Molecular Understanding of Aβ Peptide Interaction with Isoflurane, Propofol, and Thiopental: NMR Spectroscopic Study[†]

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ABSTRACT: Aβ peptide is the major component of senile plaques (SP), which accumulate in the brain of a patient with Alzheimer's disease (AD). A recent report indicated that isoflurane enhanced Aβ oligomerization (micro-aggregation) and subsequent cytotoxicity of the Aβ peptide. A separate study showed that a clinically relevant concentration of isoflurane induces apoptosis and increases Aβ production in a human neuroglioma cell line. *In vitro* studies have indicated that halothane interacts specifically with Aβ peptide to induce oligomerization and that Aβ42 oligomerizes faster than Aβ40. The specific interactions of isoflurane, propofol, and thiopental with uniformly ^{15}N labeled Aβ40 and Aβ42 peptide were investigated using multidimensional nuclear magnetic resonance (NMR) experiments. We found that isoflurane and propofol (at higher concentration) interact with Aβ40 peptides and induce Aβ oligomerization. Thiopental does not interact with specific residues (G29, A30, and I31) of Aβ40; hence, the peptide remains in the monomeric form. On the basis of our NMR study, thiopental does not oligomerize Aβ40 even at higher concentrations.

The presumed mechanism of most neurodegenerative disorders, although contentious, is an uncontrolled oligomerization of normally present protein or peptide, such as the amyloid β peptides (Aβ)[1] of Alzheimer's disease (AD) (*1*) or α-synuclein in Parkinson's disease (*2*). The major protein constituents of Aβ deposits in AD are the 40-residue containing Aβ40 and the 42-residue containing Aβ42 peptide. In Aβ42, a small elongation of the stretch of two hydrophobic residues in the C-terminal region dramatically increases the aggregation tendency of the Aβ42 peptide compared to that of the Aβ40 peptide (*3*). The Aβ42 peptide is more pathogenic than Aβ40 (*3*, *4*).

Recently, Eckenhoff and co-workers have reported that at higher concentrations, commonly used inhaled anesthetics (halothane and isoflurane) promote Aβ peptide oligomerization and cytotoxicity in rat pheochromocytoma cells (*5*). They also reported that the intravenous anesthetic, propofol modestly enhanced Aβ oligomerization at high concentrations (*5*). Tanzi and co-workers have shown that at clinically relevant concentration, isoflurane induces apoptosis, alters amyloid precursor protein (APP) processing, and increases Aβ peptide generation in a human neuroglioma cell line (*6*). However, the specific binding site of these anesthetics as well as the molecular mechanism of Aβ oligomerization was unknown. In our earlier work in a membrane mimic environment consisting of sodium dodecyl sulfate (SDS), we have shown, using nuclear magnetic resonance (NMR) spectroscopic studies, that halothane interacts with critical amino acid residues (G29, A30, and I31) of Aβ40 and Aβ42. The halothane-induced alternation of the dynamics of these three critical residues (G29, A30, and I31) connecting two helices increased the chances of bringing the two helices closer and consequently facilitated the oligomerization of Aβ40/Aβ42 (*7*). Our earlier NMR studies also indicated that Aβ42 is more prone to oligomerization than Aβ40 in the presence of halothane (*7*). In this context, it is very important to investigate the specificity of other anesthetics, both inhaled (isoflurane) and intravenous (propofol and thiopental), on Aβ40 and Aβ42 peptides in a membrane mimic environment. We wish to investigate the following: (a) Does the most commonly used inhaled anesthetic, isoflurane, interact with Aβ40 and Aβ42? (b) Do intravenous anesthetics, propofol, and thiopental interact with the Aβ40 peptide? (c) Can we derive a molecular mechanism for Aβ peptide oligomerization due to the influence of these popular anesthetics?

To discover the answers to these important questions, we have investigated a series of NMR experiments consisting of both Aβ40/Aβ42 peptide interaction with isoflurane as well as Aβ40 interaction studies with propofol and thiopental.

EXPERIMENTAL PROCEDURES

Anesthetics are used to induce reversible loss of consciousness, and they are characterized by (i) smooth and rapid

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[1] Abbreviations: APP, amyloid precursor protein; DOSY, diffusion-ordered 2D NMR spectroscopy; EC_{50}, effective concentration at which 50% of the patients are unresponsive to a standard surgical stimulus; MAC, minimum alveolar concentration; NMR, nuclear magnetic resonance spectroscopy; POCD, post-operative cognitive dysfunction; SDS, sodium dodecyl phosphate.

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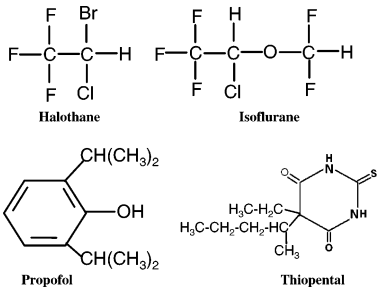

FIGURE 1: Structure of four different anesthetics. NMR studies of three anesthetics (isoflurane, propofol, and thiopental) with the Aβ peptide are reported. The structure of halothane is included in order to compare it with the three anesthetics under study.

induction of anesthesia; (ii) rapid recovery; (iii) a narrow margin of safety; and (iv) a wide range of adverse effects. This is why, anesthetics are administered by highly trained specialists during surgical procedures. Figure 1 shows the chemical structure of four anesthetics: halothane, isoflurane, propofol, and thiopental. The chemical names of isoflurane, propofol, and thiopental are 1-chloro-2,2,2-trifluoroethyl difluoromethyl, 2,6-bis(1-methylethyl)phenol, and 5-ethyl-6-oxo-5-pentan-2-yl-2-sulfanyl-pyrimidin-4-olate, respectively.

The potency of an anesthetic is defined in terms of minimum alveolar concentration (MAC) or EC_{50} at which 50% of the patients are unresponsive to a standard surgical stimulus (*8*). The MAC value of isoflurane is 1.15 (*9*). Isoflurane has a vapor pressure of 239 mm mercury at 20 °C and boils at 48.5 °C. The EC_{50} of isoflurane at 1 MAC is approximately 0.3 mM in blood (*10*−*12*). The blood/gas and brain/blood partition coefficients of isoflurane are 1.46 (*13*) and 1.60 (*14*), respectively. Isoflurane has a brain/blood coefficient of 1.6, meaning that if the gas is in equilibrium, then the concentration of isoflurane in the brain will be <0.5 mM. The intravenous anesthetic, propofol has high lipid solubility and solubilizes in a lecithin-containing emulsion (*15*). Thiopental is water-soluble, has a rapid onset of action, and provides predictable response and rapid recovery following single-dose administration. The EC_{50} value for propofol in human blood is 50 μM (*16*) and is approximately three times higher (~0.15 mM) in the brain. The EC_{50} for thiopental is 25 μM in blood (*17*) and is approximately three times higher (~0.075 mM) in the brain.

Materials. We purchased deuterated sodium dodecyl sulfate (SDS_{D25}) from Cambridge Isotope Laboratories and isoflurane, propofol, and thiopental from Sigma-Aldrich. Uniformly ^{15}N-labeled Aβ40 and Aβ42 were purchased from Recombinant Peptide Technologies (Atlanta, GA). We used an airtight microsyringe (Hamilton) for the addition of isoflurane.

