Choline acetyltransferase activity of rat synaptosomes is sensitive to enflurane, but not halothane or isoflurane†

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SUMMARY

We have examined the activity of choline acetyltransferase (ChAT) in rat cortical synaptosomes in the presence of three volatile anaesthetic agents: enflurane, halothane and isoflurane. The Michaelis constant $K_m$ for choline was reduced significantly ($P = 0.012$) in the presence of 6.5% enflurane (3 rat MAC) compared with control samples exposed to carrier air only, while maximum reaction velocity ($V_{\text{max}}$) remained unaltered. The reduction in $K_m$ was also significant at enflurane concentrations of 4.4% (2 rat MAC) ($P = 0.043$) and 2.2% (1 rat MAC) ($P = 0.043$). Halothane 3% (2.5 rat MAC) and 4.5% isoflurane (3 rat MAC) had no effect on either kinetic property. If present in vivo, an enflurane-induced alteration in acetylcholine metabolism, through modified ChAT, may contribute to the convulsive properties of this anaesthetic. (Br. J. Anaesth. 1994; 72: 577–580)

KEY WORDS


A role for the excitatory neurotransmitter acetylcholine (ACh) in the maintenance of consciousness has been suggested from observations of spontaneously released ACh from the surface of the cortex of conscious and anaesthetized animals [1-3]. The release was greater during periods of increased activity and attention and decreased during periods of rest [1]. On induction of barbiturate anaesthesia, release of ACh decreased to much lower levels than resting conscious values [1] and the level of release was related to the depth of anaesthesia [2]. The increase in output of ACh as animals regained consciousness, further supports a role for this transmitter in contributing to the level of consciousness [1].

Anaesthetic agents may modify presynaptic ACh metabolism either by reducing the synthesis or release of this transmitter or by reducing reuptake of choline into the nerve terminal [4]. At partial pressures at the top of the range administered clinically, halothane has been shown to reduce the potassium-evoked release of ACh from rat cortical synaptosomes by approximately 50% [5]. Halothane-induced reduction in high affinity choline uptake has also been observed, although it is not clear if this occurs by a competitive [5] or non-competitive mechanism [6]. While high affinity choline uptake is believed to be the rate limiting step in ACh biosynthesis [7] and thus a possible central point for anaesthetic action [5], the further possibility of additional anaesthetic modification of ACh metabolism at the level of synthesis cannot be excluded.

The committing reaction in ACh biosynthesis is catalysed by choline acetyltransferase (ChAT) and involves the transfer of an acetyl group from acetyl coenzyme A (acetyl-CoA) to choline [8]. This enzyme has been reported to be insensitive to halothane at concentrations of 3 and 4% [5]. We have investigated further the effects of the halogenated anaesthetic agents enflurane, halothane and isoflurane on ChAT activity in rat cortical synaptosomes.

MATERIALS AND METHODS

Preparation of synaptosomes

Synaptosomes were prepared from the cerebral cortex of female Wistar rats (200-250 g) using a method modified from that described by Gray and Whittaker [9]. Rats were killed by decapitation and the cortex removed. All subsequent procedures were performed at 4°C. The cortex was washed with ice cold sucrose 0.32 mol litre$^{-1}$ (10 ml/g tissue) by 12 strokes in a motor driven Potter homogenizer (Teflon glass). The homogenate was centrifuged at 5000 x$^g$ for 10 min. The supernatant was retained and the pellet re-homogenized and centrifuged at 20 000 x$^g$ for 60 min. The second pellet was discarded and the two supernatants were combined and centrifuged at 20000 x$^g$ for 60 min. The supernatant was discarded and the pellet re-suspended in sucrose 0.32 mol litre$^{-1}$, potassium phosphate 5 mmol litre$^{-1}$ (pH 7.4) at an approximate...
protein concentration of 1 mg ml\(^{-1}\). The suspension was layered onto discontinuous sucrose gradients consisting of sucrose 0.6, 0.8, 1.0 and 1.2 mol litre\(^{-1}\), in potassium phosphate 5 mmol litre\(^{-1}\) (pH 7.4). Centrifugation of gradients was for 105 min at 100000 \(\times\) g at 4 °C.

Synaptosomes were recovered from the 1.0/1.2 mol litre\(^{-1}\) interface, resuspended in sucrose 0.32 mol litre\(^{-1}\), potassium phosphate 5 mmol litre\(^{-1}\) at a protein concentration of 0.4 mg ml\(^{-1}\) and centrifuged at 150000 \(\times\) g for 30 min. The supernatant was discarded and the pellet taken up in sucrose 0.32 mol litre\(^{-1}\), potassium phosphate 5 mmol litre\(^{-1}\) to a final protein concentration of 5–12 mg ml\(^{-1}\). Protein concentration was measured by the method of Lowry and colleagues [10] using bovine serum albumin as standard.

