

Comprehensive Nuclear Magnetic Resonance Studies on Interactions of A β with Different Molecular Sized Anesthetics

Pravat K. Mandal* and Manisha Ahuja

Neurospectroscopy and Neuroimaging Laboratory, National Brain Research Centre, Manesar, Gurgaon, India

Abstract. Laboratory research on anesthetic-induced structural changes of amyloid beta (A β) peptide, from normal monomeric α -helix to the micro-aggregated form, has generated much interest in the scientific community as A β oligomerization is considered a key step in Alzheimer disease pathogenesis. A comprehensive review of the interactions of A β peptide with anesthetics of different molecular sizes is summarized as follows. Smaller sized anesthetics could access and perturb the cavity containing crucial amino acid residues G29, A30 and I31 of A β peptide leading to A β oligomerization. However, bulkier sized anesthetics are sterically hindered from accessing the cavity containing these crucial residues and do not initiate A β oligomerization. Notably, when a small sized anesthetic is co-administered with a larger sized one, the latter does not prevent access of the small sized anesthetic to the cavity. The results of these biophysical studies are supported by animal model studies which indicate that inhaled small molecular anesthetics induce enhanced A β plaque deposition in transgenic mice with AD pathology. In this review, a molecular pathway for the A β -anesthetic interaction at the atomic level is presented.

Keywords: Anesthetics, molecular size, NMR, A β , oligomerization, Alzheimer disease

INTRODUCTION

Anesthetic-induced structural changes of amyloid beta (A β) peptide from normal monomeric α -helix to the toxic oligomeric form is a significant area of research to the scientific community as A β oligomerization is a crucial event in Alzheimer disease pathogenesis. Light scattering experiments have shown that inhaled anesthetics enhance toxicity in cultured neuronal cells [1]. Reports suggest that some of the commonly used inhaled anesthetics may cause brain damage that accelerates the onset of Alzheimer's disease [2]. Synthetic A β oligomers have been reported to impair long-term memory in animal models [3]. Halothane treated transgenic mice (Tg2576) present more amyloidopathy compared to isoflurane treated transgenic

mice (Tg2576) [4]. Cognitive impairment due to these anesthetics is also reported in non-transgenic mice [4]. Based on a further *in vivo* study using isoflurane, it has been concluded that isoflurane may promote AD neuropathogenesis [5]. Interestingly, repeated administration of inhaled anesthesia (dose: twice a week, for 3 months) to 7–10 months old wild type and APP^{Swe} transgenic mice revealed that the deleterious impact of isoflurane on behavior, survival, neuronal cell death and processing of proteins involved in neurodegeneration is restricted to mice with special susceptibility [6].

Hence, it is important to trace the molecular mechanism for anesthetic-induced A β oligomer formation, through biophysical studies, in order to reveal the molecular process of amyloidogenesis. In this global search for 'better' anesthetics, *in vitro* biophysical experiments (e.g. NMR) offer one channel of investigation to study the A β peptide interactions with different sized anesthetic molecules. This review assimilates the present knowledge available to suggest a novel course

*Correspondence: Dr. Pravat K. Mandal, Additional Professor (Scientist V), Neurospectroscopy and Neuroimaging Laboratory, E-mail: pravat.mandal@gmail.com.