Preparation of Aβ *Peptide Solution in SDS.* We used deuterated SDS (SDS_{D25}) for NMR studies since the intense ^{1}H signals from the lipid were absent because of the substitution of lipid protons by deuterium. Hence, NMR signals originated exclusively from the Aβ peptide under study. The required amount of SDS_{D25} was added in 200 μL of PBS buffer at pH 7.2. The solution was slowly mixed and sonicated for a few seconds. It was kept undisturbed for a few minutes after which it became clear and was ready for addition to the Aβ peptide. We added 1 mg of uniformly ^{15}N-labeled Aβ40 or Aβ42 to the SDS_{D25} solution and gently mixed it. It took a few minutes to dissolve all of the Aβ40 or Aβ42 peptide in the micelle environment. The pH for all NMR solutions was adjusted to 7.2. A sodium 3-trimethylsilyl-[2,2,3,3-$^2H_4$]-1-propionate (TSP) solution was prepared with D_2O (50 μL) and was added to the Aβ40 or Aβ42 solution. TSP was used as a chemical shift reference compound ($\delta_{CH3} = 0$) in all NMR experiments. D_2O provided the field/frequency lock for the NMR spectrometer. Each NMR tube contains 0.1% sodium azide to prevent any bacterial contamination and fungal growth. The final volume of each NMR solution containing Aβ40 or Aβ42 was 500 μL. The final concentration of the Aβ40 or Aβ42 peptide was ~0.5 mM, and the final concentration of SDS_{D25} was 100 mM. The final TSP concentration was 0.4 mM. All NMR samples were freshly prepared prior to experiments.

NMR Experiments and Data Analysis. We performed all NMR experiments using a Bruker DRX spectrometer operating at a proton frequency of 500.132 MHz using a 5 mM TXI probe (Bruker, Germany). The NMR experiments were performed at 27 °C to maintain the stability of the isoflurane complex with Aβ40 or Aβ42. Heteronuclear single quantum coherence (HSQC) experiments (*18*) are extremely useful to identify all amide protons of the ^{15}N-labeled Aβ peptide without any ambiguity, and the specific interactions of Aβ40/Aβ42 with anesthetics will be reflected by the chemical shift alteration of the ^{15}N and NH protons of Aβ40/Aβ42. For the sequence-specific assignment of all amide protons of Aβ40/Aβ42, various NMR experiments were performed. These NMR experiments were as follows: homo-nuclear Overhauser enhancement spectroscopy (homo-NOESY), total correlation spectroscopy (TOCSY), HSQC (*18*), and ^{15}N-filtered 3D NOESY experiments. After adding the anesthetics to the sealed NMR tube, we gently mixed it and left it for 30 min to reach equilibrium. HSQC experiments were performed to monitor any changes in chemical shift due to the anesthetic. One-dimensional ^{19}F spectra (for isoflurane only) were performed after each addition of isoflurane to measure the concentration of isoflurane in solution. The time course study for each anesthetic is shown in Figure 2.

Determination of Isoflurane Concentration in Aβ-Isoflurane Complex by ^{19}F NMR. One-dimensional ^{19}F NMR experiments were carried out in the same Bruker DRX spectrometer operating at 470.92 MHz using a multinuclear probe. Isoflurane was added directly to the NMR sample tube using an airtight microsyringe (Hamilton) in steps ranging from 3 to 9 μL. The equilibrium concentrations of isoflurane in the Aβ-isoflurane complex was experimentally determined by ^{19}F NMR with reference to two external standards containing 0.50 and 2.19 mM trifluoroacetic acid (TFA) in 5 and 10 mm NMR tubes, respectively. The 10 mm standard was coaxially used with the 5 mm sample tube during concentration calibration. The external TFA resonance served as a frequency reference for chemical shift measurements.

Oligomerization of Aβ Peptide Determination by NMR Spectroscopy. HSQC is an extremely powerful experiment to identify the oligomerization of uniformly labeled peptides or proteins. In normal conditions, uniformly ^{15}N-labeled Aβ40 peptides (monomeric form) will show 40 unique amide peaks in the 2D (*X*-axis represents the amide proton (^{1}H)

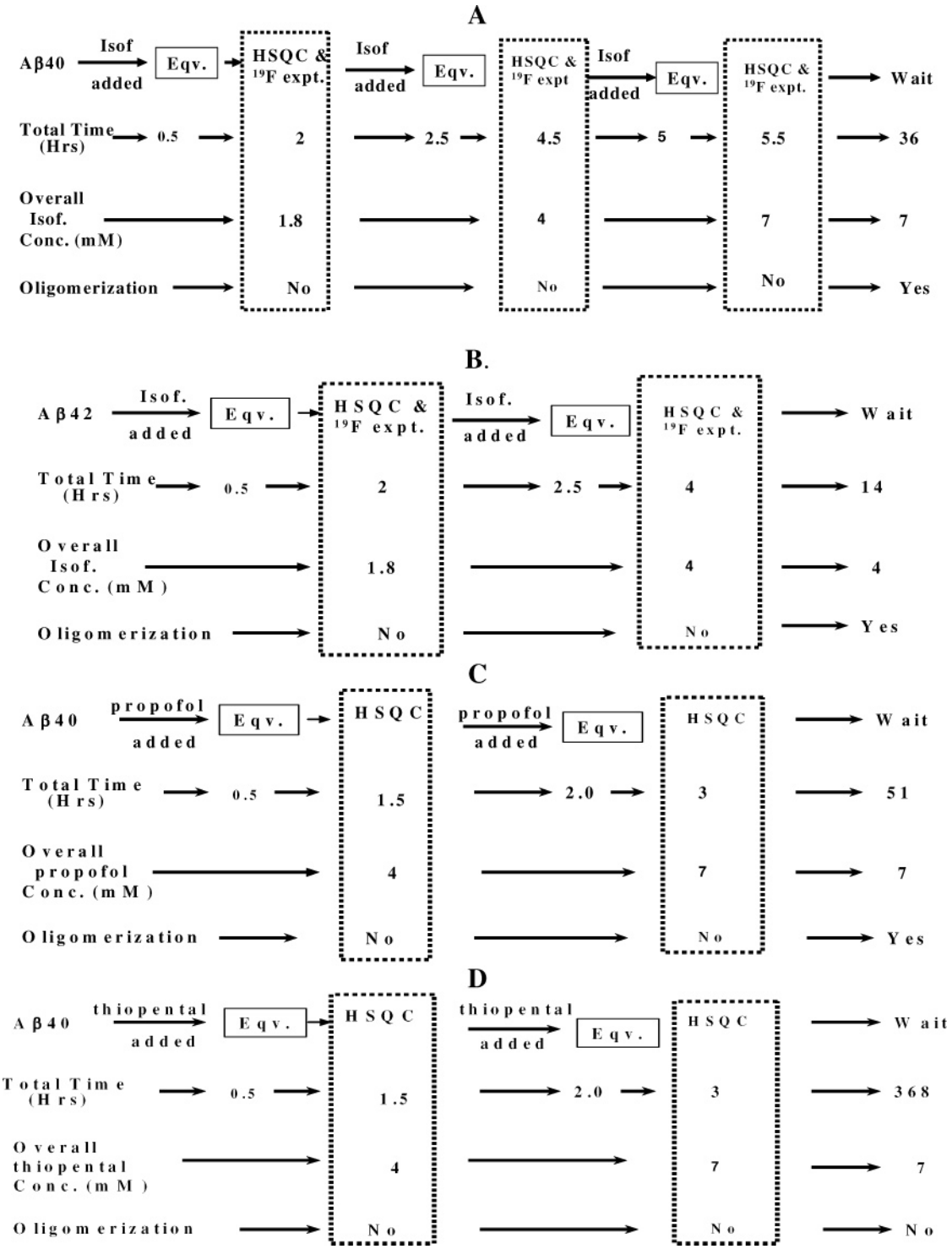

FIGURE 2: Flow chart of the time dependence NMR study. (A) Aβ40 with isoflurane; (B) Aβ42 with isoflurane; (C) Aβ40 with propofol; and (D) Aβ40 with thiopental. Eqv. refers to the anesthetic and Aβ peptide equilibrium in solution.

chemical shift, and *Y*-axis represents the ^{15}N chemical shifts of the Aβ peptide) HSQC spectra. The chemical shift range of amide protons is generally 6.5 to 10 ppm; however, the ^{15}N chemical shift range is generally 100 to 130 ppm. Because of the wide dispersion of the ^{15}N chemical shift range, the HSQC spectrum is less crowded for a small peptide like Aβ40 or Aβ42. In the event of the oligomerization of Aβ40 or Aβ42, HSQC spectra show additional amide peaks (*19*), and the intensity of these additional peaks is generally lower because of aggregation and the slow tumbling of the molecules. The number of additional amide peaks indicates the extent of aggregation (e.g., dimeric, trimeric, tetrameric, etc.), which exclusively depends on the systems under study. All NMR data from the Bruker instrument were converted into the nmrPIPE format and processed by nmrPipe programs (*21*) using a silicon graphics computer. Data were analyzed using PIPP (*22*) and SPARKY (*23*) programs using different in-house scripts.

