Enhanced choline acetyltransferase activity does not explain the action of inhaled convulsants

R. Griffths, P. Ionescu, Z. Fang, D. D. Koblin and E. I. Eger

Summary
Enhancement of choline acetyltransferase (ChAT) activity and increased intraneuronal acetylcholine (ACh) may explain the convulsant activity of some inhaled compounds. Enflurane, for example, enhances such activity. Accordingly, we measured choline acetyltransferase (ChAT) activity in rat cortical synaptosomes in the presence of two inhaled convulsants, flurothyl (CF₃CH₂OCH₂CF₃) and 1,2-dichlorohexafluorocyclobutane at partial pressures below and greatly exceeding those which produce convulsions in vivo. Neither agent changed the kinetic parameters, maximum velocity (vmax) or Michaelis constant (Km). The vmax for controls in the flurothyl series was 0.16 (0.06) nmol mg⁻¹ min⁻¹ and the Km was 0.23 (0.11) mmol litre⁻¹. For the 1,2-dichlorohexafluorocyclobutane series of experiments the results for the controls were vmax 0.23 (0.10) nmol mg⁻¹ min⁻¹ and Km 0.20 (0.08) mmol litre⁻¹. Modification of ChAT activity did not contribute to the excitatory effects of these agents. (Br. J. Anaesth. 1997; 79: 389–391).

Key words

Acetylcholine (ACh) is an excitatory neurotransmitter important for arousal and maintenance of consciousness.¹ The central cholinergic system may also play a role in the genesis of seizure.²

Enflurane enhances the activity of the enzyme responsible for ACh synthesis, choline acetyltransferase (ChAT).³ To determine if this effect applies broadly, we investigated the effects of two volatile compounds with known convulsant properties, flurothyl and 1,2-dichlorohexafluorocyclobutane, on the kinetics of ChAT in rat cortical synaptosomes.

Methods and results
With approval from the University of California San Francisco Committee on Animal Research, we studied 52 male (300–430 g), specific pathogen-free Sprague Dawley rats from Charles River Laboratories. Four rats were placed in individual plastic cylinders and the tail end of each cylinder had a rubber stopper with multiple holes. The tail and a rectal temperature probe passed through two of these holes, and the tail was secured with tape to a plastic extension of each cylinder.

Each cylinder was inserted into a slightly larger individual plastic chamber. A catheter for sampling inspired gas in the chamber and another tube for delivery of gases traversed the stopper at the “head” end of the chamber. Oxygen was flushed through the tube before beginning the study. The tubes at the tail ends were connected to a carbon dioxide adsorbent canister containing 200 g of fresh soda lime. To complete the system, the adsorbent canister was connected to a circulating fan that led to the head-end tubes. To permit delivery of compounds, we added a “T” connector between the carbon dioxide adsorbent and the tail end of the four chambers. Finally, the system was connected with a demand oxygen supply (bypass flow approximately 50 ml min⁻¹) for delivery of oxygen after closure of the system.

We initially flushed the system until the oxygen concentration was 95% or higher. Oxygen concentration was measured with a Pauling (polarographic) meter (Beckman model E2). After closing the system (oxygen supplied on demand), flurothyl (>99% purity, PCR Inc., Gainesville, FL, USA) or 1,2-dichlorohexafluorocyclobutane (>97% purity, PCR Inc., Gainesville, FL, USA) was introduced in sequentially increasing concentrations as liquid aliquots followed by a flush of oxygen. For each concentration, a 20-min equilibration period was allowed, during which we observed the rats for convolution. If no convolution occurred, we increased the concentration by 15–25%. The ED₅₀ for convolution was defined as the mean of the partial pressures in the chamber immediately below and just causing a convolution.

Richard Griffiths*, MD, FRCA, University Department of Anaesthesia, Leicester Royal Infirmary, Leicester LE1 5WW. Pompilieu Ionescu, MD, Zenzu Fang, MD, Edmond I. Eger II, MD, Department of Anesthesia, University of California, San Francisco, CA, USA. Donald D. Koblin, PhD, MD, Anesthesiology Service, Department of Veterans Affairs Medical Center, San Francisco, CA, USA. Accepted for publication: April 25, 1997.

*Present address for correspondence: Department of Anaesthesia, Peterborough District Hospital, Peterborough PE3 6DA.
Table 1  Effect of flurothyl and 1,2-dichlorohexafluorocyclobutane on choline acetyltransferase (ChAT) kinetic parameters, maximal velocity ($e_{\text{max}}$) and Michaelis constant ($K_m$) (mean (sd)). Actual flurothyl concentrations measured at the end of the experiments were, mean 0.11 (sd 0.0035), 0.21 (0.0064), 0.43 (0.017), $n=20$. Actual 1,2-dichlorohexafluorocyclobutane concentrations measured at the end of the experiments were 2.11 (0.15), 4.13 (0.28), 8.86 (0.52), $n=20$

<table>
<thead>
<tr>
<th>Kinetic parameter</th>
<th>Flurothyl (target concentrations)</th>
<th>1,2-dichlorohexafluorocyclobutane (target concentrations)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Air</td>
<td>0.125 %atm</td>
</tr>
<tr>
<td>$e_{\text{max}}$ (nmol mg$^{-1}$ min$^{-1}$)</td>
<td>0.16 (0.06)</td>
<td>0.16 (0.06)</td>
</tr>
<tr>
<td>$K_m$ (mmol litre$^{-1}$)</td>
<td>0.23 (0.11)</td>
<td>0.22 (0.09)</td>
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</table>

The convulsive ED$_{50}$ values for flurothyl and 1,2-dichlorohexafluorocyclobutane, respectively, were mean 0.156 (sd 0.004) %atm, n = 24 and 5.54 (0.01) %atm, n = 28.

