At Clinically Relevant Concentration Isoflurane and Desflurane Induce Abeta Oligomerization. Molecular Details from NMR Spectroscopy

ARTICLE *in* BIOPHYSICAL JOURNAL · FEBRUARY 2009

Impact Factor: 3.97 · DOI: 10.1016/j.bpj.2008.12.289

2 AUTHORS, INCLUDING:

Vincenzo Fodale

Università degli Studi di Messina

90 PUBLICATIONS **992** CITATIONS

SEE PROFILE

Biochemical and Biophysical Research Communications 379 (2009) 716–720

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Isoflurane and desflurane at clinically relevant concentrations induce amyloid β-peptide oligomerization: An NMR study ☆

Pravat K Mandal [a,*], Vincenzo Fodale [b]

[a] Neurospectroscopy and Neuroimaging Laboratory, National Brain Research Centre, Manesar, Gurgaon, India
[b] Department of Neurosciences, Psychiatric and Anesthesiological Sciences, University of Messina, Policlinico G. Martino, Via C. Valeria, I-98125 Messina, Italy

ARTICLE INFO

Article history:
Received 5 December 2008
Available online 29 December 2008

Keywords:
Aβ
amyloid β-peptide
Desflurane
Isoflurane
Neurodegeneration
Oligomerization

ABSTRACT

Current understanding on Alzheimer's disease (AD) reveals that soluble amyloid β-peptide (Aβ) oligomeric formation plays an important role in AD pathophysiology. A potential role for several inhaled anesthetics in promoting Aβ oligomer formation has been suggested. Using a nuclear magnetic resonance (NMR) study, we previously demonstrated that at a high concentration (higher than clinically relevant concentrations), the inhaled anesthetics halothane and isoflurane, interact with specific amino acid residues (G29, A30, and I31) and induce Aβ oligomerization. The present study confirms this is true at a clinically relevant concentration. Isoflurane and desflurane induce Aβ oligomerization by inducing chemical shift changes of the critical amino acid residues (G29, A30, and I31), reinforcing the evidence that perturbation of these three crucial residues indeed plays an important role in oligomerization. These findings support the emerging hypothesis that several commonly used inhaled anesthetics could be involved in neurodegeneration, as well as risk factor for accelerating the onset of AD.

Alzheimer's disease (AD) is the most common cause of progressive dementia. The molecular mechanism for the disease-promoting effect has been difficult to pinpoint, but emerging views point toward amyloid β-peptide (Aβ) oligomeric formation, which plays an important role in the pathogenesis of this disease [1].

Several anesthetics could promote Aβ oligomeric formation, suggesting a potential association between anesthesia and AD [2–6]. At clinically relevant concentrations, the inhaled anesthetics halothane and isoflurane-induced Aβ oligomerization, although molecular details were not well known [4,7]. Using NMR spectroscopic study, we have previously shown that these anesthetics, at a high concentration, interact with a specific region of the Aβ peptide and induce Aβ oligomerization [5]. In transgenic mice, in comparison with control group animals, more amyloid plaques were reported after administration of halothane and isoflurane [8]. It was also inferred that several commonly used inhaled anesthetics may cause brain damage which accelerates the onset of AD [3]. All these observations warrant further studies on Aβ peptide interaction with current inhaled anesthetics at clinically relevant concentrations which are used for anesthesia and sedation in intensive care units.

Recent research supports the use of volatile agents isoflurane and desflurane as the ideal sedative agents in intensive care units (ICU) because of their low blood solubility, metabolism less than 1%, and elimination independent of renal or hepatic function [9]. In addition, desflurane seems to be a promising new alternative to intravenous anesthetics for sedation of ventilated adult patients in ICU [10], and isoflurane is a safe and efficacious agent for inhalational sedation in ICU, with short wake-up times after termination of administration [11].

A consistent part of the population admitted to ICU present high levels of Aβ in the CNS (elderly and head-injured patients). The overall aim of our study was to investigate whether isoflurane and desflurane, at clinically relevant concentrations, interact with Aβ. The secondary objective was to show the time dependence for Aβ oligomerization, if any, due to these inhaled anesthetic agents.

Materials and methods

To address the two important above mentioned objectives, NMR experiments were designed to investigate time-dependent studies for Aβ peptide interaction with isoflurane and desflurane at a clinically relevant concentration.