Test-Retest Reproducibility. We performed NMR experiments twice for each anesthetic (e.g., isoflurane, propofol, and thiopental) to study Aβ-anesthetic interactions. NMR-derived results related to the chemical shift change of amide

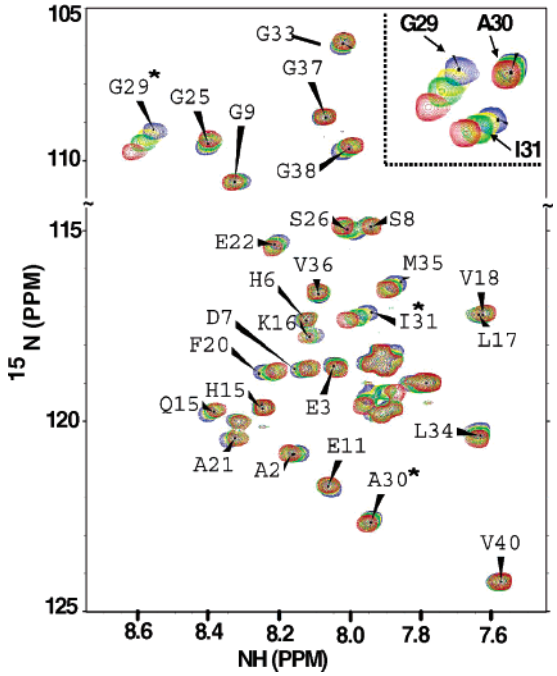

FIGURE 3: Overlay of ^{15}N/H HSQC spectra of the uniformly labeled Aβ40 in SDS micelles with/without isoflurane. Each peak in the HSQC spectra corresponds to the amide proton of Aβ40. The *Y*-axis represents the chemical shift position of ^{15}N of the amide proton, and the *X*-axis represents the ^{1}H chemical shift of the amide protons. The sequence specific assignments are performed on the basis of different NMR experiments as mentioned in the text. The color coding of the amide protons represents different conditions: (1) only Aβ40 peptide (blue); (2) Aβ40 + 1.8 mM (4 MAC) isoflurane (yellow); (3) Aβ40 + 4 mM (~8 MAC) isoflurane (green); and (4) Aβ40 + 7 mM (14 MAC) isoflurane (red). The critical residues involved in Aβ oligomerization are indicated by asterisks (*) and highlighted in the inset.

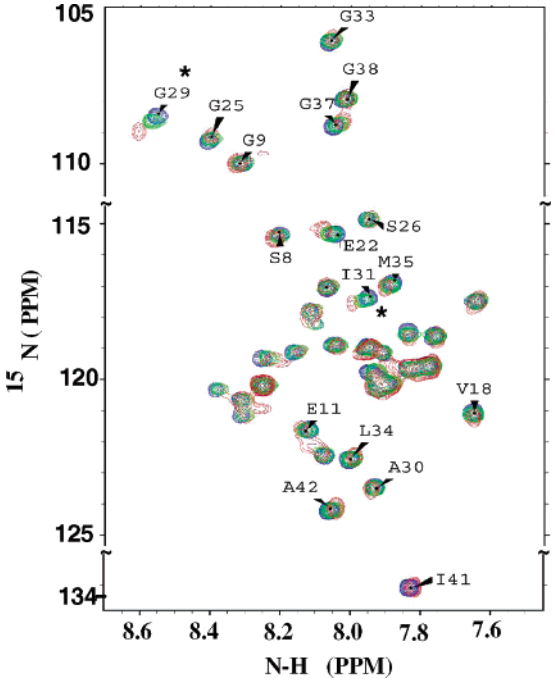

FIGURE 4: Overlay of ^{15}N/H HSQC spectra of uniformly labeled Aβ42 in SDS micelles with/without isoflurane. Each peak in the HSQC spectra corresponds to the amide proton of Aβ42. The *Y*-axis represents the chemical shift position of ^{15}N of the amide proton, and *X*-axis represents the ^{1}H chemical shift of the amide protons. The sequence specific assignments are performed on the basis of different NMR experiments as mentioned in the text. The color coding of the amide protons represents different conditions: (1) Only Aβ42 peptide (blue); (2) Aβ42 + 1.8 mM (4 MAC) isoflurane (green); and (3) Aβ42 + 4 mM (8 MAC) isoflurane (red). The critical residues involved in Aβ oligomerization are indicated by asterisks (*).

protons of Aβ peptide due to anesthetics is highly reproducible. All chemical shifts of amide protons are in absolute scale.

RESULTS AND DISCUSSION

We have studied the influence of isoflurane on Aβ40 and/or Aβ42 peptides and also the influence of propofol and thiopental on Aβ40 structure at various concentrations of these anesthetics in a membrane mimic SDS_{D25} medium.

Isoflurane Interaction with Aβ40. Figure 3 is an overlay of four HSQC spectra of uniformly ^{15}N-labeled Aβ40 without and with 3, 6, and 9 μL of isoflurane, respectively. These NMR experiments were designed to serve two purposes: (1) to investigate the concentration dependence of isoflurane interactions with Aβ40 peptide and (2) to investigate the time dependence of isoflurane interactions with Aβ40 peptide. The sequence specific assignments of the amide protons of Aβ40 (without isoflurane) were marked with blue color. The amide protons of Aβ40 were marked with different colors with the addition of 3 μL (yellow), 6 μL (green), and 9 μL (red) of isoflurane, respectively. The change in chemical shift (>20 Hz) was observed for the amide proton of amino acid residues, G29 and I31, and minimal (<10 Hz) chemical shift for A30 because of the addition of isoflurane (Figures 3 and 6). Seven amides protons (e.g., A2, A21, G25, Q15, F20, L34, and V36) show chemical shift changes (between 5 and 22 Hz) due to the addition of isoflurane. Isoflurane concentration was measured to be 1.8 mM with the addition of 3 μL of isoflurane, 4 mM with the addition of another 3 μL of isoflurane, and 7 mM after the addition of another 3 μL of isoflurane. Aβ40 remained monomeric (verified by HSQC spectra) for 30.5 h in the presence of 9 μL (7 mM) of isoflurane and then oligomerized (data not shown).

We have also performed Aβ40 interaction studies at 35 °C with the addition of isoflurane in a similar manner. We have found the isoflurane-induced chemical shift changes of different amino residues of Aβ40 at 35 °C (data not shown) to be similar to those at 27 °C. Aβ40 peptide oligomerizes in a similar period of time (~28 h) after the addition of 7 mM isoflurane at 35 °C.

Isoflurane Interactions with Aβ42. Figure 4 is an overlay of three HSQC spectra of uniformly ^{15}N-labeled Aβ42 without and with 3 and 6 μL of isoflurane, respectively. The sequence specific assignments of the amide protons of Aβ42 without isoflurane are marked by blue color (control). The amide protons of Aβ42 are indicated with the addition of 3 μL (green) and 6 μL mM (red) of isoflurane, respectively. We have found that Aβ42 oligomerized immediately in the presence of 9 μL of isoflurane. Hence, after the addition of 6 μL of isoflurane, we kept the Aβ42-isoflurane solution to monitor oligomerization by performing HSQC experiments at different time points. Isoflurane concentration was measured to be 1.8 mM with the addition 3 μL of isoflurane and 4 mM with the addition of another 6 μL of isoflurane, respectively. We have found that Aβ42 oligomerizes after 10 h in the presence of 4 mM isoflurane.