With approval from the UCSF Committee on Animal Research, we prepared synaptosomes from the cortex of male Fisher rats (200–250 g) using the method described by Dunkley and colleagues.4

ChAT activity was determined using the method of Fonnum.5 Synaptosomes were suspended (0.5 ml) in a medium containing NaCl 300 mmol litre$^{-1}$, ethylenediaminetetra-acetic acid (EDTA) 20 mmol litre$^{-1}$, neostigmine 1 mmol litre$^{-1}$, 0.5% (w/v) Triton X-100 and sodium phosphate 50 mmol litre$^{-1}$ (pH 7.4), to a protein concentration of 0.5 mg ml$^{-1}$. The mixture was placed in a 10-ml gas-tight syringe containing either air or air and one of the test gases, and incubated in a water bath at 37°C for 10 min. Constant shaking allowed equilibration of the test gas with the synaptosome suspension. A substrate mixture (0.5 ml) containing choline bromide at concentrations of 0.05–2.00 mmol litre$^{-1}$, acetyl coenzyme A 2 mmol litre$^{-1}$, and [3H]acetyl coenzyme A 0.5$^\mu$Ci in the incubation medium but without Triton X-100 was added to the synaptosome suspension, shaken gently and returned to the water bath for 30 min. Immediately after addition of the substrate mixture a 100-$\mu$l aliquot was withdrawn and ACh extracted using Kalignost (Koch-Light Lab Ltd, Bucks, UK).5 These samples were used as time zero controls for background subtraction of radioactivity from the test samples. After 30 min, 100-$\mu$l samples were obtained in duplicate and ACh content measured by scintillation counting after Kalignost extraction.5

The concentration of test gas in the syringes at the end of the incubation was measured by gas chromatography. The synaptosome preparations were exposed to air with flurothyl at 0.125, 0.25 and 0.5%atm. The concentrations of 1,2-dichlorohexafluorocyclobutane in air were 2.5, 5.0 and 10.0%atm. Controls, exposed to air only, were included in each set of experiments.

Michaels–Menten kinetic parameters, maximum reaction velocity ($e_{\text{max}}$) and Michaelis constant ($K_m$) were calculated for ChAT in each experiment using rectangular hyperbola curve fit in SigmaPlot for Windows. Results were compared using Student’s paired t test. $P<0.05$ was considered statistically significant.

Neither compound altered the kinetics of ChAT at any concentration tested (table 1).

Comment

Our previous work demonstrated that anaesthetizing concentrations of enflurane decreased the $K_m$ of ChAT for choline bromide.3 These findings led to our hypothesis that the convulsant effect of inhaled convulsants might result from enhancement of ChAT activity. The cholinergic system has been implicated in the genesis of seizures.2 These findings are consistent with our hypothesis.

In conflict with our hypothesis, our results demonstrated that flurothyl and 1,2-dichlorohexafluorocyclobutane did not alter the kinetics of ChAT from rat cortical synaptosomes. There is a remote possibility that the lack of effect seen with the synaptosome preparation resulted from the different strain of rat used for the ChAT part of the experiment. Test concentrations of both agents were distributed either side of the convulsive ED$_{50}$ for these agents in vivo in rats. It remains possible that ChAT kinetics were affected in amounts too small to be measured. However, in previous studies with enflurane,1 a 35% decrease in the $K_m$ of ChAT was detected, and a greater decrease would be expected in our experiments if ChAT kinetics were important.

Many other transmitter systems are also involved in the genesis of seizures and it may be that discrete mechanisms explain the epileptic capacity of each convulsant. At the crab neuromuscular junction, flurothyl selectively blocks inhibitory transmission (GABA) more than excitatory transmission (glutamate).6

Although the synaptosome preparation contains the machinery needed for neurotransmitter studies, the preparation has limitations which may influence the interpretation of this study. The preparation produces synaptosomes from nerve endings secreting diverse transmitters, not just ACh. Thus there is still the possibility of non-physiological crosstalk that...
could obscure drug effects. To summarize, the heterogeneous nature of the synaptosome preparation, together with the potential presence of more than one transmitter in cholinergic neurones, limits the interpretation of results from our system.

Although we found that modulation of ChAT activity does not contribute to the excitatory effects of volatile convulsants, this does not exclude ACh metabolism as a cause of the effects of such compounds and future studies may focus on other aspects of cholinergic function.

Acknowledgement

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References