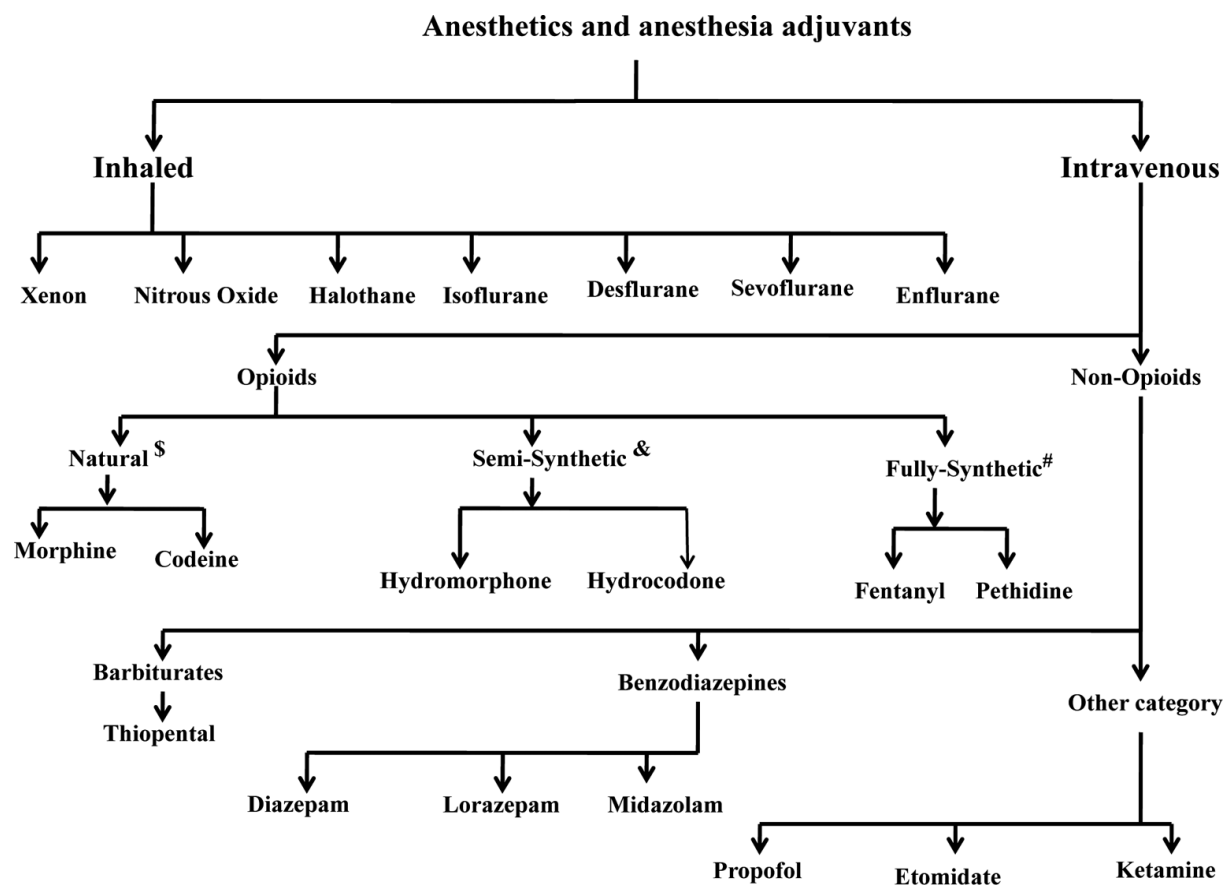


Fig. 1. Detailed overview of anesthetics and anesthesia adjuvants. Two representative substances of each group of opioids are shown due to space limitations. Further members of the respective groups are abbreviated as follows: \$ thebaine; & oxycodone, oxymorphone, desomorphine, diacetylmorphine (heroin), and nicomorphine; # methadone, tramadol, and dextropropoxyphene. Analogues fentanyl compounds are sufentanil, alfentanil, and remifentanil.

of scientific research for testing a new anesthetic drug before it is chosen for animal or clinical trial.

BIOPHYSICAL AND BIOCHEMICAL CHARACTERISTIC OF ANESTHETICS

Anesthetics are broadly classified into two categories, inhaled and intravenous. A detailed classification of various anesthetics and anesthesia adjuvants are provided in Fig. 1. Certain features of size, structure and chemical properties of some anesthetics are detailed below. The inhaled anesthetic halothane belongs to the halo-alkane group, whereas isoflurane, desflurane, sevoflurane and enflurane are halo-ethers. Xenon is a noble gas and nitrous oxide is an inorganic gas. Boiling points of these inhaled anesthetics range from 23° to 58°C and the boiling points of nitrous oxide and xenon are -89° C and -107°C, respectively.

Morphine is a T-shaped molecule. Propofol, a non-opioid anesthetic, consists of substituted phenol while thiopental consists of a cyclic ring with electronegative sulphur and oxygen atoms.

The molecular size of halothane and isoflurane are 110 Å³ [7] and 144 Å³ [7], respectively. The molecular size of propofol belonging to the non-opioid category is 191 Å³ [8]. The molecular size of opioids is much larger when compared to all inhaled and non-opioid category. Among the opioids, the fully synthetic opioid (e.g. fentanyl), is generally larger in size than natural opioid (e.g. morphine). The exact molecular mechanism of anesthesia is not understood but it is well accepted that anesthetics act on different receptors (e.g. *N*-methyl-d-aspartate (NMDA), γ -aminobutyric acid (GABA), glycine and acetylcholine etc.).