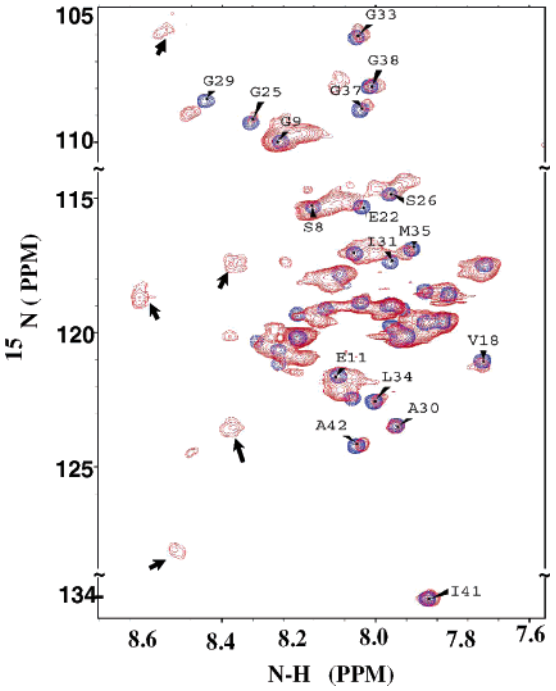

FIGURE 5: Oligomerization of Aβ42 in the presence of 4 mM (~8 MAC) isoflurane after 6 h. Overlay of ^{15}N/H HSQC spectra of uniformly labeled Aβ42 in SDS micelles with/without isoflurane. Each peak in the HSQC spectra corresponds to the amide proton of Aβ42. The *Y*-axis represents the chemical shift position of ^{15}N of the amide proton, and the *X*-axis represents the ^{1}H chemical shift of the amide protons. The sequence specific assignments are performed on the basis of different NMR experiments as mentioned in the text. The color coding of the amide protons represents different conditions: (1) Aβ42 peptide only (blue) and (2) Aβ42 + 6 μL (8 MAC) isoflurane (red) (after 6 h). The additional broad amide peaks marked by arrows indicate the oligomerization due to isoflurane.

Figure 5 shows the oligomerization of Aβ42 in the presence of isoflurane (6 μL) after 10 h. For comparison purposes, an overlay of the HSQC spectra of Aβ42 (blue) and that with 6 μL of isoflurane (red) after 10 h is shown in Figure 5. Because of the isoflurane-induced oligomerization of Aβ42, many new amide peaks (marked by arrows) are observed in the HSQC spectra of the Aβ42-isoflurane complex. With the progress of time, the Aβ42-isoflurane solution becomes viscous, and the amide peaks of Aβ42 in the HSQC spectra become very broad and eventually disappear.

A plot of the chemical shifts due to isoflurane of critical residues G29, A30, and I31 of both Aβ40 and Aβ42 are presented in Figure 6. It is noted that in the presence of the same isoflurane concentration, these critical residues shows higher chemical shift changes in Aβ42 compared to those in Aβ40.

Propofol Interactions with Aβ40. Figure 7 is an overlay of three HSQC spectra of uniformly ^{15}N-labeled Aβ40 without and with 4 mM and 7 mM propofol, respectively. The sequence specific assignments of the amide protons of Aβ40 are marked with blue color (control). The amide protons of Aβ40 are indicated by different colors in the presence of 4 mM (green) and 7 mM (red) propofol concentrations, respectively. The change in chemical shift observed for amino acid residues G29, A30, and I31 were similar to that for Aβ40-isoflurane as shown in Figure 3.

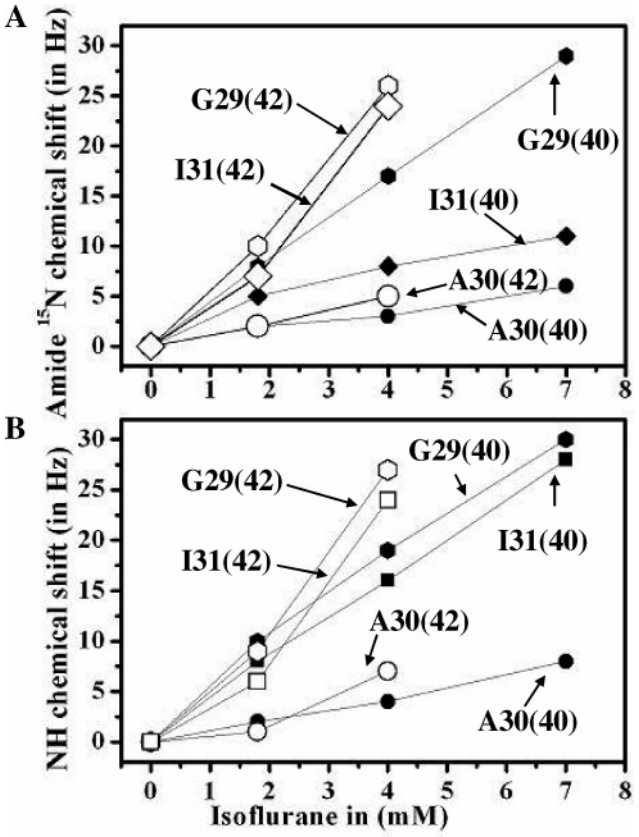

FIGURE 6: Plot of the chemical shift changes (in Hz) of ^{1}H (A) and ^{15}N (B) of three critical residues (G29, A30, and I31) in Aβ40 and Aβ42 with the addition of different amounts of isoflurane.

Aβ40 remains in monomeric form (verified by HSQC spectra at different time intervals) for 48 h in the presence of 7 mM propofol and oligomerizes. After oligomerization, the solution become viscous, and eventually, it becomes very cohesive.

Seven amides protons (e.g., A2, A21, G25, Q15, F20, L34, and V36) show chemical shift changes (between 5 and 20 Hz) due to the addition of propofol, similar to those due to isoflurane.

Aβ42 interacts with anesthetics, isoflurane, and halothane in a manner similar to that of Aβ40. The only difference we found was that Aβ42 oligomerizes faster than Aβ40. Aβ40 serves as a better system compared to Aβ42 as long time dependent studies with anesthetics can be performed with Aβ40. Hence, we have not performed Aβ42-propofol interactions studies.

Thiopental Interactions with Aβ40. Figure 8 is an overlay of three HSQC spectra, one without thiopental (Aβ only, blue) and the others with 4 mM (green) and 7 mM (red) thiopental concentration, respectively. No chemical shift change was observed for the critical amino acid residues G29, A30, and I31 due to thiopental. Aβ peptide remains in monomeric form (verified by HSQC spectra every 12 h) in the presence of 7 mM thiopental for 366 h, and no oligomerization of Aβ40 was observed. However, seven amides protons (e.g., A2, A21, G25, Q15, F20, L34, and V36) show chemical shift changes (between 5 and 20 Hz) due to the addition of thiopental. We are planning to perform NMR experiments with Aβ42 peptide at various thiopental concentrations in the future.

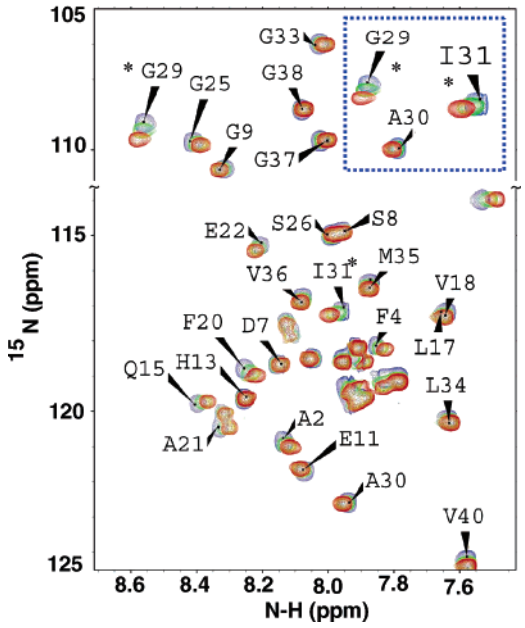

FIGURE 7: Overlay of ^{15}N/H HSQC spectra of uniformly labeled Aβ40 in SDS micelles with/without propofol. Each peak in the HSQC spectra corresponds to the amide proton of Aβ40. The *Y*-axis represents the chemical shift position of ^{15}N of the amide proton, and the *X*-axis represents the ^{1}H chemical shift of the amide protons. The sequence specific assignments are performed on the basis of different NMR experiments as mentioned in the text. The color coding of the amide protons represents different conditions: (1) only Aβ40 peptide (blue); (2) Aβ40 + 4 mM propofol (green); and (3) Aβ40 + 7 mM propofol (red). The critical residues involved in Aβ oligomerization are indicated by asterisks (*) and highlighted in the inset.