**Anaesthetic apparatus**

Anaesthetic agents were delivered to synaptosome samples via a manifold of 10 outlets, each delivering 60 ml min\(^{-1}\) of humidified air. The flow of air was controlled by a flowmeter block and each outlet was calibrated using a bubble flowmeter. Anaesthetic agents were delivered at a partial pressure equivalent to four times the human MAC for each agent (6.5 % halothane, 3 % enflurane and 4.5 % isoflurane).

Air only was delivered to control samples.

**Choline acetyltransferase assay**

ChAT activity was determined using the method of Fonnum [11]. Synaptosomes were suspended in an incubation medium containing NaCl 300 mmol litre\(^{-1}\), ethylenediaminetetra-acetic acid (EDTA) 20 mmol litre\(^{-1}\), neostigmine 1 mmol litre\(^{-1}\), 0.5 % Triton X-100 and sodium phosphate 50 mmol litre\(^{-1}\) (pH 7.4), to a protein concentration of 0.5 mg ml\(^{-1}\). A substrate mixture (0.5 ml) containing choline bromide at concentrations of 0.2–4.0 mmol litre\(^{-1}\), acetyl CoA 2 mmol litre\(^{-1}\) and \(^{[3]}\)Hacetely-CoA 0.5 \(\mu\)Ci in the above medium, without Triton X-100, was placed in a 10-ml plastic test tube and exposed to air or air with anaesthetic agent to equilibrate the solutions at 37 °C. After 10 min, 0.5 ml of synaptosome suspension was added to the substrate mixture and incubated at 37 °C. Immediately after addition of the synaptosomal suspension, a 100-\(\mu\)l aliquot was withdrawn and ACh extracted using kalignost [9]. These samples were used as time zero controls for background subtraction from the test samples. After incubation for 60 min, 300-\(\mu\)l samples were obtained in duplicate and the ACh content measured by scintillation counting after kalignost extraction [9].

**Kinetic parameters**

Michaelis–Menten kinetic parameters, maximum reaction velocity (\(V_{\text{max}}\)) and Michaelis constant (\(K_{\text{m}}\)) were calculated for ChAT in each experiment using Pharm/Pcs computer software [12]. Results were compared using analysis of variance. Student’s paired \(t\) test and Wilcoxon’s signed rank test. A value of \(P < 0.05\) was considered statistically significant.

**RESULTS**

In the absence of anaesthetic agents, ACh synthesis was directly proportional to time during the period of the assay used in this study (\(r = 0.99\)). \(V_{\text{max}}\) and \(K_{\text{m}}\) for ChAT activity were determined for synaptosomes exposed to air only or air containing either 3 % halothane, 6.5 % enflurane or 4.4 % isoflurane (table I).

Enflurane 6.5 % reduced significantly (\(P = 0.012\)) the \(K_{\text{m}}\) for choline determined for ChAT by 37 % while \(V_{\text{max}}\) was not altered significantly (fig. 1, table I). In contrast, at equivalent human anaesthetic doses, 3 % halothane and 4.5 % isoflurane did not produce any significant effect on \(V_{\text{max}}\) or \(K_{\text{m}}\) of the synaptosomal ChAT activity (table I). \(V_{\text{max}}\), determined in the presence of all three anaesthetic agents, remained the same compared with air treated controls (table I).

\(V_{\text{max}}\) and \(K_{\text{m}}\) values were determined also for synaptosomes exposed to air or air containing 1.1 % enflurane, 2.2 % enflurane and 4.4 % enflurane. Enflurane at 2.2 % and 4.4 % reduced significantly the \(K_{\text{m}}\) determined for ChAT (\(P = 0.043\)) by 13 % and 26 %, respectively. The \(K_{\text{m}}\) for ChAT was reduced also by 1.1 % enflurane (ns). The relation-

<table>
<thead>
<tr>
<th>Agent</th>
<th>(V_{\text{max}}) (mmol mg(^{-1}) min(^{-1}))</th>
<th>(K_{\text{m}}) (mmol litre(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>0.80 (0.04)</td>
<td>0.26 (0.04)</td>
</tr>
<tr>
<td>Halothane</td>
<td>0.84 (0.11)</td>
<td>0.26 (0.04)</td>
</tr>
<tr>
<td>Enflurane</td>
<td>0.80 (0.04)</td>
<td>0.24 (0.03)</td>
</tr>
<tr>
<td>Isoflurane</td>
<td>0.80 (0.04)</td>
<td>0.24 (0.03)</td>
</tr>
</tbody>
</table>