The calculated cerebral concentration of the inhaled anesthetics are in the range of 0.26–0.57 mmol l⁻¹ [9]. On the other hand, the calculated cerebral concentra-

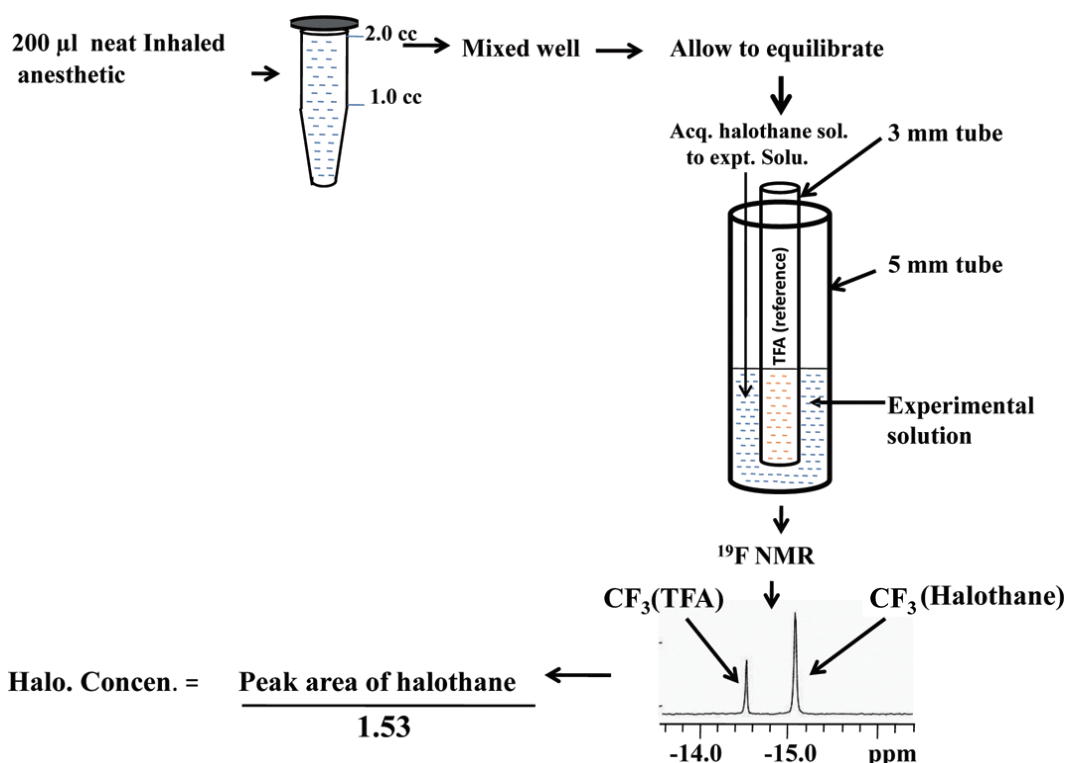


Fig. 2. Experimental flowchart for preparation of clinically relevant concentration and measurement of inhaled anesthetics. In this setup, an experimental solution is kept in a 5 mm NMR tube and the reference solution trifluoro acetic acid (TFA) is kept in the 3 mm tube which is coaxially arranged with the 5 mm tube. The final inhaled anesthetic concentration upon dilution from aqueous solution is determined by measuring the peak area of the CF_3 moiety of TFA and the CF_3 moiety of inhaled anesthetics present in the experimental solution [12].

tions of intravenous anesthetics (propofol and thiopental) are approximately 0.15 mmol l^{-1} and $0.075 \text{ mmol l}^{-1}$, respectively [9]. The clinically relevant concentration (CRC) of opioids is lower than CRC of the non-opioids anesthetics in the intravenous anesthetic family.

AMYLOID BETA PEPTIDE ($\text{A}\beta$)

$\text{A}\beta$ is primarily a 40 or 42 amino acid containing peptide and is the constituent of amyloid plaques in the brain. $\text{A}\beta$ is produced by the proteolytic cleavage of the amyloid precursor protein (APP) located on the plasma membrane, trans-Golgi network, endoplasmic reticulum (ER) and endosomal, lysosomal and mitochondrial membranes [10].

It has been suggested that low-molecular weight $\text{A}\beta$ oligomers could be the fundamental building blocks of larger oligomers [11]. Synthetic $\text{A}\beta$ spontaneously aggregates into β -sheet-rich fibrils, resembling those in plaques [3]. This provides the basis for investigating

the influence of different agents (e.g. certain anesthetics) which may induce the formation of $\text{A}\beta$ oligomers. NMR provides an excellent experimental tool to investigate the molecular pathway for $\text{A}\beta$ oligomerization by different anesthetics at clinically relevant concentration range.