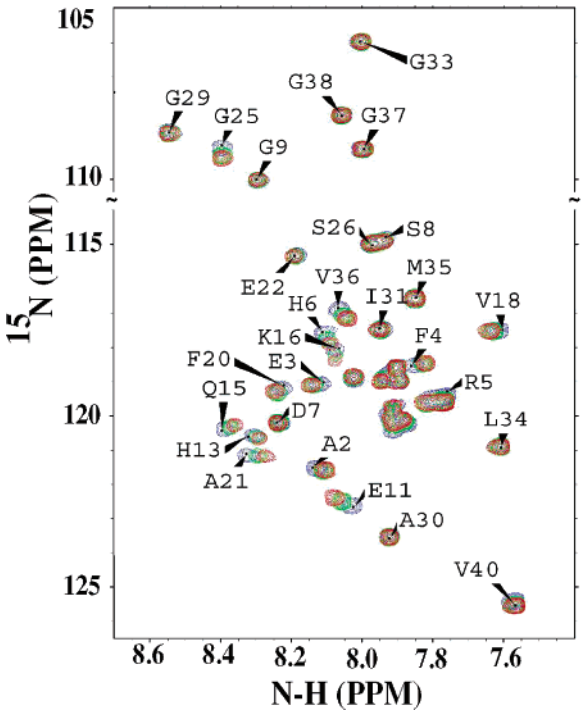

FIGURE 8: Overlay of ^{15}N/H HSQC spectra of uniformly labeled Aβ40 in SDS micelles with/without thiopental. Each peak in the HSQC spectra corresponds to the amide proton of Aβ40. The *Y*-axis represents the chemical shift position of ^{15}N of the amide proton, and the *X*-axis represents the ^{1}H chemical shift of the amide protons. The sequence specific assignments are performed on the basis of different NMR experiments as mentioned in the text. The color coding of the amide protons represents different conditions: (1) only Aβ40 peptide (blue); (2) Aβ40 + 4 mM thiopental (green); and (3) Aβ40 + 7 mM thiopental (red).

Biophysical studies of Aβ peptide are plagued by many difficulties (*25*). The biggest problem relates to their time-dependent aggregation of Aβ peptide in aqueous solution, which is a major problem for NMR experiments because higher concentrations (0.8−1.5 mM) are generally required for NMR studies. This difficulty has clearly precluded NMR studies of the naturally occurring Aβ40 and Aβ42 peptides in water solutions. The SDS micelle environment not only provides an appropriate membrane mimicking system for NMR but also prevents the aggregation of Aβ40 and Aβ42 peptides (*26*). In fact, the more aggregation prone Aβ42 is stable at relatively high concentrations (up to 1 mM) in aqueous SDS solutions for 2 months. The stability and lack of precipitation of the Aβ42 indicates that SDS provides an amenable environment for detailed structural analysis by high-resolution NMR methods. The temperature for all NMR experiments presented here was set to 27 °C. This temperature, 27 °C, is a typical setting of anesthesia for elderly patients, except in the case of cardiac surgery.

We have shown for the first time the specific interactions of isoflurane and propofol with Aβ peptide. These amide protons of G29 and I31 have the same orientation in the kink (bent) region of the Aβ peptide and the amide proton of A30 in the opposite direction compared to that of G29 and/or I31 amide protons. Hence, these two residues (G29 and I31) show appreciable chemical shifts, whereas the A30 amide proton shows minimal chemical shift because of the orientation of the amide proton. The chemical shift change of the critical residues (e.g., G29, A30, and I31) due to isoflurane is higher in Aβ42 compared to that in Aβ40 with the same concentration of isoflurane (Figure 6). We have found similar observations in the case of halothane interactions with the Aβ40/Aβ42 peptide (*7*). Aβ40 oligomerizes in the presence of 7 mM isoflurane within 30.5 h, whereas Aβ42 oligomerizes in the presence of 4 mM isoflurane after 10 h (Figure 2). These observations clearly indicate that Aβ42 is more prone to oligomerization than Aβ40 at a much lower concentration of isoflurane. Aβ42 is more sensitive to halothane compared to isoflurane, where Aβ42 oligomerizes in the presence of 4 mM halothane after a 6 h period as reported in our earlier study (*7*).

The chemical shift change of the G29 or I31 amide proton is an important indicator of the Aβ oligomerization process. Apart from halothane and isoflurane, we have also found that G29 or I31 residues show chemical shift alteration in the presence of propofol (Figure 7). However, in the case of thiopental, these two critical residues of Aβ40 do not show any chemical shift change (Figure 8).

The extent of the chemical shift change of G29 or I31 due to anesthetic interaction may correlate the time dependence of Aβ40 oligomerization. For this purpose, we have plotted the chemical shift change of G29 and I31 of Aβ40 with the same concentration of four anesthetics (e.g., halothane, isoflurane, propofol, and thiopental) (Figure 9A). Figure 9A shows that G29 or I31 amide protons shift more profoundly in halothane and isoflurane, the least in propofol, whereas there is no chemical shift change in thiopental. Halothane data (*7*) was taken from our earlier work for comparison purposes. The chemical shift change of the G29 or I31 amide proton for a particular concentration of

