 51 |
| 7     | gi|22090|4|obj|BA00911.1 | subunit d of mitochondrial H-ATP synthase | 18.8 | 27 | 5.8 | 5.7 | 33.5 | 68 |
| 8     | gi|71620|pir|ATRTTC | actin beta | 41.8 | 48 | 5.3 | 5.1 | 54.7 | 2BTF | 63 |
| 9     | gi|27721|5|1|ref|XP_217406.1 | ubiquinol-cytochrome c reductase core protein 1 | 27.8 | 54 | 5.1 | 5.1 | 34.9 | 70 |
| 10    | gi|13670|8|sp|P20788|UCR1_RAT | ubiquinol-cytochrome c reductase Fe-S subunit | 27.7 | 31 | 8.9 | 7.7 | 43.4 | * |
| 11    | gi|13342|4|emb|CA37654.1 | HSP60 protein | 57.9 | 66 | 5.4 | 5.1 | 54.3 | 1KP8 | 36 |
| 12    | gi|02930|pir|A25113 | tubulin beta chain 15 | 49.9 | 63 | 4.8 | 4.5 | 61.3 | ITUB | 55 |
| 13    | gi|67299|3|pdb|1MAB|A | chain A F1-Atpase | 55.3 | 57 | 8.3 | 7.9 | 59.0 | 1MAB | 54 |
| 14    | gi|69809|5|pdb|NP_036702.1 | glutamate dehydrogenase | 61.4 | 57 | 8.1 | 7.5 | 45.0 | 1HWY | 32 |
| 15    | gi|27732|0|3|ref|XP_215806.1 | creatine kinase | 47 | 49 | 8.6 | 8 | 46.4 | 1QKI | 47 |
| 16    | gi|27732|0|3|ref|XP_215806.1 | creatine kinase | 47 | 49 | 8.6 | 8 | 46.4 | 1QKI | 47 |
| 17    | gi|69786|9|ref|NP_036661.1 | creatine kinase | 42.7 | 49 | 5.3 | 5.2 | 47.8 | * |
| 18    | gi|67559|6|ref|NP_035824.1 | voltage dependant anion channel 1 | 30.8 | 37 | 8.6 | 8.4 | 58.0 | * |
| 19    | gi|67559|6|ref|NP_035824.1 | voltage dependant anion channel 1 | 30.8 | 37 | 8.6 | 8.4 | 58.0 | * |
| 20    | gi|67559|6|ref|NP_035824.1 | voltage dependant anion channel 1 | 30.8 | 37 | 8.6 | 8.4 | 58.0 | * |
| 21    | gi|13786|2|2|ref|NP_112644.1 | voltage dependant anion channel 2 | 31.7 | 37 | 7.4 | 7.3 | 43.1 | 64 |
| 22    | gi|27690|9|1|ref|XP_221202.1 | ribosomal protein L12 | 21.7 | 26 | 9.1 | 5.1 | 23.2 | 1DD3 | 100 |
| 23    | gi|12867|5|sp|P19234|NUHM_RAT | NADH-ubiquinone oxireductase 24 kDa subunit | 26.5 | 31 | 6.0 | 5.1 | 38.2 | 36 |
| 24    | gi|95070|1|ref|NP_062257.1 | CTD binding SR like | 124.8 | 17 | 9.6 | 4.4 | 38.2 | 1LB2 | * |
| 25    | gi|83340|9|ref|NP_058797.1 | peptidylprolyl isomerase A | 17.9 | 17 | 8.3 | 8 | 42.7 | * |
| 26    | gi|27668|2|47|ref|XP_234701.1 | RIKEN cDNA 2900003E13 | 47.3 | 44 | 7.6 | 6.5 | 40.5 | * |
| 27    | gi|27666|1|5|ref|XP_215197.1 | NADH dehydrogenase:ubiquinone Fe-S protein 8 | 24 | 30 | 5.9 | 4.9 | 36.8 | * |
| 28    | gi|16341|6|gb|AAA30663.1 | NADH dehydrogenase | 30 | 32 | 6.4 | 5.6 | 23.7 | * |
| 29    | gi|57029|emb|CA39599.1 | H+ transporting ATP synthase | 25.7 | 28 | 7.0 | 7 | 29.4 | 1MAB | * |