Materials. In this study, desflurane (Baxter), isoflurane (Lancaster Synthesis Inc., USA), Deuterated SDS_{D25} (Cambridge Isotope Laboratories), and ^{15}N-labeled Aβ40 peptide (Recombinant Peptide Technologies, Atlanta, GA, USA) have been used. NMR data have been recorded using 5 and 3 mm NMR tubes purchased from Wilmad Lab Glass.

☆ Part of the results will be presented at Biophysical Society meeting, Boston, Feb. 2009.

* Corresponding author.
E-mail address: Pravat.mandal@gmail.com (Dr. P.K. Mandal).
URL: http://www.nbrc.ac.in (P.K Mandal).

0006-291X/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2008.12.092

Preparation of Aβ peptide solution in SDS. One mg Aβ40 lyophilized powder (uniformly ^{15}N labeled) was added to SDS_{D25} solution and gently mixed. The final concentration of ^{15}N labeled Aβ40 peptide was ~0.22 mM, and 10% of D_2O provided the field/frequency lock for the NMR spectrometer. In all the NMR studies, pH of the Aβ peptide solution was adjusted and measured at 7.2 before addition of anesthetics.

NMR experimental setup. Two coaxial tubes (3 and 5 mm) were used for all NMR studies. Aβ40 peptide solution was kept in a 5 mm NMR tube, and the 3 mm NMR tube contained a standard 0.5 mM TFA solution. Heteronuclear single quantum coherence (HSQC) experiments were performed for three sets of Aβ40 solution (the first for Aβ alone as control, the second for isoflurane, and third for desflurane). To study Aβ-isoflurane interaction, an 80 μl saturated aqueous isoflurane solution was added to the 5 mm tube containing Aβ40 solution. The 3 mm NMR tube containing 0.5 mM TFA was inserted in the 5 mm NMR tube and kept coaxially till all necessary experiments were performed. Both NMR tubes were sealed with Teflon to prevent evaporation of isoflurane and/or TFA. Similarly, to study Aβ-desflurane interaction, 85 μl saturated aqueous desflurane solution was added to Aβ40 solution and sealed immediately. Experimental flowchart is presented in Fig. 1. All NMR experimental conditions were kept identical while monitoring two peptide-anesthetics systems. The advantage of the coaxially arranged NMR tubes is that it allows HSQC experiments to be performed to monitor the influence of anesthetics on Aβ peptide and anesthetics concentration determination using the ^{19}F NMR experiment, at any time point, without disturbing the system under investigation [12].

NMR experiment details and data analysis. Determination of anesthetic concentration by ^{19}F NMR. We used two coaxial NMR tubes (5 and 3 mm) for the determination of anesthetic concentration by ^{19}F NMR experiments using a 500 MHz NMR spectrometer (Bruker, Germany). A detailed procedure for calculating the anesthetic concentration from the ^{19}F spectra is provided in our earlier work [12]. The concentrations of isoflurane and desflurane were determined at two time points, one initially after adding the respective anesthetic and another one at the end of all HSQC experiments [13].

HSQC experiments. All HSQC experiments were performed using a 600 MHz Bruker spectrometer (Bruker, Germany). Processing and analysis of the NMR data were carried out using NMRPipe [14] PIPP [15] and SPARKY [16] software on an Octane Silicon Graphics computer. Assignments of the amide peaks were made according to our earlier work [5].

Results and discussion

Fig. 1 indicates the experimental flowchart of the present study. After addition of isoflurane and desflurane into respective Aβ solutions, the concentrations of the anesthetics measured were 0.32 and 0.29 mM compared to 0.5 mM standard TFA solution.

Isoflurane and desflurane interactions with Aβ

Fig. 2A shows the overlay of HSQC spectra of Aβ and Aβ with isoflurane. We found a substantiated Aβ oligomerization after 9