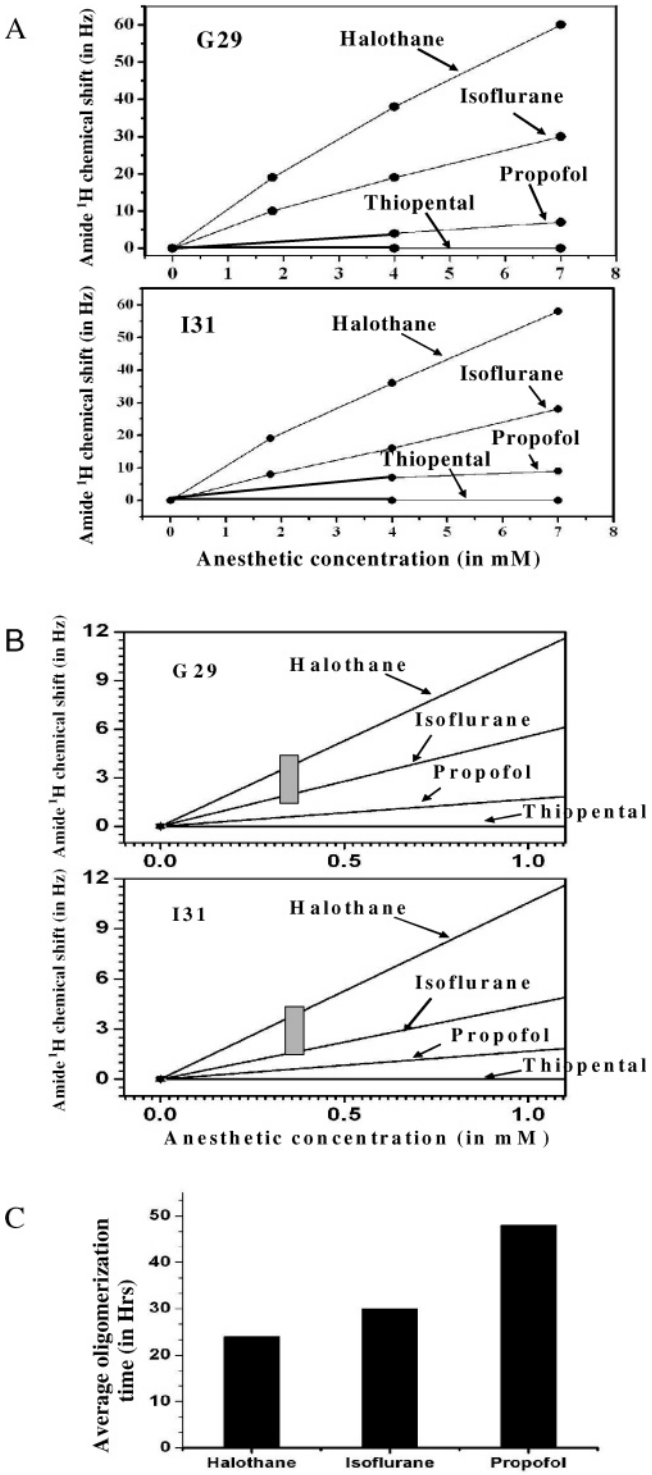

FIGURE 9: (A) Plot of the ^{1}H chemical shift of G29 and I31 in different anesthetic concentrations. (B) Expanded region of plot A to highlight the ^{1}H chemical shift of G29 and I31 at low anesthetic concentrations (below 0.3 mM). The shaded area indicates the clinically relevant concentration (0.3 mM) of halothane and isoflurane. The clinically relevant concentrations of propofol and thiopental are much lower than those of halothane or isoflurane. (C) Plot of an average Aβ40 oligomerization time in the presence 7 mM concentrations different anesthetics (halothane, isoflurane, and propofol). Aβ40 does not oligomerize in the presence of thiopental even at much higher concentrations. From our earlier work, we selected the chemical shift and average oligomerization of G29 and I31 in the presence of halothane (*7*).

anesthetics (e.g., halothane, isoflurane, propofol, and thiopental) follow the order halothane $\gg$ isoflurane $>$ propofol. In order to highlight the chemical shift changes below 1 mM anesthetic concentration, Figure 9B is presented. The average Aβ40 oligomerization time is also plotted at the same 7 mM anesthetic concentration for comparative analysis (Figure 9C). We have found that the anesthetic efficiency to induce Aβ40 oligomerization is in the following order (halothane $>$ isoflurane $\gg$ propofol), which is an excellent correlation between the extent of the perturbation of the G29 or I31 chemical shift with the average oligomerization time of the Aβ40 peptide.

Eckenhoff and co-workers reported a comparative study for measuring anesthetic efficiency to induce Aβ42 oligomerization. In their study, using a light scattering technique, they have measured the percentage of monomeric Aβ42, available much lower in the presence of halothane and higher in propofol, and the efficiency of different anesthetics to oligomerize Aβ42 were in the following order (halothane$>$ isoflurane $\gg$ propofol). It is worthwhile to note that our NMR derived results indicating the propensity of various anesthetics for Aβ oligomerization follow a similar hierarchal pattern (i.e., halothane $\gg$ isoflurane $>$ propofol) as reported by Eckenhoff and co-workers (*5*).

The other seven residues (e.g., A2, A21, G25, Q15, F20, L34, and V36) do show chemical shift changes (between 4−20 Hz) in isoflurane, propofol, and thiopental. For two of these amide residues (e.g., Q15 and F20), the chemical shift changes are plotted with anesthetic concentration (Figure 10). Halothane data is taken for comparison purposes from our earlier work (*7*). It is important to note that Q15 shows similar chemical shifts in all four anesthetics, whereas F20 shows similar chemical shifts in halothane, isoflurane and propofol, but opposite shifts in thiopental. Although A2, A21, G25, Q15, F20, L34, and V36 residues show chemical shifts in thiopental, propofol, isoflurane, and halothane, these residues are not involved in Aβ peptide oligomerization.

Hypothesis of Aβ Oligomerization. It has been shown that bound volatile general anesthetics alter both local protein dynamics and global protein stability (*27*−*29*). Both Aβ40 and Aβ42 peptides have two α-helices (α-helix-I (residues 15−23), α-helix-II (residues 32−36) connected by a more flexible kink region. The dynamics of the kink (bent) region of Aβ peptide is altered because of the interaction with anesthetics as indicated by the profound chemical shift change of G29 and I31. This may likely bring closer the two helices of Aβ, and it might initiate the cascade of events of Aβ peptide oligomerizaton. NMR data indicate that isoflurane and propofol interact with critical residues G29, A30, and I31; however, thiopental does not interact with those residues. The volume of the anesthetics is in the following order: halothane $<$ isoflurane $<$ propofol $<$ thiopental. Halothane, being smaller in size (Figure 1), is very effective in interacting in the sensitive loop region containing G29, A30, and I31 of the Aβ peptide and induces oligomerization faster. Thiopental is bulkier (because of the extended size of the chain) than isoflurane, and propofol may not fit in the loop region, and that may explain why we did not observe any chemical shift change of G29, A30, or I31 of the Aβ40 peptide by thiopental (Figure 8). Hence, no Aβ40 oligomerization was observed in the presence of thiopental.

The schematic diagram of isoflurane/propofol-induced oligomerization of Aβ peptide in a SDS membrane mimic

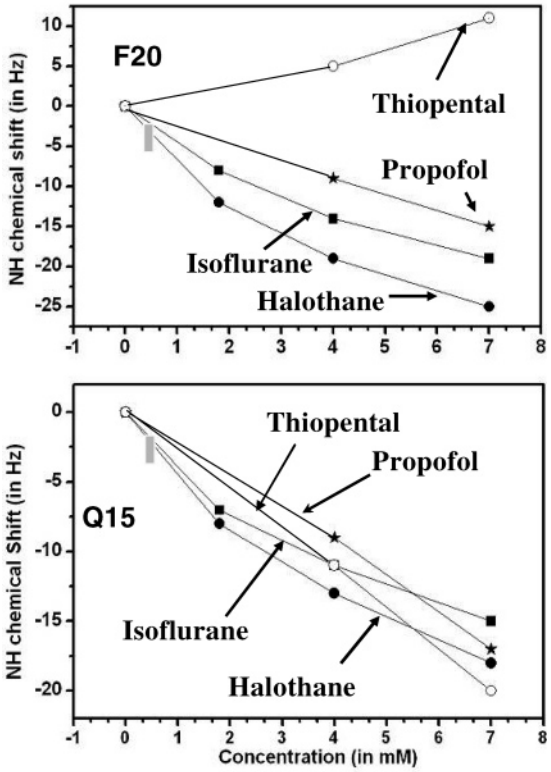

FIGURE 10: Plot of the ^{1}H chemical shift of F20 and Q15, depicting appreciable chemical shifts in thiopental, propofol, isoflurane, and halothane concentrations. The shaded area indicates the clinically relevant concentrations (0.3 mM) of halothane and isoflurane. The clinically relevant concentrations of propofol and thiopental are much lower than those of halothane or isoflurane.

system is presented in Figure 11. The Aβ structure is taken from the PDB (1BA4) (*26*). The diagrammatic representation of the membrane and anesthetics is not indicative of specific conformational details but is a pictorial representation of anesthetic binding sites with the Aβ40 peptide in the membrane-aqueous environments.

Relevance of the Anesthetic Concentration. It has been suggested that anesthesia might result from molecular interactions at the interface between aqueous and nonpolar media (e.g., membrane) (*30*). This suggestion is supported by NMR and gas chromatography studies of clinical anesthetics with macromolecular surfaces (*31*). Recent progress in computational studies of membrane-aqueous interfaces has made it possible to determine concentration profiles of anesthetics across these interfaces by computer simulations (*32*). The Aβ40/Aβ42 peptide is located in a membrane-aqueous environment as determined by earlier studies using backbone dynamics studies (*24*). Isoflurane/propofol is generally located in the membrane-aqueous region (*27*, *29*). The anesthetic concentrations used in our NMR studies were higher relative to those in a clinical setting. The clinically used concentrations of halothane and isoflurane are approximately 0.3 mM (*10*−*12*). In our study, we have systematically increased the anesthetic concentration and monitored the chemical shift changes of the critical residues (G29, A30, and I31). There is no clinical relevance for the use of 7 mM concentration in this study; however, we wanted to compare the structural changes of Aβ40 in a similar anesthetic concentration with isoflurane, propofol, and thiopental.

