Laboratory Investigations

In vitro effects of fluoride and bromide on pseudocholinesterase and acetylcholinesterase activities

Jay R. Kambam MD,* Winston C.V. Parris MD,*
Rebecca J. Naukam BS,* John J. Franks MD,*
B. V. Rama Sastry PhD†

The in vitro effects of two metabolites of inhalational anaesthetics, fluoride and bromide, on pseudocholinesterase (PCHE) and acetylcholinesterase (ACHE) activities in the blood samples of seven healthy patients were studied. The PCHE and ACHE activities were determined by kinetic spectrophotometric methods. Fluoride at the levels achieved with clinical concentrations of enflurane and sevoflurane ($25-75 \mu\text{M} \cdot \text{L}^{-1}$) inhibited PCHE activity by 28-65 per cent ($P < 0.01$) and ACHE activity by less than five per cent ($P > 0.05$). Bromide at the levels achieved with clinical concentrations of inhalational anaesthetics had no significant effect on either PCHE or ACHE activity. We recommend caution when succinylcholine and/or ester type local anaesthetics are used in the immediate postoperative period following enflurane or sevoflurane anaesthesia. We also recommend that blood drawing for PCHE activity be delayed at least until 24 hr following enflurane or sevoflurane anaesthesia.

Key words

ANAESTHETICS, VOLATILE: enflurane, halothane, methoxyflurane, sevoflurane;

ENZYMES: acetylcholinesterase, pseudocholinesterase;

IONS: bromide, fluoride;

METABOLISM.

From the Departments of Anesthesiology* and Pharmacology† Vanderbilt University Medical Center, Nashville, Tennessee.

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Address correspondence to: Dr. J.R. Kambam, Department of Anesthesiology, Vanderbilt University Hospital, Nashville, TN 37232-2125.

Les effets in vitro de deux métabolites anesthésiques d'inhalation, le fluorure et le bromure sur les activités de la pseudochoolinesterase (PCHE) et l'acetylcholinesterase (ACHE) ont été étudiés sur les échantillons sanguins de sept patients en bonne santé. Les activités de la PCHE et l'ACHE ont été déterminées par spectrophotométrie. Les niveaux de fluorure atteints avec des concentrations d'enflurane et de sevoflurane à des concentrations utilisées en clinique ($25-75 \mu\text{M} \cdot \text{L}^{-1}$) ont inhibé l'activité de la PCHE de 28-65 pour cent ($P < 0,01$) et l'activité de l'ACHE de moins de cinq pour cent ($P > 0,05$). Les niveaux de bromure atteints aux concentrations anesthésiques utilisées en clinique n'ont eu aucun effet significatif sur les activités de la PCHE ou de l'ACHE. On recommande la précaution lors de l'utilisation de la succinylcholine et/ou les anesthésiques locaux dans la période post-opératoire immédiate après l'anesthésie à l'enflurane ou le sevoflurane. On recommande aussi que la prise d'échantillon sanguin pour l'activité de la PCHE soit retardée d'au moins 24 heures après l'anesthésie à l'enflurane ou la sevoflurane.

Bromide and fluoride ions are two of the many metabolites that can be measured in blood following the administration of enflurane, halothane, methoxyflurane, and sevoflurane anaesthesia.¹⁻⁶ Patients who receive these anaesthetics sometimes receive a second general anaesthetic within a short time for a variety of indications including bleeding and thrombus formation, or regional analgesia with an ester type local anaesthetic for postoperative pain relief. During the recovery phase blood samples may be drawn for pseudocholinesterase assay to determine the cause of postoperative apnoea. Also, succinylcholine may be administered for brief relaxation at the end of surgery or for reintubation of the trachea in the recovery room. We examined the effects of bromide and fluoride

TABLE I Effect of fluoride on pseudocholinesterase (PCHE) and acetylcholinesterase activities ($n = 7$, each concentration)

Fluoride concentration $\mu\text{M} \cdot \text{L}^{-1}$	PCHE (units)		ACHE (units)	
	Mean $\pm$ SEM	%Inhibition	Mean $\pm$ SEM	%Inhibition
Control	5.53 $\pm$ 0.33	—	10.85 $\pm$ 1.34	—
5	5.06 $\pm$ 0.26*	8.3	—	—
10	4.62 $\pm$ 0.20*	15.8	10.67 $\pm$ 1.28	1.4
50	2.40 $\pm$ 0.08*	56.0	10.42 $\pm$ 1.25	3.6
100	1.48 $\pm$ 0.06*	73.0	10.34 $\pm$ 1.23	4.5
500	0.43 $\pm$ 0.02*	92.0	8.68 $\pm$ 1.02*	19.5
1000	0.26 $\pm$ 0.02*	95.0	7.76 $\pm$ 0.80*	27.5
5000	—	—	3.32 $\pm$ 0.24*	69.2

*Multifactor analyses of variance ($P < 0.05$).

on pseudocholinesterase (PCHE) and acetylcholinesterase (ACHE) activities which had not been studied previously.