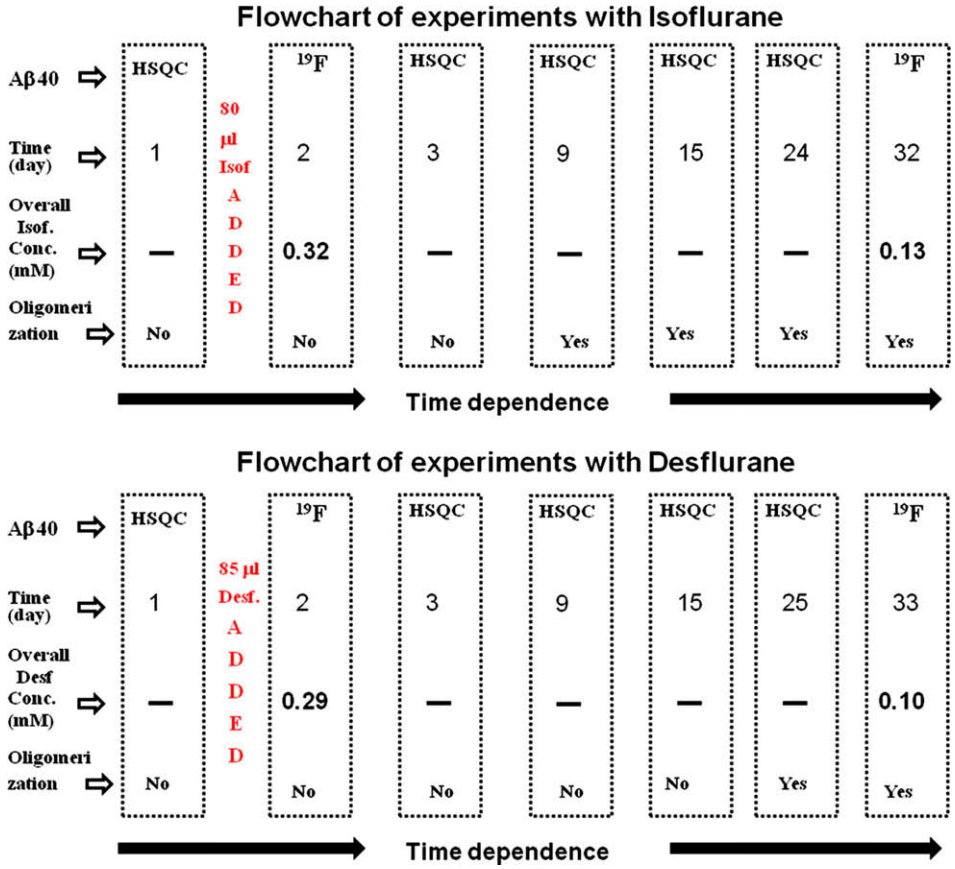

Fig. 1. Flowcharts of NMR experiments for Aβ interaction studies with isoflurane and desflurane at a clinically relevant concentration. After addition of aqueous isoflurane or desflurane solution, it was left for equilibrium, and respective anesthetic concentration was measured by ^{19}F NMR. Aβ-anesthetic interactions were monitored at different time points. Anesthetic concentration at the end was also measured. Due to natural process of evaporation, both isoflurane and desflurane evaporated, despite Teflon-sealing of the NMR tube.