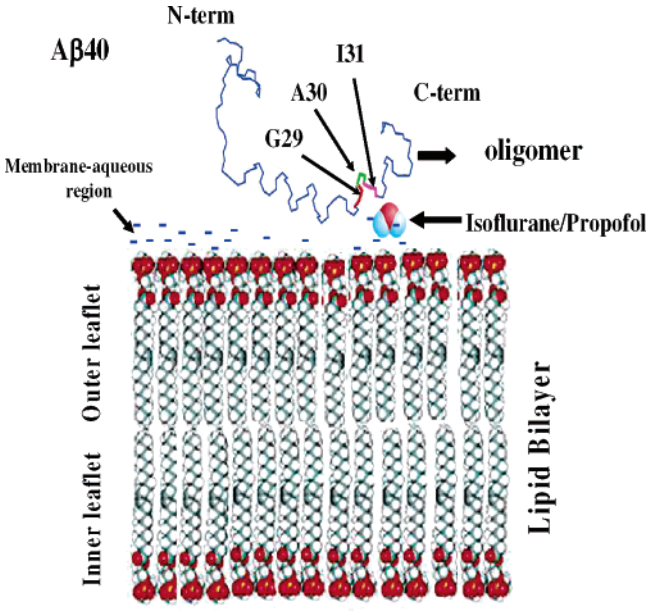

FIGURE 11: Schematic diagram of Aβ−anesthetic interaction on the extracellular side of the plasma membrane. Aβ is represented by a backbone trace of an NMR determined structure in the protein data bank (1BA4). The critical amide protons in the loop regions involved in Aβ oligomerization are marked with different colors as follows: G29 (red), A30 (green), and I31 (magenta). Other amino acid residues, such as A2, A21, G25, Q15, F20, L34, and V36, also show chemical shifts by thiopental, propofol, and isoflurane. However, these seven residues are not involved in the Aβ oligomerization process.

In our NMR studies, the Aβ peptide concentration of approximately 0.5 mM is much higher than that in an *in vivo* situation (21 ± 7 μM) (*33*). In a clinical situation, the ratio of Aβ40/anesthetic is in the order of 0.083 (considering the clinically used 0.3 mM anesthetic concentration). In our *in vitro* NMR studies, Aβ40/anesthetic was 0.078 (Aβ40 peptide concentration is 0.5 mM and anesthetic concentration was 7 mM). An extrapolation of the response curve (chemical shift change vs anesthetic concentration) will help us to determine any chemical shift change in the clinically relevant concentration. From the extrapolation of the chemical shift below the clinically relevant anesthetic concentration (0.3 mM), we have found that isoflurane and halothane induce chemical shift changes between 1.5 and 3 Hz for both G29 and I31 amide protons. In the case of propofol, the chemical shift changes of G29 and I31 are not observed below the clinically relevant concentration of propofol, which is in the micromolar range (Figure 9B). On the basis of the chemical shift change below the clinically relevant concentration, we could infer that the oligomerization propensity of the anesthetics would be in the following order: halothane > isoflurane ≫ propofol. This important observation supports the usefulness of NMR methodology compared to that of other biophysical techniques.

Relevance of this Biophysical Study. A number of clinical reports have suggested a possible link between anesthesia and the subsequent onset of senile dementia. Bedford described cases of postoperative disorientation with progression toward incontinence, mutism, and death, which are consistent with the diagnosis of vascular dementia (*34*). In a retrospective study of 147 cases of senile dementia, Ritchie and co-workers (*35*) found 19 patients who, according to family members, demonstrated either the first signs of senile

dementia or a significant acceleration of pre-existing symptoms following general anesthesia; however, the type of dementia was not specified. Moller and co-workers indicated post-operative cognitive dysfunction (POCD) in non-cardiac surgery (*36*). They have studied 1218 patients, aged at least 60 years old after non-cardiac surgery, and found that POCD occurred in 27% and 10% of patients 1 week and 3 months after anesthesia and surgeries, respectively (*36*). These findings also indicated that increasing age, duration of anesthesia, poor education, second operations, post-operative infections, and respiratory complications were risk factors for early POCD. In contrast, only age was a risk factor for late POCD. There have been numerous reports of delayed and long-lasting post-operative cognitive dysfunction following cardiac surgery (*37*, *38*). Newman and co-workers studied 261 patients who underwent cardiac coronary artery bypass surgery for potential POCD incidence. Neurocognitive tests were performed pre-operatively, before discharge, 6 weeks, 6 months, and 5 years after the surgery. They found that the POCD rate was 53% at discharge, 36% at 6 weeks, 24% at 6 months, and 42% at 5 years, respectively, and cognitive function at discharge was a significant predictor of long-term function ($p < 0.001$) (*38*). The molecular mechanism underlying POCD is largely unknown. It is also reported that AD patients deteriorated after exposure to anesthesia (*39*).

Epidemiological studies have failed to demonstrate a relationship between frequency and/or duration of exposure to anesthesia and increased risk of AD (*40*, *41*). Bohnen and colleagues (*42*) found that increased exposure to general anesthetics (particularly barbiturates and halogenated agents) and spinal anesthesia early in life appear to be associated with an earlier age of onset of dementia. From the registry of the Alzheimer's Disease Research Center at the University of Pittsburgh, we have found that among 1053 probable AD subjects participating in AD research, 53 subjects have had major surgery. All these important clinical reports support the relevance of this biophysical study.

CONCLUSIONS

In conclusion, we stress that the extent of interactions of inhaled and intravenous anesthetics with Aβ peptide are different and that inhaled anesthetics (halothane and isoflurane) are more potent for Aβ peptide oligomerization. Although the intravenous anesthetic propofol induces the oligomerization of the Aβ peptide at a much higher concentration, it would be less likely to oligomerize the Aβ peptide below the clinically relevant concentration. On the contrary, thiopental does not oligomerize the Aβ peptide even at a much higher concentration. The extent of oligomerization can be determined by diffusion-ordered 2D NMR spectroscopy (*20*) (DOSY), which is also planned for future studies. Although we provide the first NMR spectroscopic report to explain the molecular mechanism of Aβ oligomerization due to certain anesthetics, further biophysical studies in different membrane environments as well as both animal and clinical studies are warranted.

