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Acetylcholine receptor-enriched membrane vesicles in response to ethanol: Activity and microcalorimetric studies

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Abstract

The activity of acetylcholinesterase (AChE) in acetylcholine receptor (AChR)-enriched membrane vesicles isolated from electric organ of *Torpedo californica* exhibited a biphasic response to ethanol action. Below an ethanol concentration of 35 m*M*, AChE activity increased with increasing concentration of ethanol. At ethanol concentrations greater than 35 m*M*, the activity was found to decrease montonically. In contrast, ethanol (35–400 m*M*) increased the activity of soluble AChE. This biphasic behavior was consistent with the proposed important role of ethanol–membrane interaction. Microcalorimetric measurements revealed that the enthalpy change in acetylcholine (ACh) hydrolysis reaction was 586 J/mol in association with membrane-bound AChE in AChR-enriched membrane vesicles, as compared to −544 J/mol with the isolated soluble AChE. This discrepancy was attributed to the presence of membranes. Unlike its action on the enzyme activity, ethanol did not affect the enthalpy change in ACh hydrolysis reaction catalyzed by either membrane-bound or soluble AChE. Comparison of results on activity and heat measurements suggested that the interaction of ethanol with membrane vesicles was nonspecific with no ethanol-induced membrane structural or conformational change.

Keywords: Membrane vesicles; Ethanol effect; Acetylcholinesterase activity; Microcalorimetry

1. Introduction

One approach to investigating ethanol toxicity in neurotransmission is to focus on whether ethanol alters the level of neurotransmitters in various brain regions. For example, the release of acetylcholine (ACh) in the cerebral cortex is decreased in response to ethanol exposure [1,2]. Furthermore, the levels of several enzymes, substrates and receptors associated with neurotransmission have been found to change along with the neurotransmitter [3,4]. However, the relationships between these changes are difficult to establish in whole-tissue studies. A complementary approach to clarifying ethanol toxicity is to study the effects of ethanol on proteins involved in neurotransmission, such as acetylcholinesterase (AChE) and acetylcholine receptor (AChR). These two proteins are required for synaptic transmission. This subject was recently reviewed by our laboratory [5].

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To probe the structural aspect of this effect, we previously studied the action of ethanol on the structural stability of AChR-enriched membrane vesicles isolated from the electric organ of *Torpedo californica* [6]. In the presence of ethanol up to 200 mM, the thermogram of these membranes exhibited no significant decrease in the temperature of receptor transition at 57°C, but a decrease in the enthalpy change (ΔH_d), indicating an ethanol-induced structural perturbation. A decrease in ΔH_d of the receptor transition was also observed in the presence of α-bungarotoxin. The addition of ethanol did not cause a recovery of the transition, suggesting that ethanol interacted with the receptor noncompetitively.

To elucidate mechanisms of alcohol–membrane interaction, a biphasic action of ethanol on the activity of membrane-bound enzymes has attracted increasing interest [7–11]. It has been suggested that a low concentration of ethanol could increase the activity of these enzymes by enhancing lipid fluidity. Sublethal concentrations of ethanol could suppress the function of membrane-bound enzymes by disrupting membrane lipid–protein interaction. Such a biphasic effect was well demonstrated for (Na^+–K^+)-ATPase [10], but not established for AChE yet. It has been shown that ethanol decreased the activity of isolated membrane-bound AChE from the electric organ [6] and an insensitivity to a low concentration of ethanol was found for AChE prepared from synaptic plasma membranes of rat cerebral cortex [10]. However, a biphasic ethanol effect on membrane-bound AChE prepared from bovine erythrocytes was recently observed in our laboratory [11]. Such a discrepancy on membrane-bound AChE in response to ethanol requires further investigations.

Ethanol-dependent disordering of lipids in AChR-enriched membrane vesicles is correlated with some effects on AChR activity, such as desensitization [12]. AChR-enriched membrane vesicles are known to retain the ligand-binding and ion-permeability-control properties expected from AChR [13,14]. These membrane vesicles contain membrane-bound AChE, providing a useful system for the studies of ethanol action on membrane-bound AChE.