M1 gi|11750|sp|P01841|CRTC_RAT | Calreticulin [Precursor] | 48 | 63 | 4.3 | 4.4 | 4.4 |
M2 gi|12157|4|sp|P06761|GR87_RAT | 78kDa glucose-regulated protein | 72.3 | 89 | 5.1 | 4.7 | 69 |
M3 gi|11936|2|sp|P08113|ENPL_MOUSE | Endoplasmic [Precursor] | 92.5 | 97 | 4.7 | 4.6 | 100 |
M4 gi|12365|1|sp|P08109|HSTC_MOUSE | Heat shock cognate 71 kDa protein | 70.9 | 80 | 5.4 | 5.1 | 1HXI | 62 |
M5 gi|13518|5|sp|P2000|ACON_BOVIN | Aconitatehydratase, mitochondrial[Precursor] | 85.4 | 86 | 8.1 | 7.9 | 1ACO | 100 |
Smaller such differences were noted in the voltage-dependent anion channel-1 (VDAC-1) and in creatine kinase.

**Labeling Quantitation**—Fig. 2A shows only the relative labeling intensity as a ratio of autoradiogram spot volume to stain volume (where volume = area of the spot × its optical density). To convert this relative labeling intensity to binding stoichiometry, proteins with known binding character were labeled under identical conditions, included in identical gels and autoradiograms, and used for calibration purposes (Fig 2B). Human serum albumin has a halothane-binding affinity of ~1 mM and a stoichiometry of ~7:1 (halothane:albumin) (7, 15, 16). The halothane-hemoglobin affinity is similar, but the stoichiometry is approximately 1:1 (subunit)² depending on the halothane concentration and the experimental approach. Thus, occupancy of one site per protein should produce a spot ratio range of approximately 2–4. This crude analysis suggests that approximately half of the labeled proteins have one or more halothane molecules bound at 0.5 mM halothane. Smaller spot ratios are interpreted as partial occupancy of presumably lower affinity binding sites. Similarly, it is also possible that ratios higher than 4 indicate partial occupancy of many low affinity sites. The single concentration of halothane used in this study does not allow discrimination between stoichiometry and affinity, although the displacement of labeling in most identified proteins by 5 mM non-radioactive halothane indicates limited capacity and therefore a limited degree of specificity. These estimates must be viewed as tentative because of the nonequilibrium nature of the necessarily brief photolabeling binding experiments using ultraviolet light.

**Common Protein Structural Features**—A total of 11 proteins identified from this study (and the three standards) have coordinates deposited in the Protein Data Bank (PDB) for the same or a similar species. These data permitted an initial correlation of labeling intensity with specific three-dimensional features. Because we (17) and others have previously provided evidence for internal cavities as favored sites in some proteins, we first analyzed surface features using CASTp (cast.engr.uic.edu/cast/)(18, 19) to obtain cavity and pocket volume and opening (mouths) data and then related these to the labeling data shown in Fig. 1. Each protein was categorized by the number of cavities (defined as a pocket with no openings, or 0 mouths) with molecular surface volumes of between 100 and 400 Å³ (the molecular volume of most volatile anesthetics is between 100 and 200 Å³) and with pockets (defined as having ≥1 opening, or mouth) of the same volume range. The results are shown in Fig. 3 and indicate that there is a significant relationship between labeling and the number of cavities of appropriate volume. Pockets (1 mouth) alone correlated less well, and adding pockets to cavities reduced the significance, suggesting that surface-exposed pockets, despite the apparently better solvent access, are a less attractive feature for anesthetic binding than buried cavities. Dynamic fluctuations in the structure presumably provide access to these cavities (20), and the improved binding relative to pockets is likely because of optimized engagement of the anesthetic surface area in van der Waals interactions.