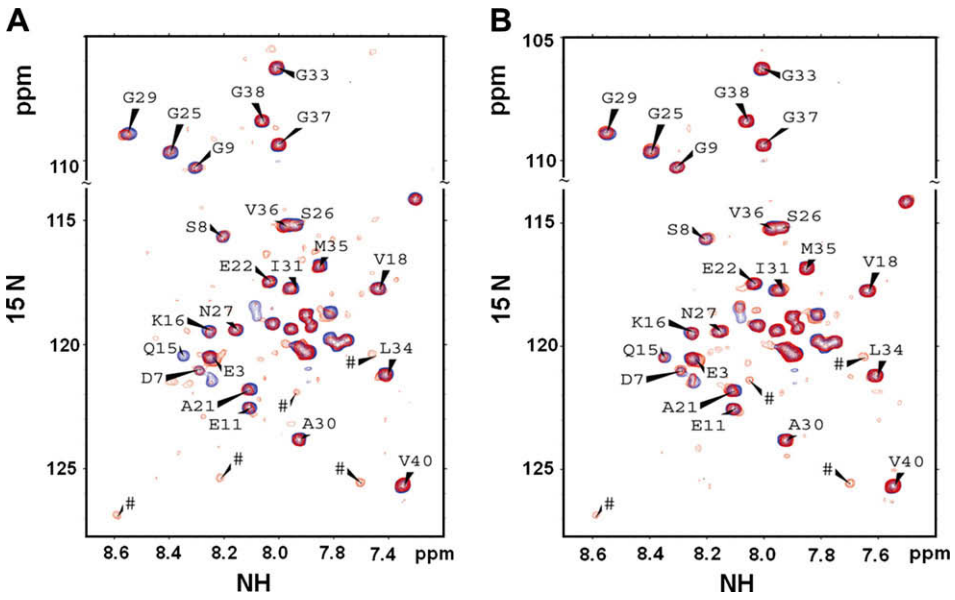

Fig. 2. (A) HSQC spectra of Aβ40 peptide in the presence of 0.32 mM isoflurane. The HSQC spectra (control, Aβ40 without isoflurane) are shown in blue, and HSQC spectra after 9 days in the presence of isoflurane are shown in red. It is important to note that G29 also shows 9 Hz chemical shift due to interaction with isoflurane. Due to Aβ oligomerizaton, additional amide peaks are seen (marked by # sign). (B) HSQC spectra of Aβ40 peptide in the presence of 0.29 mM desflurane. Blue denotes the HSQC spectra (control, Aβ40 without isoflurane) and red denotes the HSQC spectra of Aβ40 with desflurane after 25 days. Aβ oligomerizaton (evidenced by additional amide peaks as indicated by # sign) takes place more slowly with desflurane as compared to isoflurane.

days in the presence of isoflurane at a clinically relevant concentration. Additional amide peaks appear in the NMR spectrum due to isoflurane-induced Aβ oligomerization, and these are marked by the sign (#) in Fig. 2A.

Fig. 2B shows the overlap of HSQC spectra of Aβ40 and Aβ40 + desflurane. Oligomerization of Aβ is also seen (as evidenced by additional amide peaks marked by the sign (#)). It is important to note that Aβ oligomerization due to desflurane progresses slowly, and profound Aβ oligomerization was observed only after 25 days (Fig. 2B).

In the presence of isoflurane, G29 moiety shows chemical shifts of 5 Hz for ^{15}N and 9 Hz for NH, while the I31 amide peak shows shifts of 4 Hz for ^{15}N and 4 Hz for NH. In the presence of desflurane, G29 moiety shows chemical shifts of 4 Hz for ^{15}N and 1 Hz for NH, while the I31 amide NH shows a 7 Hz shift (Fig. 3).

Importance of the chemical shift of G29 and I31

We have previously shown that isoflurane and halothane, in the presence of thiopental, induce chemical shifts of G29 and I31 signals in the range of 60 Hz, while A30 shows around a 10 Hz chemical shift change [5]. Furthermore, Aβ oligomerization was observed within 28 h in the presence of thiopental in combination with halothane. In all the above cases, the concentration of anesthetics was much higher than what would be clinically relevant. What is encouraging, however, is that Aβ peptide did not oligomerize in the presence of thiopental alone, even at higher concentration. However, one crucial observation was that in the presence of thiopental (at higher concentration), the G29, A30, and I31 amide peaks did not shift, and no Aβ oligomerization was observed. However, in the presence of thiopental, other residues such as Q15 did show chemical shift changes, indicating interaction of thiopental with Aβ.

In this present study, dealing with clinically relevant concentrations, we have observed chemical shifts of G29 and I31 in a much smaller range (Fig. 3). What is significant is the observation that at a clinically relevant concentration, Aβ oligomerization is observed after a longer period (9 days for isoflurane and 25 days in the case of desflurane).

Characteristics of the anesthetics

Isoflurane and desflurane have similar types of physiochemical characteristics. Both these inhaled anesthetics belong to the