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REFERENCES

1. McKeon-O'Malley, C., and Tanzi, R. (2001) Etiology, Genetics, and Pathogenesis of Alzheimer's Disease, *Functional Neurology of Aging*, pp 333−348, Academic Press, New York.
2. Duda, J. E., Lee, V. M. Y., and Trojanowski, J. Q. (2000) Neuropathology of synuclein aggregates: New insights into mechanisms of neurodegenerative diseases, *J. Neurosci. Res. 61*, 121−127.
3. Jarrett, J. T., Berger, E. P., and Lansbury, P. T. (1993) The carboxy terminus of the beta-amyloid protein is critical for the seeding of amyloid formation-implications for the pathogenesis of Alzheimers-disease, *Biochemistry 32*, 4693−4697.
4. Hardy, J. (1997) The Alzheimer family of diseases: many etiologies, one pathogenesis? *Proc. Natl. Acad. Sci. U.S.A. 94*, 2095−2097.
5. Eckenhoff, R. G., Johansson, J. S., Wei, H. F., Carnini, A., Kang, B. B., Wei, W. L., Pidikiti, R., Keller, J. M., and Eckenhoff, M. F. (2004) Inhaled anesthetic enhancement of amyloid-beta oligomerization and cytotoxicity, *Anesthesiology 101*, 703−709.
6. Xie, Z. C., Dong, Y. L., Maeda, U., Culley, D. J., Crosby, G., and Tanzi, R. E. (2006) The common inhalation anesthetic isoflurane induces apoptosis and increases amyloid beta protein levels, *Anesthesiology 104*, 988−994.
7. Mandal, P. K., Pettegrew, J. W., McKeag, D. W., and Mandal, R. (2006) Alzheimer's disease: halothane induces abeta peptide to oligomeric form-solution NMR studies, *Neurochem. Res. 31*, 883−890.
8. Eger, E. I., II, Saidman, L. J., and Brandstater, B. (1965) Minimum alveolar anesthetic concentration: a standard of anesthetic potency, *Anesthesiology 26*, 756−763.
9. Stevens, W. D., Dolan, W. M., Gibbons, R. T., White, A., Eger, E. I., Miller, R. D., DeJong, R. H., and Elashoff, R. M. (1975) Minimum alveolar concentrations (MAC) of isoflurande with and without nitrous oxide in patients of various ages, *Anesthesiology 42*, 197−200.
10. Franks, N. P., and Lieb, W. R. (1993) Selective actions of volatile general-anesthetics at molecular and cellular-levels, *Br. J. Anaesth. 71*, 65−76.
11. Eckenhoff, R. G., and Johansson, J. S. (1999) On the relevance of "clinically relevant concentrations" of inhaled anesthetics in in vitro experiments, *Anesthesiology 91*, 856−860.
12. Eckenhoff, R. G., and Johansson, J. S. (1997) Molecular interactions between inhaled anesthetics and proteins, *Pharmacol. Rev. 49*, 343−367.
13. Lerman, J., Gregory, G. A., Willis, M. M., and Eger, E. I., II. (1984) Age and solubility of volatile anesthetics in blood, *Anesthesiology 61*, 139−143.
14. Eger, E. I., II. (1992) Desflurane animal and human pharmacology: aspects of kinetics, safety, and MAC, *Anesth. Analg. (Baltimore) 75*, S3−S7; discussion S8−S9.
15. Knopes, K. D. (2002) *Geriatric Anesthesiology*, pp 29−30, American Geriatrics Society.
16. Mazoit, J. X., and Samii, K. (1999) Binding of propofol to blood components: implications for pharmacokinetics and for pharmacodynamics, *Br. J. Clin. Pharmacol. 47*, 35−42.
17. Coates, K. M., Mather, L. E., Johnson, R., and Flood, P. (2001) Thiopental is a competitive inhibitor at the human alpha 7 nicotinic acetylcholine receptor, *Anesth. Analg. (Baltimore) 92*, 930−933.
18. Mandal, P. K., and Majumdar, A. (2004) A comprehensive discussion of HSQC and HMQC pulse sequences, *Concepts Magn. Reson., Part A 20*, 1−23.
19. Mandal, P. K., and Pettegrew, J. W. (2004) Alzheimer's disease: Soluble oligomeric Aβ(1−40) peptide in membrane mimic environment from solution NMR and circular dichroism studies, *Neurochem. Res. 29*, 2267−2272.
20. Jerschow, A., and Muller, N. (1998) Diffusion-separated nuclear magnetic resonance spectroscopy of polymer mixtures, *Macromolecules 31*, 6573−6578.
21. Delaglio, F., Grzesiek, S., Vuister, G. W., Zhu, G., Pfeifer, J., and Bax, A. (1995) NMRPipe: a multidimensional spectral processing system based on UNIX pipes, *J. Biomol. NMR 6*, 277−293.

22. Garrett, D. S., Powers, R., Gronenborn, A. M., and Clore, G. M. (1991) A common sense approach to peak picking two-, three- and four-dimensional spectra using automatic computer analysis of contour diagrams, *J. Magn. Reson. 95*, 214−220.
23. Goddard, T. D., and Kneller, D. G. (1994) *SPARKY 3*, University of California, San Francisco, CA.
24. Mandal, P. K., and Pettegrew, J. W. (2004) Alzheimer's disease: NMR studies of asialo (GM1) and trisialo (GT1b) ganglioside interactions with Aβ(1−40) peptide in a membrane mimic environment, *Neurochem. Res. 29*, 447−453.
25. Teplow, D. B. (1998) Structural and kinetic features of amyloid beta-protein fibrillogenesis, *Amyloid 5*, 121−142.
26. Coles, M., Bicknell, W., Watson, A. A., Fairlie, D. P., and Craik, D. J. (1998) Solution structure of amyloid beta-peptide(1−40) in a water-micelle environment. Is the membrane-spanning domain where we think it is? *Biochemistry 37*, 11064−11077.
27. Johansson, J. S., Zou, H., and Tanner, J. W. (1999) Bound volatile general anesthetics alter both local protein dynamics and global protein stability, *Anesthesiology 90*, 235−245.
28. Pidikiti, R., Zhang, T., Mallela, K. M. G., Shamim, M., Reddy, K. S., and Johansson, J. S. (2005) Sevoflurane-induced structural changes in a four-alpha-helix bundle protein, *Biochemistry 44*, 12128−12135.
29. Pidikiti, R., Shamim, M., Mallela, K. M. G., Reddy, K. S., and Johansson, J. S. (2005) Expression and characterization of a four-alpha-helix bundle protein that binds the volatile general anesthetic halothane, *Biomacromolecules 6*, 1516−1523.
30. Pohorille, A., Cieplak, P., and Wilson, M. A. (1996) Interactions of anesthetics with the membrane-water interface, *Chem. Phys. 204*, 337−345.
31. Yokono, S., Ogli, K., Miura, S., and Ueda, I. (1989) 400 MHz two-dimensional nuclear Overhauser spectroscopy on anesthetic interaction with lipid bilayer, *Biochim. Biophys. Acta 982*, 300−302.
32. Balasubramanian, S. V., Campbell, R. B., and Straubinger, R. M. (2002) Propofol, a general anesthetic, promotes the formation of fluid phase domains in model membranes, *Chem. Phys. Lipids 114*, 35−44.
33. Carnini, A., Lear, J. D., and Eckenhoff, R. G. (2006) Inhaled anesthetics modulation of amyloid beta(1−40). Assembly and growth, *Curr. Alzheimer Res.*, in press.
34. Bedford, P. D. (1955) Adverse cerebral effects of anasthesia in old people, *Lancet* 259−263.
35. Ritchie, K., Nargoet, C., and Herguetta, T. (1992) Family observation of heterogeneity in early stage senile dementia of the Alzheimer type, *Revue de Geriatrie 17*, 251−258.
36. Moller, J. T., Cluitmans, P., Rasmussen, L. S., Houx, P., Rasmussen, H., Canet, J., Rabbitt, P., Jolles, J., Larsen, K., Hanning, C. D., Langeron, O., Johnson, T., Lauven, P. M., Kristensen, P. A., Biedler, A., van Beem, H., Fraidakis, O., Silverstein, J. H., Beneken, J. E., and Gravenstein, J. S. (1998) Long-term postoperative cognitive dysfunction in the elderly ISPOCD1 study, *Lancet 351*, 857−861.
37. Selnes, O. A., and McKhann, G. M. (2002) Cognitive changes after coronary artery bypass surgery, *Curr. Opin. Psychiatry 15*, 285−290.
38. Newman, M. F., Kirchner, J. L., Phillips-Bute, B., Gaver, V., Grocott, H., Jones, R. H., Mark, D. B., Reves, J. G., Blumenthal, J. A., and Cardi, N. O. R. G. (2001) Longitudinal assessment of neurocognitive function after coronary-artery bypass surgery, *N. Engl. J. Med. 344*, 395−402.
39. Bone, I., and Rosen, M. (2000) Alzheimer's disease and anaesthesia, *Anaesthesia 55*, 592−593.
40. Bohnen, N. I. L. J., Warner, M. A., Kokmen, E., Beard, C. M., and Kurland, L. T. (1994) Alzheimers-disease and cumulative exposure to anesthesia—a case-control study, *J. Am. Geriatr. Soc. 42*, 198−201.
41. Kokmen, E., Beard, C. M., Chandra, V., Offord, K. P., Schoenberg, B. S., and Ballard, D. J. (1991) Clinical risk factors for Alzheimer's disease: a population-based case-control study, *Neurology 41*, 1393−1397.
42. Bohnen, N., Warner, M. A., Kokmen, E., and Kurland, L. T. (1994) Early and midlife exposure to anesthesia and age of onset of Alzheimer's disease, *Int. J. Neurosci. 77*, 181−185.

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