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Inhaled Anesthetic Potency in Aged Alzheimer Mice

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Abstract

BACKGROUND—The number of elderly patients with frank or incipient Alzheimer's disease (AD) requiring surgery is growing as the population ages. General anesthesia may exacerbate symptoms of and the pathology underlying AD, so minimizing anesthetic exposure may be important. This requires knowledge of whether the continuing AD pathogenesis alters anesthetic potency.

METHODS—We determined the induction potency and emergence time for isoflurane, halothane, and sevoflurane using the minimum alveolar anesthetic concentration for loss of righting reflex as an end point in 12- to 14-mo-old triple transgenic Alzheimer (3xTgAD) mice and wild type C57BL/6 controls. 3xTgAD mice model AD by harboring three distinct mutations: the APP^{Swe}, Tau, and PS1 human transgenes, each of which has been associated with familial forms of human AD.

RESULTS—The 3xTgAD mice exhibited mild resistance (from 8% to 30%) to volatile anesthetics but displayed indistinguishable emergence patterns from all three inhaled anesthetics.

CONCLUSIONS—These results show that the genetic vulnerabilities and neuropathology associated with AD produce a small but significant decrease in sensitivity to the hypnotic actions of three inhaled anesthetics. Emergence times were not altered.

Alzheimer's disease (AD) is a progressive neurodegenerative disorder primarily affecting memory through the hippocampus, but ultimately disturbing all areas and functions of the

brain. Currently, AD afflicts an estimated 25 million people worldwide, with an expected prevalence of 100 million by 2050.¹ General anesthesia is also administered to large numbers of patients worldwide, and this is skewed toward the older population. Therefore, preclinical and diagnosed Alzheimer patients will unavoidably be exposed to anesthetics with increasing frequency in the coming years. Not only are patients with AD considered to be at risk for some anesthesia side effects (e.g., delirium) but also there is now concern that inhaled general anesthesia is a risk factor for AD with *in vitro* and *in vivo* studies suggesting that inhaled anesthetics may promote and intensify Alzheimer's neuropathogenesis.²⁻⁶ Despite these recent concerns, there is an absence of information on the sensitivity of patients with AD to the hypnotic actions of these drugs. Patients with AD have disrupted sleep behavior,⁷ as mimicked by some mouse models of AD.⁸ Should patients with AD be abnormally sensitive to anesthetics, then lower concentrations could be used as a strategy to minimize deleterious effects. Thus, we determined the anesthetic sensitivity of triple transgenic Alzheimer (3xTgAD) mice engineered with three different mutations and which have been shown to produce the hallmark lesions of AD (intracellular neurofibrillary tangles and extracellular plaque) in an age-dependent manner that also replicates the characteristic decrement in learning and memory.⁹

METHODS

Mice

In accordance with National Institutes of Health guidelines and with approval of the Institutional Animal Care and Use Committee at the University of Pennsylvania, we used 3xTgAD mice, consisting of the homozygous human APP_{Swe}, human tau_{p301L}, and PS1_{M146V} transgenes in a 129/C57BL6 hybrid background. Because we were unable to identically match the hybrid background of 3xTgAD mice, we chose age-matched C57BL/6 mice as wild type controls for all studies, which were obtained from the National Institute on Aging. Animals of both genders were used in approximately equal proportions. Mice were housed under a 12-h light/dark cycle and were given access to food and water *ad libitum*. For the minimum alveolar anesthetic concentration for loss of righting reflex (MAC_{LORR}) experiments, fourteen 12- to 14-mo-old 3xTgAD mice and fifteen age-matched wild type controls were used. At this age, the 3xTgAD mice show significant cognitive loss,⁹ but are otherwise healthy and not of different body mass than wild type.

Habituation

Mice were placed in custom-designed individual controlled environment chambers, which permit simultaneous exposure of 24 mice to anesthetics or other gases, as described previously.¹⁰ Matched gas flow rates were chosen to give time constants for gas turnover of 2 min in an open circuit design. Oxygen and carbon dioxide concentrations were monitored in the effluent chamber gas. The chambers were actively warmed by partial submersion in a circulating 37°C water bath. Mouse rectal temperature was measured daily, immediately before placement in the anesthetic chambers and immediately upon completion of the habituation run. Mice were habituated in the chambers for 2 h per day over 4 consecutive days.