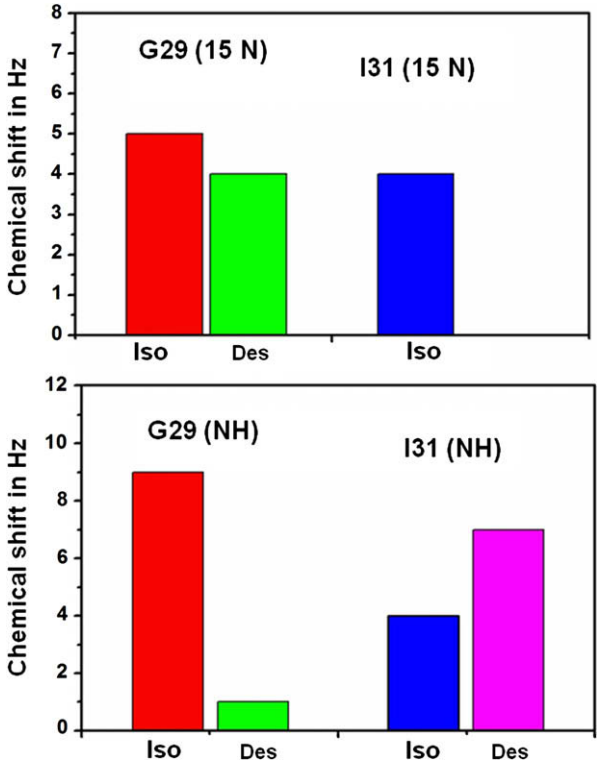

Fig. 3. Plot of chemical shift changes of the residues G29 (^{15}N and NH) and I31 (NH) due to interaction with isoflurane and desflurane. It is to be noted that, for the I31 residue, no ^{15}N chemical shift changes occur due to isoflurane, although, we do observe I31 (NH) chemical shift changes due to desflurane. Experiments with high anesthetic concentration also indicated that G29 shows the highest chemical shift, followed by an intermediate shift for I31; the lowest chemical shift was observed in A30.

haloether category and have similar molecular size. The molecular volume of isoflurane is 144 $Å^3$ [17]. However, there are certain differences between these two anesthetics. Desflurane is more volatile than isoflurane. Desflurane structurally differs from isoflurane due to the replacement of a chlorine atom by a fluorine atom. In desflurane, fluorination rather than chlorination increases vapor pressure, decreases intermolecular attraction, enhances molecular stability, and decreases potency. The blood/gas partition coefficients of isoflurane and desflurane are 1.4 and 0.42, respectively, while the brain/blood partition coefficients of isoflurane and desflurane are 1.60 and 1.30, respectively [18]. The potency of an inhaled anesthetic is defined in terms of minimum alveolar concentration (MAC) or EC_{50}, a value at which 50% of patients are unresponsive to a standard surgical stimulus [19]. The EC_{50} of most inhaled anesthetics is between 0.2 and 0.3 mM [20,21]. The brain/blood coefficient of 1.6 for isoflurane means that if the gas is in equilibrium, the concentration of isoflurane in the brain should be <0.5 mM. As desflurane has a brain/blood coefficient of 1.3, the concentration of desflurane in the brain should be <0.4 mM.

The biophysical data obtained using a light-scattering experiment indicated that inhaled anesthetics may be involved by inducing oligomerization of Aβ peptide [4]. Using NMR spectroscopic studies, we have further established that inhaled anesthetics (halothane, isoflurane) interact with three critical residues (G29, A30, and I31) and induce Aβ oligomerization. It was also inferred that size of anesthetic was important since it must fit into the pocket loop region of the Aβ peptide to induce oligomerization [5,22]. In all the cases, the anesthetics were at much higher concentrations than clinically relevant concentrations. Hence, this model needs to be validated at clinically relevant concentrations.

A working model for inhaled anesthetic and Aβ peptide interaction

Our earlier studies in this area [2,5,6,22] helped us map chemical shift changes of the crucial amino acid residues that are involved in the oligomerization process. As indicated earlier [5], topological coexistence of anesthetics and Aβ peptide support interaction of Aβ with anesthetics. A plausible mechanism of anesthetic-induced Aβ oligomerization is presented in Fig. 4. Aβ peptide is generated due to the action of β and γ secretase on amyloid precursor protein (APP) and more Aβ peptide is available in the aged brain. Thus, excessive Aβ peptide load in the elderly is susceptible to interaction with inhaled anesthetics in a specific manner to induce "neurotoxic" oligomeric Aβ. Eventually these oligomers may form plaques.

Clinical implications of this research

Translated from bench to bedside, data from our research could potentially have an important clinical relevance. As previously stated, isoflurane or desflurane are preferred as ideal sedative agent in ICU [9–11]. However, we have demonstrated that both desflurane and isoflurane, at clinically relevant concentrations, comparable to those administered in patients in ICU for prolonged inhalational sedation, oligomerize Aβ peptide. What is significant is that Aβ oligomerization was observed within the clinical range of the duration of sedation [23].