Clinically relevant concentration (CRC) for inhaled anesthetics

Generally, neat inhaled anesthetics are too concentrated and the addition of these into the experimental solution yield more than the CRC of the respective inhaled anesthetic. In order to attain CRC levels of inhaled anesthetic in the experimental solution, it is preferable to add the desired amount of aqueous inhaled anesthetics to the experimental solution. The determination of the final concentration of the inhaled anesthetic in the experimental solution is schematically presented in Fig. 2, which is based on our earlier work [12]. The advantage of this setup is that the effective concentration of the inhaled anesthetic in the experimental solution

can be measured at different time points without disturbing the reference sample and experimental solution as both NMR tubes are coaxially aligned and remain in this position throughout the experimental period.

INTERACTION PATTERN OF A β PEPTIDE WITH ANESTHETICS

We hereby review the specific effect of inhaled (halothane, isoflurane, desflurane etc.) and intravenous (propofol, diazepam and thiopental) anesthetics on A β peptide at various time points by one, two and three dimensional heteronuclear NMR experiments as well as by two dimensional heteronuclear single quantum coherence (HSQC) experiments [13].

A β Peptide Interaction Studies with different sized Anesthetics

The smaller sized anesthetic isoflurane, at high concentration, induces chemical shift change of three critical residues (G29, A30 and I31) of A β peptide, which is linearly correlated with the addition of isoflurane. The NH peak chemical shift change due to isoflurane was 50 Hz, 45 Hz and 12 Hz for G29, I31 and A30, respectively [14]. However, at clinically relevant concentration (CRC) of isoflurane the chemical shifts of the critical residues show NH chemical shift change 9 Hz and 4 Hz for G29 and I31, respectively. A β oligomerization was found after 9 days in the presence of CRC of isoflurane [15] (Fig. 3), whereas A β oligomerization was observed after 25 days in the presence of CRC of desflurane [15].

Intermediate sized anesthetic, propofol, at high concentration, induced NH chemical shift changes of the same critical residues (I31, G29 and A30) and oligomerization of A β peptide was observed [14]. However, at CRC of propofol, no chemical shift change of the critical residues and no subsequent oligomerization of A β was observed even after 69 days [16].

Interestingly, a larger anesthetic thiopental, even at very high concentration, did not perturb the critical residues (G29, A30 and I31) and no A β oligomerization was observed [14]. A similar effect was observed with another bigger sized anesthetic, namely diazepam where, again, no A β oligomerization was observed. It is important to note that these two bigger sized anesthetics, thiopental and diazepam, do not cause in vitro A β peptide oligomerization at any concentration or time frame.

However, certain other amino acid residues (i.e. F20 and Q15) are perturbed and show chemical shift change

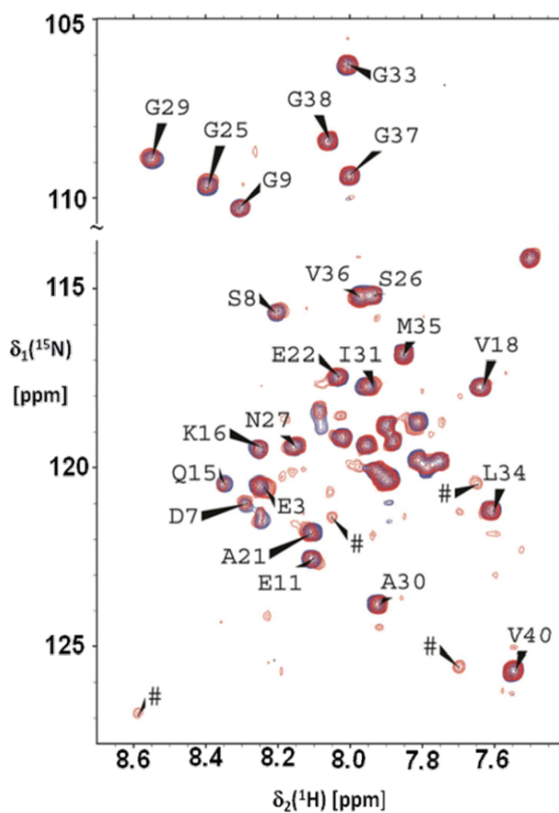


Fig. 3. Overlap of two dimensional [^{15}N , ^1H] HSQC spectra of A β peptide (blue) and in the presence of clinically relevant concentration of isoflurane (red). Backbone amides of residues G29, A30 and I31 show systematic change in chemical shift upon addition of isoflurane [15]. A β oligomerization was observed after 9 days indicated by symbol (#). Reproduced with permission.

due to addition of thiopental but these did not influence the process of A β oligomerization. This finding indicates that perturbations of the three critical residues G29, A30 and I31 is essential for the induction of A β oligomerization.