The sequence and label incorporation data for all 34 proteins and peptides in this study allowed an attempt at correlating labeling with hydrophobicity, predicted stability, and aromatic content. None were found to correlate significantly, giving confidence that the labeling is not simply a nonspecific feature of hydrophobicity ($r^2 = 0.01, p = 0.6$) stability ($r^2 = 0.02, p = 0.4$) or a photochemical preference for aromatic residues ($r^2 = 0.003, p = 0.8$).

### DISCUSSION

**Selectivity**—A combination of halothane photolabeling, two-dimensional gel electrophoresis, and MALDI-MS/tryptic fingerprinting allowed an in situ identification of a set of brain membrane proteins that binds the inhaled anesthetic halothane. Of the 265 spots detected on the stained blots, only 90 (34%) were labeled sufficiently to be detectable on the autoradiograms. Of these 90 spots, most had some evidence of specificity (inhibition of label incorporation with non-radioactive halothane) with 25% (67 spots) showing greater than 50% label displacement by this limited 10-fold excess concentration of halothane.

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2 J. Xi, R. Liu, G. Reid Asbury, M. F. Eckenhoff, and R. G. Eckenhoff, unpublished results.
non-radioactive halothane. We previously estimated that 15% of PDB entries have cavities large enough to bind halothane (10). The larger percentage in this study is possibly because of the fact that membrane and oligomeric proteins have larger and more numerous cavities than the small soluble ones that comprise most of the PDB entries to date (21). Binding specificity is further demonstrated by the subunit preferences in the case of mitochondrial respiratory complexes III and IV and ATP synthase. These instances of selectivity indicate that, although they are promiscuous, the small inhaled anesthetic molecules do favor a unique architecture within those targets.

The 14 proteins with high resolution structures provide some evidence that an important molecular feature is the presence of internal protein cavities of appropriate volume.

**State-dependent Binding**—State- or conformation-dependent binding is difficult to demonstrate with the methods used in this study, in that a change in size and charge of the protein does not always accompany conformational transitions. In some cases, conformational transitions are stabilized by the binding of other ligands, producing a change neither in molecular weight nor isoelectric point. But in other cases, phosphorylation or other conformational modifications alter both the state and the charge, allowing detection in the pI dimension of two-dimensional gels. Thus, the ATP synthase subunit d is found at the same molecular weight at a pI of both 5.3 and 6.9 (probably a phosphorylation), but there is an almost 4-fold difference in the amount of label incorporation. There are smaller but also significant differences in label incorporation between different charge states of the VDAC-1 and creatine kinase. These examples provide direct evidence for state-dependent binding of an inhaled anesthetic to native brain proteins, a necessary feature but not sufficient for a molecular target to be of pharmacological importance.

**Mitochondria, a Target Organelle for Anesthetics?**—Mitochondria have been proposed as an anesthetic target, largely because of the early and plausible evidence for alterations in oxidative phosphorylation (22, 23). In addition, using electron probe microanalysis, we showed that mitochondria are a preferred and saturable organelle site for halothane (24). Nevertheless, interest waned as it became clear that the anesthetic concentrations required for significant decreases in ATP levels were generally higher than that associated with unconsciousness and that inhaled convulsant compounds also decreased mitochondrial activity (23). However, the possibility for tight feedback control of cellular energy production and usage, combined with some recent in vivo studies (see below), suggests that these previous studies might not be definitive. In the present study, we found that halothane labels at least three components of the respiratory chain, complexes I, III, and IV, in both a subunit- and state-dependent manner. We could not identify complex II in our gels, suggesting that this component of the respiratory chain is either not a strong halothane-binding target or is lost in the preparation. That halothane binding to respiratory complex proteins has a physiological consequence is suggested by functional assays in isolated mitochondria, where state 3 oxidative phosphorylation was very sensitive to anesthetics (23). Also, complex I activity in isolated cardiac mitochondria was inhibited by several inhaled anesthetics, including halothane (25). More importantly, mutations in mitochondrial complex I dramatically enhanced anesthetic sensitivity in Caenorhabditis elegans (26) and in humans (27), suggesting that mitochondrial effects contribute to anesthetic action.