Methods

With the approval of our Institutional Review Board, ten ml of heparinized venous blood were collected from seven different ASA physical status I and II patients (both sexes aged 31–60 yr). Plasma was separated from the red cells and both plasma and red cells were stored in a refrigerator and used for cholinesterase assays.

Determination of PCHE activity

A kinetic method was used in the determination of PCHE and dibucaine number (DN).⁷ The reaction mixture contained benzoylcholine as substrate in phosphate buffer with a pH of 7.40. The hydrolysis of benzoylcholine was monitored at 240 nm using a Beckman model 24 recording spectrophotometer at room temperature. Duplicate cholinesterase activities were measured for each patient's plasma and the mean was reported. The PCHE activity was expressed as units $\cdot$ mg⁻¹ of plasma proteins, where one unit of PCHE was defined as hydrolysis of one nanomol substrate $\cdot$ min⁻¹ after an extinction coefficient for benzoylcholine had been determined. Activities in the presence of fluoride and bromide were determined in the same manner using concentrations of sodium fluoride of 5–1000 μM and sodium bromide of 10–1000 μM , respectively. The DN were also measured to determine the normal genotype using a final concentration of 10 μM dibucaine.

Determination of ACHE activity

A colorimetric method was used in the determination of red cell ACHE activity.⁸ The reaction system consisted of acetylthiocholine as substrate in 0.1 M sodium phosphate buffer and 5,5-dithiobis-2-nitrobenzoate (DTNB) with a pH of 8.0. Thiocholine produced by hydrolysis of

acetylthiocholine reacts with DTNB to yield the yellow anion. The absorbance, corresponding to the amount of acetylthiocholine hydrolyzed, was monitored at 412 nm using a Beckman model 24 recording spectrophotometer at room temperature. Duplicate cholinesterase activities were measured for each patient's plasma and the mean was reported. The effects of fluoride and bromide were also studied using final concentrations of sodium fluoride of 10–5000 μM , and sodium bromide of 10–1000 μM , respectively. The ACHE activity was expressed as units $\cdot$ mg⁻¹ protein, where one unit was defined as the hydrolysis of one micro mole of acetylthiocholine $\cdot$ min⁻¹.

Multifactor analysis of variance (ANOVA) and Tukey's multiple-range testing were used to analyze the data. Linear regression analyses between log concentrations of fluoride, and bromide and PCHE, and ACHE activities were performed and R (correlation coefficient) values with corresponding P values were calculated. A P -value of less than 0.05 was considered statistically significant.

Results

PCHE activity

All samples had a normal DN indicating that enzymes were of a normal phenotype. Fluoride, at concentrations of 5 to 1000 μM , inhibited PCHE activity by 8–95 per cent ($P < 0.05$; Table I) and bromide at concentrations of 10–1000 μM inhibited PCHE activity less than one per cent ($P > 0.05$; Table II).

ACHE activity

Fluoride at concentrations of 10–5000 μM inhibited ACHE activity by 1.42–69 per cent ($P < 0.05$; Table I) and bromide at concentrations of 10–1000 μM inhibited ACHE activity less than one per cent ($P > 0.05$; Table II).

Plots of per cent inhibition of PCHE and ACHE activities vs log molar concentrations of fluoride and bromide are shown in the Figure. The mean concentrations

FIGURE Effects of fluoride and bromide on pseudocholinesterase (PCHE) and acetylcholinesterase (ACHE) activities. Fluoride at concentrations of 42 μM and 3000 μM inhibited 50 per cent (I_{50}) of the PCHE and ACHE activities, respectively ($P < 0.001$, and $r = 0.95$ for PCHE; $P < 0.001$, and $r = 0.9$ for ACHE).

of fluoride required to inhibit 50 per cent (I_{50}) of PCHE and ACHE activities were 42 μM and 3000 μM , respectively ($P < 0.001$, and $r = 0.95$ for PCHE; $P < 0.001$, and $r = 0.9$ for ACHE).