Aβ is naturally present in the CNS, with higher levels in the elderly. Moreover, deposits of Aβ are widely distributed in the brains of 30% of fatally head-injured patients after a survival time of only 4 h, without correlation between its presence and cerebral contusions, intracranial haematoma, axonal injury, ischemic brain damage, brain swelling or the pathology of raised intracranial pressure, suggesting that the deposition of Aβ is a consequence of the acute phase response of nerve cells to stress in susceptible individuals [24–27]. We may therefore consider that in the brains of a majority of the ICU population, there are higher amounts of Aβ, readily available to interact with anesthetics during prolonged sedation. Consequently, the direct administration of general inhaled anesthetics which affect the rate at which Aβ bind together, could increase risk in a patient. Other available information applicable to clinical situations must be taken into serious consideration. However, treatment with 12% desflurane (surgical levels of anesthesia commonly range between 5% and 10%) in combination with hypoxia for 6 h, induces increased Aβ generation in human cells lines. Neither 12% desflurane nor hypoxia in isolation affect Aβ generation [28]. Often, ICU patients require surgery and related administration of anesthetics. In these subjects, the association of desflurane and hypoxia could be a major concern in terms of risk of developing cognitive dysfunctions and AD.

More specific studies are required to establish the possible relationship between the deposition of Aβ following (i) head injury, (ii) anesthetics with Aβ oligomerization properties given in ICU setting for prolonged inhalational sedation, and (iii) molecular neuropathology of AD [29]. Our concern is based on recent reports suggesting that further study on memory and cognitive recovery after prolonged isoflurane sedation is warranted [11]. Pending *in vivo* confirmation, it may be prudent to avoid the combination of clinically relevant concentrations of desflurane plus hypoxia in the more vulnerable groups namely, those with excessive levels of cerebral Aβ, including AD patients, individuals with down

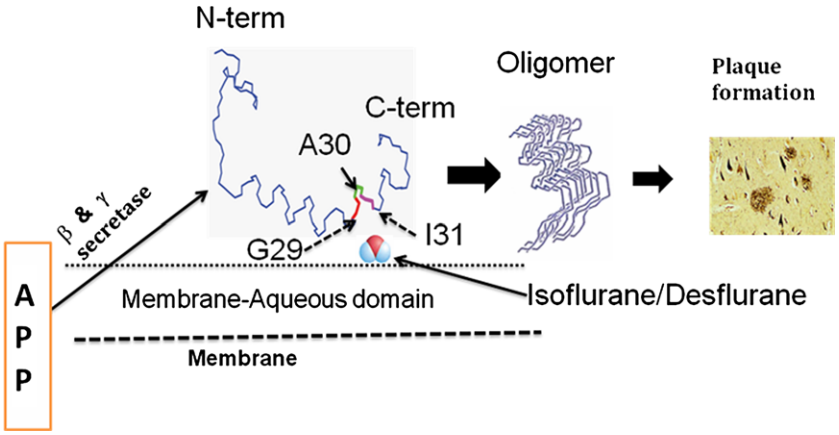

Fig. 4. A schematic diagram for Aβ interactions with isoflurane and/or desflurane at a clinically relevant concentration that leads to oligomeric Aβ formation. Aβ peptide is generated by the amyloid precursor protein (APP), by the action of β and γ secretase by natural process, and the inhaled anesthetic interacts with three specific residues (G29, A30, and I31), initiating the formation of neurotoxic oligomeric Aβ formation. This oligomer may be responsible for plaque formation as seen in AD patients on biopsy.

syndrome, and asymptomatic carriers of AD-associated mutations/variants in APP, presenilin 1 and 2, and APOE-ε4 [28].

Our previous study had limitations as it was performed in the presence of high anesthetic concentration, which could raise some queries. In order to overcome this problem, we have developed an experimental methodology for measuring the isoflurane and desflurane concentration at clinically relevant concentration. This is well described in our earlier work [12].

Secondly, oligomeric Aβ formations were detected by using the NMR spectroscopic technique: this technique is comprehensive and a reliable method for characterizing oligomeric Aβ formation [30], and in particular interaction of Aβ with the anesthetics agents. In this study, Aβ-anesthetic interactions were also followed by performing HSQC experiments at different time points. Our experimental model using NMR spectroscopy could serve as a test proposal for drugs which are used during surgery, to test whether these drugs have any potential neurotoxic effects.