Anesthesia and MAC_{LORR} Determination

After habituation, the mice were given a 2-day rest and then returned to the warmed chambers for MAC_{LORR} determinations. Oxygen (100%) flowed through a calibrated agent-specific vaporizer containing isoflurane, halothane, or sevoflurane and, subsequently, through each chamber connected in parallel via equalizing resistors. Average initial and peak anesthetic concentrations were 0.59% and 1.05% for isoflurane, 0.61% and 1.22% for halothane, and 0.94% and 2.08% for sevoflurane. Every 15 min, anesthetic concentrations were increased by an average of 0.04% for isoflurane, 0.05% for halothane, or 0.10% for sevoflurane to construct anesthetic dose-response curves. Anesthetic gas concentrations were determined in triplicate during the last 2 min of each step using a Riken FI-21 refractometer (AM Bickford, Wales Center, NY). At the 14-min mark, each chamber was rotated 180 degrees to place the mouse on its back. If the mouse remained on its back with at least three paws up in the air for 60 s, the righting reflex was recorded as lost. At the end of the 15-min period, the entire process was repeated for the next concentration. Full dose-response curves were generated three times for each anesthetic using the same mice with a minimum of 2 days between exposures.

Emergence Time

After all mice lost their righting reflex, the anesthetic was increased by one last step before being discontinued. Emergence from anesthesia was defined as the time that elapsed from turning off the vaporizer until the righting reflex returned, monitored with a VB-C50iR network video camera (Canon U.S.A., Lake Success, NY).

Statistical Analysis

Hill plots for induction of anesthesia were calculated for 3xTgAD and control mice for each anesthetic. Ninety-five percent confidence intervals were calculated using Prism 5.0 (GraphPad Software, San Diego, CA) as previously described.^{10,11} A Fisher's exact test determined whether the best-fit values for MAC_{LORR} and Hill slope differed significantly between genotypes. Emergence times were calculated. Differences were analyzed using a two-way analysis of variance (volatile anesthetic by genotype). Bonferroni *post hoc* corrections were applied when appropriate.

RESULTS

Determination of Volatile Anesthetic Dose-Response Curves

For all tested volatile anesthetics, MAC_{LORR} (an anesthetic 50% effective dose value) and Hill slope best-fit constants differed significantly between 3xTgAD and wild type control mice (Fig. 1, Table 1). In each instance, 3xTgAD mice displayed mild resistance to induction of anesthesia compared with age-matched controls. Hill slope constants were significantly less steep in 3xTgAD mice exposed to halothane suggesting increased variability in 3xTgAD mice compared with controls. This trend of shallower Hill slope was also observed for 3xTgAD mice exposed to isoflurane and sevoflurane, although they failed to reach statistical significance.

Emergence

As shown in Figure 2, emergence times for 3xTgAD mice were not significantly different from those of wild type controls for any of the anesthetics determined by two-way analysis of variance. As expected, there was a significant effect of the anesthetic drug itself on emergence. Average rectal temperatures ($\pm$ SD) recorded immediately after emergence confirmed euthermia ($37.0^{\circ}\text{C} \pm 1.5^{\circ}\text{C}$) in all mice.

DISCUSSION

The current incidence of AD is expected to quadruple by 2050.¹ When combined with the predicted requirement for perioperative care of the elderly, the need to understand the interaction between neurodegeneration and anesthesia becomes apparent. This is even more urgent given the concern that anesthetics may accelerate neurodegeneration.¹² The aim of this study was to determine whether hypnotic potency and emergence times for some common inhaled anesthetics were altered in an animal model of Alzheimer's pathogenesis. Although we predicted that the synaptic dysfunction in this model would mimic that produced by anesthetic exposure, ultimately resulting in something like additivity, we were surprised to find that the anesthetic requirement for hypnosis is slightly higher in the AD compared with wild type animals. This suggests that normal synaptic function facilitates anesthetic sensitivity. Although highly significant, the small difference in hypnotic potency would be difficult to detect in the perioperative setting. Indeed, it is possible that the conventional MAC response (movement in response to a noxious stimulus) is unaltered, because it involves reflex loops in the spinal cord, a region of the central nervous system not vulnerable to AD neuropathology.