When a smaller sized anesthetic, halothane, is co-administered with a bigger sized anesthetic, diazepam or thiopental, the critical residues (G29, A30 and I31) show chemical shifts and subsequent A β oligomerization. However, in the presence of either diazepam [17] or thiopental [14] alone, no change in chemical shift of those critical residues and no subsequent A β oligomerization was observed.

DISCUSSION

Biophysical studies (e.g. size exclusion chromatog-

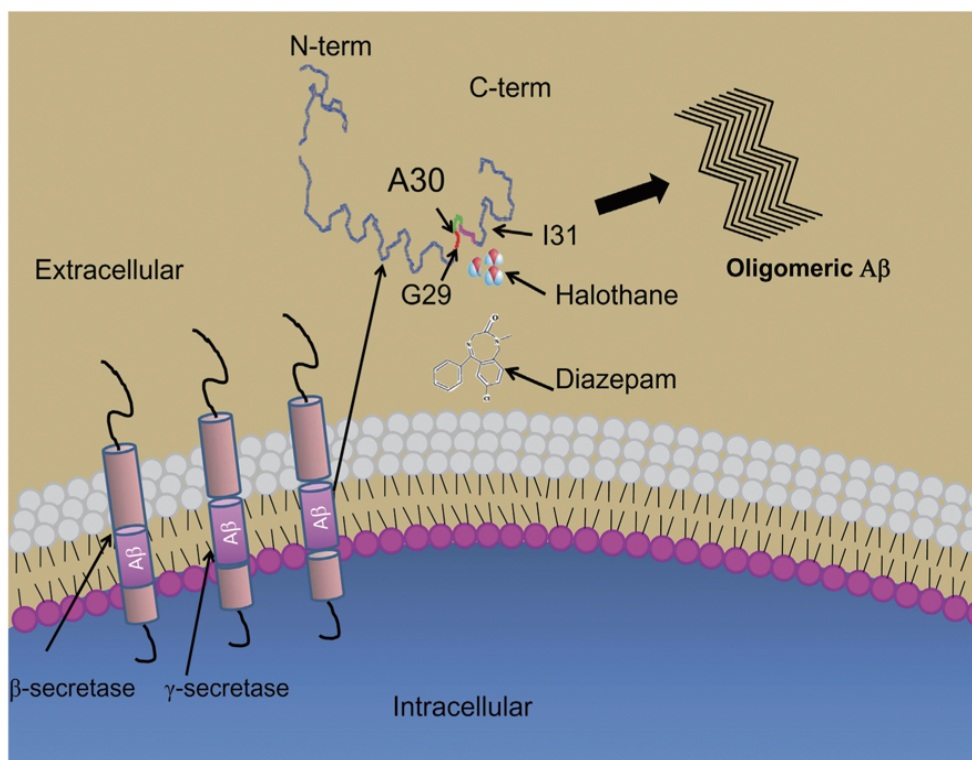


Fig. 4. Schematic diagram for interaction studies of A β peptide with bigger sized anesthetic (e.g. diazepam) co-administered with smaller sized anesthetic (e.g. halothane). Due to steric hindrance, diazepam could not access the helix-loop-helix region containing critical residues G29, A30 and I31. Diazepam also could not block the entry of the smaller sized halothane. Hence A β oligomerization was initiated when halothane is co-administered with diazepam. This model works in a same fashion with inhaled anesthetics alone or other bigger sized anesthetics (e.g. thiopental and propofol) when halothane is co-administered with them separately. The figure is adapted and revised from our earlier works [15–17].

raphy, fluorescence, analytical ultracentrifugation, etc.) have shown that halothane and isoflurane favor intermediate A β oligomer formation [1]. Light scattering studies indicate that inhalational anesthetics induce oligomeric A β formation, though the molecular mechanism for the oligomerization has not been identified. Similarly, it was also shown that propofol at lower concentration stabilizes A β peptide but at a higher concentration, oligomerization of A β was enhanced [1]. To understand anesthetic-induced A β oligomerization, and for providing molecular details of active interacting domain of anesthetics, their binding nature and the molecular pathway for A β oligomerization, employing NMR as a tool is very useful.