Conclusions

This biophysical study provides molecular details for the oligomerization of Aβ by isoflurane and desflurane at a clinically relevant concentration, and also provide a possible explanation of increased plaque load noted in transgenic mice with AD after administration of halothane and isoflurane [31]. Our data are also correlated with recent reports suggesting that inhaled anesthetics have durable adverse effects on cognition [32] and that some commonly used inhaled anesthetics may cause brain damage which in turn could accelerate the onset of AD [3]. We believe that research in this field needs to be extended to the clinical setting to identify safer anesthetics for elderly patients, as well as patients with AD and other neurodegenerative disorders.

Acknowledgments

Professor Partha Raghunathan and Dr. Subbulakshmy Natarajan are thanked for critical comment and suggestions. Research support from National Brain Research Centre, Department of Biotechnology, Government of India as well as a grant from the Italian Ministry for University and Research, Program for the Development of Research of National Interest (PRIN Grant No. 2007H84XNH - Scientific coordinator: V. Fodale) was very appreciated. Thanks to Ms. Ruchi Bansal for assistance to prepare Fig. 4.

References

[1] K. Blennow, M.J. de Leon, H. Zetterberg, Alzheimer's disease, Lancet 368 (2006) 387–403.
[2] P.K. Mandal, D. Schifilliti, F. Mafrica, V. Fodale, Inhaled anesthesia and cognitive performance, Drugs of Today, 45 (2009), in press.
[3] B.M. Kuehn, Anesthesia-Alzheimer disease link probed, JAMA 297 (2007) 1760.
[4] R.G. Eckenhoff, J.S. Johansson, H.F. Wei, A. Carnini, B.B. Kang, W.L. Wei, R. Pidikiti, J.M. Keller, M.F. Eckenhoff, Inhaled anesthetic enhancement of amyloid-beta oligomerization and cytotoxicity, Anesthesiology 101 (2004) 703–709.
[5] P.K. Mandal, J.W. Pettegrew, Abeta peptide interactions with isoflurane, propofol, thiopental and combined thiopental with halothane: a NMR study, Biochim. Biophys. Acta 1778 (2008) 2633–2639.
[6] V. Fodale, D. Quattrone, C. Trecroci, V. Caminiti, L.B. Santamaria, Alzheimer's disease and anaesthesia: implications for the central cholinergic system, British Journal of Anaesthesia 97 (2006) 445–452.
[7] A. Carnini, J.D. Lear, R.G. Eckenhoff, Inhaled anesthetic modulation of amyloid beta(1-40) assembly and growth, Curr. Alzheimer Res. 4 (2007) 233–241.
[8] S.L. Bianchi, T. Tran, C. Liu, S. Lin, Y. Li, J.M. Keller, R.G. Eckenhoff, M.F. Eckenhoff, Brain and behavior changes in 12-month-old Tg2576 and nontransgenic mice exposed to anesthetics, Neurobiol. Aging (2007).
[9] G.A. McLeod, Analgesia and sedation in the critically-ill. The Royal College of Surgeons of Edinburgh SELECT Programme. Available from: <http://www.rcsed.ac.uk/eselect/cc4.htm#1>, 2008 (accessed 24.08.08) 6424–6429.
[10] A. Meiser, C. Sirtl, M. Bellgardt, S. Lohmann, A. Garthoff, J. Kaiser, P. Hugler, H.J. Laubenthal, Desflurane compared with propofol for postoperative sedation in the intensive care unit, Br. J. Anaesth. 90 (2003) 273–280.
[11] P.V. Sackey, C.R. Martling, C. Carlsward, O. Sundin, P.J. Radell, Short- and long-term follow-up of intensive care unit patients after sedation with isoflurane and midazolam—a pilot study, Crit. Care Med. 36 (2008) 801–806.
[12] P.K. Mandal, J.W. Pettegrew, Clinically relevant concentration determination of inhaled anesthetics (halothane, isoflurane, sevoflurane, and desflurane) by (19)F NMR, Cell Biochem. Biophys. 52 (2008) 31–35.