**Fig. 1.** Representative Lineweaver–Burk plot of choline acetyltransferase activity in the absence (■) and presence (□) of 6.5 % enflurane. Eleven similar experiments were performed in air controls and in the presence of enflurane.
FIG. 2. Effect of enflurane on choline acetyltransferase activity (mean (SEM)). Values for $K_m$ (A) and $V_{max}$ (B) are given in Table I. There were five determinations for each point up to 4.4% enflurane and 12 determinations for 6.5% enflurane and air alone. *$P < 0.05$ compared with control.

ship between the partial pressure of enflurane and the reduction in $K_m$ for ChAT is shown in figure 2A. $V_{max}$ did not alter significantly at any of the concentrations (fig. 2B).

DISCUSSION

This study has demonstrated that enflurane, at concentrations of 6.5%, lowered the $K_m$ of ChAT without altering $V_{max}$, while 3% halothane and 4.5% isoflurane had no effect on either variable. These doses of anaesthetic agents correspond to approximately four human MAC. However, these doses of agents correspond to 3 rat MAC for enflurane and isoflurane and 2.5 rat MAC for halothane [13]. A subsequent dose–response analysis of the enflurane-induced effect on $K_m$ showed a progressive reduction from a concentration of 1.1% (0.5 rat MAC) up to 6.5% (3 rat MAC), although the reduction at 0.5 MAC was not statistically significant.

For this study, we used a synaptosome preparation from rat cortex. Synaptosomes are produced by homogenization of brain tissue so that isolated nerve terminals are produced. The synaptosomes contain the necessary machinery for neurotransmitter uptake, synthesis and release [14]. However, there are some limitations with the preparation, the most notable being the heterogeneous population of transmitter types obtained. This may lead to non-physiological communication between types of synapses when one particular transmitter is being investigated. During this investigation, however, the synaptosomes were lysed with detergent in order to gain access to the intracellular enzyme, ChAT, which removed the possibility of cellular cross-talk in this experiment. As intra- and extracellular components were still present in the preparation, an indirect secondary effect on ChAT cannot be excluded.

There are few studies on the effects of anaesthetic agents on presynaptic ACh metabolism. However, a previous study [5] indicated that halothane had no effect on ChAT activity in concentrations up to 4%, which corresponds to 3.2 rat MAC. Our observations on halothane agree with this study.

Our results strongly suggest a selective and direct effect of enflurane on ChAT. The molecular basis of this apparent selectivity of enflurane for ChAT remains to be elucidated. It is possible that there is molecular selectivity at the level of ChAT. Stereospecificity of enantiomers of isoflurane has been reported for anaesthetic-induced increase in $K^+$ efflux from Lymnea stagnalis neurones via a potassium channel [15]. It seems unlikely that the reduction in $K_m$ could be a simple chemical concentration effect of the anaesthetic agents in solution, as 4.5% isoflurane (1.08 mmol litre$^{-1}$) and 3% halothane (0.84 mmol litre$^{-1}$) showed no effect, whereas statistically significant effects of enflurane were observed at a concentration of 2.2% (0.68 mmol litre$^{-1}$).

Enflurane has been implicated in the development of EEG abnormalities and in the genesis of seizure [16]. The appearance of these abnormalities in the presence of enflurane is related to the dose of agent received by the patient and is particularly evident at high inspired enflurane concentrations [17]. Involvement of ACh in seizure activity is suggested by increases in ACh concentration in rat brain regions that has been demonstrated in experimental models of seizure activity [18]. A link between enflurane-induced seizure and cholinergic transmission is suggested by the reduction in enflurane-induced spike activity by hyoscine [19]. Measurement of ACh turnover under enflurane anaesthesia has been shown to be reduced in cortical but not subcortical structures in the brain, whereas halothane reduces ACh turnover in both areas [20]. If present in vivo, an enflurane-induced reduction in $K_m$ of ChAT
could lead to facilitation of ACh synthesis in susceptible brain regions and may thereby contribute to the convulsant properties of enflurane. The lack of effect on $K_m$ or $V_{\text{max}}$ of ChAT at equipotent clinical concentrations of the structural isomer of enflurane, isoflurane or of halothane, which are both non-epileptogenic, is consistent with the above suggestion.

In summary, we have shown that ChAT activity is susceptible to kinetic modification in the presence of partial pressures of enflurane towards the top of the clinical relevant range. The relationship of these in vitro observations to possible in vivo mechanisms of seizure genesis remains to be investigated.

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REFERENCES