It is not clear why synaptic dysfunction and loss would make an organism resistant to the hypnotic effects of anesthetics.¹³ However, the observation that defects in sleep behavior occur in animals carrying the APP_{Swe} transgene,⁸ when combined with the well known prevalence of insomnia in Alzheimer patients,⁷ suggests that the continuing neuropathology favors arousal pathways. Thus, if the hypnotic component of the anesthetic state arises in part via enhancement of endogenous sleep-promoting neural circuits, then one might predict partial resistance to hypnosis with selective dysfunction of sleep-promoting pathways.

There are several features of this study that limit extrapolation to the clinical setting. First, these are transgenic mice, and the ability of this ensemble of mutations to fully mimic human AD can be rightly questioned. On the other hand, this particular model does develop both intracellular and extracellular amyloid plaque and intracellular neurofibrillary tangles, hallmark lesions of AD. The animal goes on to progressively develop cognitive changes much like human AD, including changes in sleep as noted above. A consistent finding in the murine models of AD is that, unlike human AD, little neurodegeneration and cell loss occur. How these similarities and differences would be predicted to alter sensitivity to anesthetic drugs, whose mechanisms of action are thought to rely on a multitude of molecular targets is, of course, not yet clear. However, it is clear that this study would be difficult to reproduce in patients, because the associated dementia makes hypnotic end points less clear and informed consent difficult to obtain. Thus, predictions from animal models require serious consideration.

The 3xTgAD mice have been maintained on a hybrid 129/C57BL6 mixed background.⁸ Genetic differences between mouse strains may alter sensitivity to anesthetics.^{14,15} We did, however, match the strains as closely as possible. The reported difference in anesthetic sensitivity between the 129 and C57BL/6J strains is small for most drugs and is statistically indistinguishable for the anesthetics used in this study.¹⁵ Although it is recognized that the hybrid could have different sensitivity than either pure strain, this behavior has not been reported for anesthetics.

In summary, we have shown that a murine model displaying the hallmark lesions and cognitive changes of AD is slightly resistant to the hypnotic actions of three inhaled anesthetics. This suggests that either the broad synaptic dysfunction produced by the AD neuropathology is functionally antagonistic to that produced by the anesthetic or that the AD neuropathology selectively affects sleep-promoting areas.

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Figure 1.

Concentration effect relationships for halothane, isoflurane, and sevoflurane in the wild type (WT) and triple transgenic Alzheimer (3xTgAD) mice. In each case, black squares are WT and open squares are 3xTgAD. Each point represents the fraction of the group obtunded at a given anesthetic concentration on a single day. Note that 50% effective concentration values are shifted significantly to the right in each case, and that Hill slopes are, in general more shallow in the 3xTgAD mice, which indicates increased variability in their anesthetic responsiveness. This is also mirrored by the somewhat lower R^2 value for the 3xTgAD relationship. Fit parameters are given in Table 1.

Figure 2.

Statistically identical patterns of anesthetic emergence appear after exposure to halothane, isoflurane, or sevoflurane. Mean time $\pm$ SE to regain righting reflex for wild type (WT) (black bars) and triple transgenic Alzheimer (3xTgAD) mice (white bars) respectively, was halothane 40.4 ± 4.1 and 29.67 ± 5.3 ; isoflurane 27.0 ± 2.6 and 24.4 ± 3.1 ; and sevoflurane 11.8 ± 2.9 and 9.9 ± 1.2 . Two-way analysis of variance found a significant effect of anesthetic ($F = 9.10$, $P < 0.02$) as predicted, but no effect of genotype ($F = 1.72$, $P = 0.30$).

Table 1

Concentration/Effect Relationship Parameters, Mean (95% Confidence Intervals)

Anesthetic	EC ₅₀ (% atm)	Hill slope	R ²	F	P
Halothane					
WT	0.72 (0.71–0.73)	18.6 (14.8–22.3)	0.96		
3xTgAD	0.93 (0.89–0.97)	8.7 (5.7–11.7)	0.77	111.4	<0.0001
Isoflurane					
WT	0.76 (0.75–0.77)	17.7 (14.7–20.6)	0.97		
3xTgAD	0.82 (0.81–0.83)	15.9 (13.2–18.5)	0.96	64.0	<0.0001
Sevoflurane					
WT	1.52 (1.48–1.56)	12.6 (8.9–16.3)	0.94		
3xTgAD	1.66 (1.62–1.71)	8.6 (6.4–10.8)	0.92	16.6	<0.0001

EC₅₀ = 50% effective dose; WT = wild type mice; 3xTgAD = triple transgenic Alzheimer mice.