**Mitochondria and Side Effects**—Halothane binding to mitochondrial proteins could also be associated with effects of halothane other than anesthesia, such as muscle relaxation or cardiovascular depression. The decrease in oxidative phosphorylation and increase in the pyruvate: lactate ratio observed in dogs given 2% or greater concentrations of halothane were interpreted as toxic effects unrelated to anesthesia (27), although these effects were fully reversible. In a comparison of muscle biopsies from patients given inhaled or local anesthetics, persistent defects in activity showed that some mitochondrial effects appear to be long-lived. Finally, halothane produces “preconditioning” against ischemia (28), presumably through interactions with mitochondrial K<sub>ATP</sub> channels and perhaps other mitochondrial proteins. Whether the binding targets identified here might contribute to these actions is not yet clear.

**Voltage-dependent Anion Channels**—The mitochondrial voltage-dependent anion channel is a fairly rapid and non-selective phosphorylation-dependent mitochondrial transporter of organic anions. That this simple and evolutionarily ancient monomeric protein could contribute to anesthetic action is suggested by halothane labeling, a rapid response to synaptic activity (29), and evidence that anesthetic steroids are bound with high affinity (30). VDAC interacts with the peripheral benzodiazepine receptor (31, 32) in the outer mitochondrial membrane, binding the benzodiazepine ligand flunitrazepam with nanomolar affinity in a peripheral benzodiazepine receptor-dependent manner. The benzodiazepines, which also produce sedation, are routinely used to supplement inhaled anesthetics during surgery and significantly reduce the EC<sub>50</sub> for inhaled anesthetics (33). Benzodiazepines and inhaled anesthetics are thought to act allosterically with γ-aminobutyric acid at γ-aminobutyric acid type A receptors. Thus, the similarities between the mitochondrial benzodiazepine complex and VDAC are...
striking and, combined with the binding data from this and other studies, suggest a role for VDAC in anesthetic action. Although not yet evaluated for inhaled anesthetics, there was no significant difference in anesthetic steroid sensitivity, based on single injection sleep times, for loss-of-righting reflex in wild type compared with VDAC-1 knockout mice (34). Although there are ambiguities with the sleep time measurement, these data suggest that anesthetic-VDAC interactions underlie actions other than the loss-of-righting reflex.

Chaperones—Several proteins involved in protein (re)folding were labeled by halothane in this study (35). HSP60, endoplasmic (an HSP90-like protein), 78-kDa glucose-regulated protein (HSP70-like), and heat shock cognate 71-kDa protein all incorporate into stoichiometry of approximately 1:1. If halothane or other inhaled anesthetics influence the activity of proteins with chaperone functions, the potential exists for uncorrected misfolding in the presence of an anesthetic during stressful events such as ischemia. Furthermore, because misfolded proteins may self-assemble into toxic oligomers, often of a fibrillar form (36), it is of interest that halothane also labels the filamentous or fibrous proteins, β-actin and β-tubulin. Inhibition of chaperone activity and preferential binding of fibrillar oligomers could set the stage for anesthetic-induced exacerbation of neurodegenerative disorders (37, 38), a possibility that has received little attention.

Protein Abundance—Unlike DNA transcript-profiling experiments, there is currently no practicable way to normalize or enhance the abundance of proteins for proteomic analysis. Coupled with the fact that low abundance proteins may have considerable physiological importance, this represents a significant limitation to the interpretation and generalization of these results. Mitochondrial proteins dominate the labeling profile for halothane, which may in part be because of their abundance. In published two-dimensional gel maps (14) of whole rat brain membranes and that many of these proteins are mitochondrial. In particular, the respiratory chain complexes and the voltage-dependent anion channel are abundant and favored binding targets for halothane. Anesthetic binding correlates with the number of internal protein cavities of appropriate volume. Finally, we provide evidence for unit- and state-dependent binding selectivity, lending credibility to the possibility that many of these targets contribute to the rich pharmacological profile of halothane.

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