Anesthetic Binding Pocket in A β

Series of NMR studies on smaller sized anesthetics (e.g. isoflurane, desflurane), intermediate sized anesthetics (e.g., propofol) and larger sized anesthetic (e.g. thiopental and diazepam) indicate that smaller sized

inhaled anesthetics could access the specific helix-loop-helix region of A β peptide containing the critical amino acid residues (G29, A30 and I31) inducing A β oligomerization [14,17]. However, due to steric hindrance, larger sized intravenous anesthetics (thiopental and diazepam) could not access the cavity containing these critical residues, and therefore no A β oligomerization was observed. Propofol, being an intermediate sized (191 Å³) intravenous anesthetic, could reach the cavities at high concentration, inducing A β oligomerization. However, at CRC of propofol, these critical residues are not disturbed and no A β oligomerization was observed [16]. Another crucial observation from these NMR studies is that although bulkier anesthetics, e.g. thiopental [14], diazepam [17] and propofol [16], do not individually interact with critical residues at CRC, they induce A β oligomerization when co-administered with smaller sized halothane. The explanation for this is that the bigger sized anesthetics do not block the access of smaller sized halothane molecules to the helix-loop-helix region containing the

three crucial residues (G29, A30 and I31). The conclusion drawn from these experiments is that oligomerization of A β is induced whenever inhaled anesthetics are given alone or co-administered with bigger sized anesthetics.

Hydrophobic Interaction between Anesthetics and Critical Amino Acid Residues

Studies in various systems have reported the hydrophobic nature of interactions between anesthetics and proteins [18]. In our dose-dependent study with inhaled anesthetic (i.e. isoflurane at four different concentrations), the three critical residues (G29, A30 and I31) were perturbed in a systematic manner. Similarly, with intermediate sized intravenous anesthetic (propofol), the critical residues were perturbed to a different extent with varying concentrations, except at CRC of propofol where no A β oligomerization was observed. The concentration dependence on the perturbation of the critical residues with propofol, clearly indicate the hydrophobic nature of interactions of the critical residues with both isoflurane and propofol. In the case of diazepam and thiopental, these critical residues were not perturbed as these bulkier anesthetics could not access the cavity containing the three critical residues due to steric hindrance.

Biophysical studies involving light scattering and fluorescence studies [1,19] could not provide specific molecular interaction pattern of the anesthetics and A β peptide. However, NMR studies with various anesthetics provide crucial additional information on the size of the cavity containing the three critical residues [16]. From published results, it is reasonable to assume that this cavity size is comparable to the propofol molecular volume which is 191 Å³. This information should serve as a significant indicator of molecular size while designing new generation anesthetics, keeping in mind that the molecular size should be equal to or slightly higher than that of propofol in order to prevent the anesthetics from reaching the cavity and causing subsequent A β oligomerization.

Molecular Pathway leading to anesthetic induced A β oligomerization

The topological co-existence of both A β and anesthetics in the extracellular space makes anesthetics available for interaction with A β peptide. Figure 4 presents the schematic diagram [9,14,15,17] for the interaction of A β peptide and diazepam (at CRC) co-

administered with halothane. A β peptide is generated via the proteolytic cleavage action of β - and γ -secretase on APP protein located in the transmembrane region. The cleavage of APP to A β peptide and its subsequent release to the extracellular domain is a normal biological process. A β peptide accumulates in abundance in the aged brain. Consequently, anesthetic interactions with A β in the older population could be more relevant than in a younger control group. This schematic model based on NMR studies provides vital information regarding the molecular pathway for anesthetic-induced A β oligomerization.

Animal Model Studies: Plaque Load and Cognition

The results of current animal model studies concur with the findings of NMR and other biophysical studies that small molecular anesthetics do indeed play a role in A β oligomerization. Transgenic mice, Tg2576 with APP Swedish mutation expressing AD pathology, on exposure to halothane at CRC showed enhanced amyloid beta plaque deposition compared to isoflurane, an anesthetic of bigger size [4]. No detectable alteration in cognitive performance of aged transgenic mice (12 months old) was found with either anesthetic but isoflurane exposure impaired cognitive function in non-transgenic mice [4]. The supportive explanation given for this ambiguous finding is that twelve months old transgenic mice were of advanced age and already had significant cognitive decline at the time of exposure to anesthetic. Consequently any further effect on the cognition due anesthetic induced A β oligomerization was difficult to detect. Quoting the study group, "The clinical correlate of this study might be those patients with a diagnosis of Alzheimer disease at the time of surgery (quite common)". It is interesting to note that the results of a controlled laboratory experiment on animals is being directly extrapolated to a clinical scenario [4] where the development of the disease is characterized by multi factorial etiology and the susceptibility to it based on a complex interaction of genetic and environmental conditions. It was thus crucial that the effect of these anesthetics be investigated on younger mice. In another study, 7 to 10 month old transgenic mice were exposed to isoflurane over a cumulative period of 8 hours spread over three months [6]. Reports show that isoflurane exposure not only produced cognitive decline in transgenic mice but was found to be life threatening in some cases, while the non-transgenic mice remained resistant to the effects of anesthesia [6]. This study highlights the nature of individual susceptibility to certain anes-