Discussion

Inorganic fluoride is one of the metabolites of halothane, enflurane, methoxyflurane, and sevoflurane. Peak levels of fluoride in plasma occur approximately four hours after enflurane and sevoflurane anaesthesia (at concentrations of about 20 $\mu\text{M} \cdot \text{L}^{-1}$) and 24 hr after methoxyflurane anaesthesia (40–50 $\mu\text{M} \cdot \text{L}^{-1}$). Serum fluoride concentrations were significantly higher following the use of halothane in obese patients (10 $\mu\text{M} \cdot \text{L}^{-1}$) compared with non-obese patients.⁹ Serum fluoride concentrations were also much higher after the administration of enflurane to an obese patient (up to 40 $\mu\text{M} \cdot \text{L}^{-1}$) and to a patient receiving isoniazid (up to 75 $\mu\text{M} \cdot \text{L}^{-1}$) compared with non-obese patients.^{10–11} Our data show that inorganic fluoride at concentrations of 20–75 $\mu\text{M} \cdot \text{L}^{-1}$ inhibits PCHE activity by 28–65 per cent, and ACHE activity by 1.4–2.5 per cent. Bromide, a normal metabolite of halothane, develops peak plasma concentrations about 24 hr following halothane anaesthesia.² Unlike fluoride, our data show that bromide inhibits neither PCHE activity nor ACHE activity significantly.

Pseudocholinesterase is a glycoprotein produced in the liver which is responsible for the metabolism of succinylcholine, a depolarizing muscle relaxant and all the ester type local anesthetics. Viby-Mogensen has shown that for patients ($n = 70$) with the genotypically normal enzyme, the duration of succinylcholine action increased with

decreasing PCHE activity.¹² Approximately one third ($n = 23$) of the Viby-Mogensen's patients had low and the remaining patients ($n = 47$) had either normal or high enzymatic activity. The duration of apnoea in patients with low enzymatic activity lasted from 5–21 min. An additional decrease of up to 65 per cent of PCHE activity caused by fluoride ion in these patients may be associated with a marked prolongation of succinylcholine action. A remarkable decrease in PCHE activity in patients with the genotypically normal enzyme can cause a desensitization block with a single dose administration of succinylcholine. We have previously described such a case of desensitization block with a single dose administration of succinylcholine in a patient with low, but genotypically normal, enzyme.¹³ Systemic toxicity caused by ester type local anesthetics has also been documented in patients with decreased PCHE activity.¹⁴ Also if PCHE activity is determined in these patients during immediate postoperative period, a very low activity may be seen. This iatrogenically induced low PCHE activity may then be mistakenly diagnosed as inherited PCHE abnormality. This may not be important in patients with normal or high PCHE activities, because a decrease of up to 65 per cent may not be associated with any noticeable effect on succinylcholine action or systemic ester type local anesthetic toxicity.

Acetylcholinesterase (also called true cholinesterase, red cell cholinesterase) is usually present in the red blood cells, the neuromuscular junction of skeletal muscles, and other nervous and non-nervous tissues. The ACHE is responsible for the rapid hydrolysis of acetylcholine which is the principal neurotransmitter released at the

TABLE II Effect of bromide on pseudocholinesterase (PCHE) and acetylcholinesterase activities ($n = 7$, each concentration)

Bromide concentration $\mu\text{M} \cdot \text{L}^{-1}$	PCHE (units)*		ACHE (units)*	
	Mean $\pm$ SEM	%Inhibition	Mean $\pm$ SEM	%Inhibition
Control	5.53 $\pm$ 0.33	—	8.40 $\pm$ 0.26	—
10	5.48 $\pm$ 0.31	0.6	8.41 $\pm$ 0.25	0.0
50	5.53 $\pm$ 0.34	0.0	8.42 $\pm$ 0.25	-0.2
100	5.53 $\pm$ 0.35	0.0	8.44 $\pm$ 0.27	-0.4
500	5.56 $\pm$ 0.35	-0.5	8.45 $\pm$ 0.25	-0.6
1000	5.55 $\pm$ 0.36	-0.4	8.43 $\pm$ 0.25	-0.4

*Multifactor analyses of variance ($P > 0.05$).

neuromuscular junction. Non-depolarizing muscle relaxants (NDMR) act by competing with acetylcholine at the neuromuscular junction. Thus any alterations in ACHE activity could influence the duration of action of NDMR. Since fluoride at concentrations of $20\text{--}75 \mu\text{M} \cdot \text{L}^{-1}$ inhibits less than five per cent of ACHE activity, the effect on the action of nondepolarizing muscle relaxants may be negligible.

The clinical implications of our data are as follows: (1) patients who undergo reoperation in the immediate recovery phase following enflurane and sevoflurane anaesthesia should have close neuromuscular monitoring if they receive succinylcholine, (2) epidural ester type local anaesthetics should be administered with caution in patients recovering from enflurane and sevoflurane anaesthesia, and (3) in patients with suspected low or abnormal PCHE activity, the PCHE assay should be delayed at least 24 hr following enflurane and sevoflurane anaesthesia.

In conclusion our data show that fluoride at concentrations achieved with enflurane, methoxyflurane, and sevoflurane anaesthesia significantly inhibits PCHE activity ($P < 0.001$). No significant effect on ACHE activity was noted with these concentrations. Bromide at concentrations achieved following halothane anaesthesia has no significant effect on either PCHE or ACHE activity.

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