[13] P.K. Mandal, A. Majumdar, A comprehensive discussion of HSQC and HMQC pulse sequences, Concepts Magn. Reson. A 20A (2004) 1–23.
[14] F. Delaglio, S. Grzesiek, G.W. Vuister, G. Zhu, J. Pfeifer, A. Bax, NMRPipe: a multidimensional spectral processing system based on UNIX pipes, J. Biomol. NMR 6 (1995) 277–293.
[15] D.S. Garrett, R. Powers, A.M. Gronenborn, G.M. Clore, A common sense approach to peak picking two-, three- and four-dimensional spectra using automatic computer analysis of contour diagrams, J. Magn. Reson. 95 (1991) 214–220.
[16] T.D. Goddard, D.G. Kneller, SPARKY 3, University of California, San Francisco, 1994.
[17] C.M. Borghese, D.F. Werner, N. Topf, N.V. Baron, L.A. Henderson, S.L. Boehm, Y.A. Blednov, A. Saad, S. Dai, R.A. Pearce, R.A. Harris, G.E. Homanics, N.L. Harrison, 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, J. Pharmacol. Exp. Ther. 319 (2006) 208–218.
[18] E.I. Eger 2nd, Desflurane animal and human pharmacology: aspects of kinetics, safety, and MAC, Anesth. Analg. 75 (1992) S3–S7. discussion S8–S9.
[19] E.I. Eger 2nd, L.J. Saidman, B. Brandstater, Minimum alveolar anesthetic concentration: a standard of anesthetic potency, Anesthesiology 26 (1965) 756–763.
[20] R.G. Eckenhoff, J.S. Johansson, Molecular interactions between inhaled anesthetics and proteins, Pharmacol. Rev. 49 (1997) 343–367.
[21] N.P. Franks, W.R. Lieb, Molecular and cellular mechanisms of general anaesthesia, Nature 367 (1994) 607–614.
[22] P.K. Mandal, J.W. Pettegrew, S. Mittal, R. Mandal, D. Mckeag, Could anesthesia be a risk factor for Alzheimer's disease: a NMR spectroscopic study, Biophys. J. (2006) (meeting abstract, platform presentation).
[23] S.M. Mirsattari, M.D. Sharpe, G.B. Young, Treatment of refractory status epilepticus with inhalational anesthetic agents isoflurane and desflurane, Arch. Neurol. 61 (2004) 1254–1259.
[24] S.M. Gentleman, M.J. Nash, C.J. Sweeting, D.I. Graham, G.W. Roberts, Beta-amyloid precursor protein (beta APP) as a marker for axonal injury after head injury, Neurosci. Lett. 160 (1993) 139–144.
[25] D.I. Graham, S.M. Gentleman, A. Lynch, G.W. Roberts, Distribution of beta-amyloid protein in the brain following severe head injury, Neuropathol. Appl. Neurobiol. 21 (1995) 27–34.
[26] G.W. Roberts, S.M. Gentleman, A. Lynch, D.I. Graham, Beta A4 amyloid protein deposition in brain after head trauma, Lancet 338 (1991) 1422–1423.
[27] J.A. Nicoll, G.W. Roberts, D.I. Graham, Amyloid beta-protein, APOE genotype and head injury, Ann. NY Acad. Sci. 777 (1996) 271–275.
[28] G. Zhang, Y. Dong, B. Zhang, F. Ichinose, X. Wu, D.J. Culley, G. Crosby, R.E. Tanzi, Z. Xie, Isoflurane-induced caspase-3 activation is dependent on cytosolic calcium and can be attenuated by memantine, J. Neurosci. 28 (2008) 4551–4560.
[29] G.W. Roberts, S.M. Gentleman, A. Lynch, L. Murray, M. Landon, D.I. Graham, Beta amyloid protein deposition in the brain after severe head injury: implications for the pathogenesis of Alzheimer's disease, J. Neurol. Neurosurg. Psychiatry 57 (1994) 419–425.
[30] P.K. Mandal, J.W. Pettegrew, Alzheimer's disease: Soluble oligomeric A beta(1-40) peptide in membrane mimic environment from solution NMR and circular dichroism studies, Neurochem. Res. 29 (2004) 2267–2272.
[31] S.L. Bianchi, T. Tran, C. Liu, S. Lin, Y. Li, J.M. Keller, R.G. Eckenhoff, M.F. Eckenhoff, Brain and behavior changes in 12-month-old Tg2576 and nontransgenic mice exposed to anesthetics, Neurobiol. Aging 29 (2008) 1002–1010.
[32] R. Eckenhoff, W. Zheng, M. Kelz, From anesthetic mechanisms research to drug discovery, Clin. Pharmacol. Ther. 84 (2008) 144–148.