Table 1
Comparative analysis of animal model studies on the effect of inhaled anesthetics

	Mena and colleagues [6]	Bianchi and colleagues [4]
Type of transgenic mice	APP SwedishTg2576	APP SwedishTg2576
Mice gender	Male	Female
Age at the time of experiment	7 to 10 months	12 months
Type of inhaled anesthetics used	Isoflurane only	Isoflurane and halothane
Anesthetic treatment	Dose: Induction with 4% isoflurane for 1 min, and then 2% isoflurane with 98% oxygen for 20 min as maintenance.	Dose: halothane (0.8–1%) or isoflurane (0.9–1%) in 30% oxygen, balanced by nitrogen
Dose and duration	Duration: repetitive anesthesia, twice a week, for 3 months, from 7 to 10 month of age Total time of exposure = 8 hours	Duration: 120 min per day for 5 days (total five exposures) Total time of exposure = 10 hours
Observation (Histopathology)	Isoflurane exposure causes increased levels and aggregation of A β peptides, increased mortality and neuronal cell apoptosis in transgenic mice, but no increase in plaque deposition found. No effect on wild type mice.	Enhanced amyloid beta plaque deposition in Tg2576 mice with exposure to halothane, but not with isoflurane. Wild-type mice do not develop plaque
Outcome in terms of cognition etc.	Isoflurane exposure produces significant reduction in cognitive behavior of Tg2576 mice compared to wild type. Continuous exposure to isoflurane (dose mentioned above) found to have deleterious impact on behavior, survival, neuronal cell death.	No detectable alteration in the cognitive performance of Tg2576 mice with either halothane or isoflurane. Decreased cognitive performance in wild-type mice exposed to isoflurane but not halothane.
Clinical relevance	The study suggests that the risk of some inhaled anesthetics may be limited to subjects with special genetic and environmental risk factors for AD; aspiring to ensure proper selection of patients, and the type of anesthetics and anesthesia to be used for them.	The condition of transgenic mice in the controlled experimental settings is being directly correlated with Alzheimer disease patients. The study group states, “The clinical correlate of this study might be those patients with a diagnosis of Alzheimer disease at the time of surgery (quite common).”

thetics rather than suggesting anesthetics as a general risk factor for AD [6]. A comprehensive analysis of the animal model studies of the two research groups is provided in Table 1. The outcome of these researches urges the need for further well-designed human studies with larger sample size to confirm the implications for the use of certain anesthetic in humans.

CONCLUSIONS

NMR studies provide valuable molecular details in detecting the A β oligomerization propensity by various anesthetic drugs. The state-of-the-art NMR technique can serve as a screening method for identifying next generation anesthetics. This biophysical research also provides additional information as guidance for the synthesis of the next generation anesthetic molecule. We believe that this research will accelerate clinical research, which in its turn will contribute to serious rethinking on the choice of “safe” anesthetics in the elderly population.

ACKNOWLEDGMENTS

Dr. Subbulakshmy Natarajan, MBBS, Ph.D and Dr. Sreedevi Sugunan, MBBS are appreciated for comments. Dr. Vincenzo Fodale, MD is highly appreciated for valuable suggestions. Dr. Mandal is thankful to National Brain Research Center for providing research support.

REFERENCES

- [1] Eckenhoff RG, Johansson JS, Wei HF, Carnini A, Kang BB, Wei WL, Pidikiti R, Keller JM, Eckenhoff MF. (2004) Inhaled anesthetic enhancement of amyloid-beta oligomerization and cytotoxicity. *Anesthesiology* **101**, 703-709.
- [2] Kuehn BM. (2007) Anesthesia-Alzheimer disease link probed. *JAMA* **297**(16), 1760.
- [3] Balducci C, Beeg M, Stravalaci M, Bastone A, Sclip A, Bisini E, Tapella L, Colombo L, Manzoni C, Borsello T and others. (2010) Synthetic amyloid-beta oligomers impair long-term memory independently of cellular prion protein. *Proc Natl Acad Sci U S A* **107**, 2295-300.
- [4] Bianchi SL, Tran T, Liu C, Lin S, Li Y, Keller JM, Eckenhoff RG, Eckenhoff MF. (2008) Brain and behavior changes in 12-month-old Tg2576 and nontransgenic mice exposed to anesthetics. *Neurobiol Aging* **29**, 1002-10.

- [5] Xie Z, Culley DJ, Dong Y, Zhang G, Zhang B, Moir RD, Frosch MP, Crosby G, Tanzi RE. (2008) The common inhalation anesthetic isoflurane induces caspase activation and increases amyloid beta-protein level in vivo. *Ann Neurol* **64**, 618-27.
- [6] Perucho J, Rubio I, Casarejos MJ, Gomez A, Rodriguez-Navarro JA, Solano RM, De Yébenes JG, Mena MA. (2010) Anesthesia with isoflurane increases amyloid pathology in mice models of Alzheimer's disease. *J Alzheimers Dis* **19**, 1245-57.
- [7] Borghese CM, Werner DF, Topf N, Baron NV, Henderson LA, Boehm SL, Blednov YA, Saad A, Dai S, Pearce RA and others. (2006) An isoflurane- and alcohol-insensitive mutant GABA(A) receptor alpha(1) subunit with near-normal apparent affinity for GABA: Characterization in heterologous systems and production of knockin mice. *Journal of Pharmacology and Experimental Therapeutics* **319**, 208-218.
- [8] Liu R, Meng Q, Xi J, Yang J, Ha CE, Bhagavan NV, Eckenhoff RG. (2004) Comparative binding character of two general anaesthetics for sites on human serum albumin. *Biochem J* **380**, 147-52.
- [9] Mandal PK, Fodale V. (2009) Smaller Molecular-sized Anesthetics Oligomerize A β Peptide Simulating Alzheimer's Disease: a Relevant Issue. *European Journal of Anesthesiology* **46**, 805-806.
- [10] Sakono M, Zako T. (2010) Amyloid oligomers: formation and toxicity of A β oligomers. *Febs J* **277**, 1348-58.
- [11] Walsh DM, Tseng BP, Rydel RE, Podlisny MB, Selkoe DJ. (2000) The oligomerization of amyloid beta-protein begins intracellularly in cells derived from human brain. *Biochemistry* **39**, 10831-9.
- [12] Mandal PK, Pettegrew JW. (2008) Clinically Relevant Concentration Determination of Inhaled Anesthetics (Halothane, Isoflurane, Sevoflurane, and Desflurane) by (19)F NMR. *Cell Biochem Biophys* **52**, 31-35.
- [13] Mandal PK, Majumdar A. (2004) A comprehensive discussion of HSQC and HMQC pulse sequences. *Concepts in Magnetic Resonance Part A* **20A**, 1-23.
- [14] Mandal PK, Pettegrew JW. (2008) A β peptide interactions with isoflurane, propofol, thiopental and combined thiopental with halothane: A NMR study. *Biochim Biophys Acta* **1778**, 2633-2639.
- [15] Mandal PK, Fodale V. (2009) Isoflurane and desflurane at clinically relevant concentrations induce amyloid beta-peptide oligomerization: an NMR study. *Biochem Biophys Res Commun* **379**, 716-20.
- [16] Mandal PK, Bhavesh NS, Chauhan VS, Fodale V. (2010) NMR investigations of A β peptide interactions with propofol at clinically relevant concentrations with and without aqueous halothane solution. *Journal of Alzheimer Disease* **21**, 1303-1309.
- [17] Mandal PK, Simplaceanu V, Fodale V. (2010) Intravenous Anesthetic Diazepam does not induce Amyloid beta-peptide Oligomerization but Diazepam Co-administered with Halothane Oligomerizes Amyloid Beta-peptide: An NMR study. *Journal of Alzheimer Disease* **20**, 127-134.
- [18] Streiff JH, Allen TW, Atanasova E, Juranic N, Macura S, Penheiter AR, Jones KA. (2006) Prediction of volatile anesthetic binding sites in proteins. *Biophysical Journal* **91**, 3405-3414.
- [19] Carnini A, Lear JD, Eckenhoff RG. (2007) Inhaled anesthetic modulation of amyloid beta(1-40) assembly and growth. *Current Alzheimer Research* **4**, 233-241.