In extension of our previous work on AChR-enriched membrane vesicles and AChE [6,11], we have performed enzyme activity assays to examine the action of ethanol on membrane-bound AChE in AChR-enriched membrane vesicles. The important role of membrane lipids in response to alcohol was demonstrated. To elucidate ethanol-membrane interaction, microcalorimetry was employed to examine the enthalpy change in ACh hydrolysis reaction catalyzed by membrane-bound and soluble AChE in the absence and presence of ethanol.

2. Materials and methods

2.1 Chemicals

The fresh electric organ from *Torpedo californica* was obtained from Pacific Biomarine (Venice, CA). Acetylcholine (ACh) chloride, acetylthiocholine iodide (ATC), 5,5′-dithiobis (2-nitrobenzoic acid), ϵ-amino-n-caproic acid, benzamidine-HCl, diisopropyl fluorophosphate (DFP) and phenylmethylsulfonyl fluoride were purchased from Sigma Chemical Company, (St. Louis, MO). Other chemicals were of reagent grade and obtained from commercial sources. Double distilled water was used in all experiments.

2.2 Preparation of AChR-enriched membrane vesicles

AChR-containing membrane vesicles were prepared according to the procedure described by Sobel et al. [15]. Typically, 500 g of the electric organ from *Torpedo californica* that had been freshly excised and frozen in liquid nitrogen was thawed overnight at 4°C. It was minced into small pieces and resuspended in a 1 : 1 volume ratio of cold medium solution containing 0.1 mM $PhMeSO_2F$ and 0.02% NaN_3. The suspension was homogenized with a Waring Blender (model 1043) for 3 min at maximal speed. The homogenate was then centrifuged at 5,000 rpm (GSR rotor) for 10 min. The supernatant was collected through four layers of cheese cloth. The pellet

was resuspended in two volumes of medium solution, homogenized and centrifuged under the same conditions to collect additional supernatant. The combined supernatants were centrifuged at 8,000 rpm for 2 h. The pellet was resuspended in about 120 ml of 32% sucrose and homogenized in a motor-driven homogenizer with a teflon pestle. About 10 ml of the homogenized pellet was layered on the top of a discontinuous sucrose gradient (10 ml at 41.5%, 8 ml at 37.5%, 6 ml at 35%, w/w) in a 35-ml tube and centrifuged at 25,000 rpm in a Beckman SW-27 rotor for 16 h. The 37.5% layer, which contains AChR-enriched membrane vesicles, was collected, diluted with one volume of medium solution and centrifuged at 40,000 rpm in a 60 Ti rotor for 30 min. The pellet was resuspended in a minimum volume of medium solution and homogenized in a motor-driven homogenizer with a teflon pestle. The homogenate was stored under liquid nitrogen for later use. All manipulations were performed at 4°C.

2.3 Preparation of soluble acetylcholinesterase (AChE)

To differentiate the characteristics of membrane bound AChE from soluble AChE in response to alcohol, the soluble enzyme was isolated from the electric organ according to Lee et al. [16]. This allows a comparison between membrane bound AChE and soluble AChE from the same source. The tissue, previously stored under liquid nitrogen, was thawed overnight at 4°C and homogenized for 3 min in 0.75 ml volume of 10 m*M* tris-HCl buffer (pH 8.0) containing 0.1 *M* NaCl, 10 m*M* EDTA, 2 m*M* benzamidine-HCl and 2 m*M* ϵ-amino-n-caproic acid, using a model 1040 Waring Blender. The homogenate was centrifuged at 12,000 rpm (GSR rotor) for 60 min. The pinkish, somewhat cloudy supernatant was centrifuged at 18,000 rpm (SS-34 rotor) for 90 min. The clear, pinkish supernatant was reported to contain a hydrophobic fraction of AChE [16]. Higher-speed centrifugation showed no loss of the activity of the supernatant, indicating the presence of soluble enzyme. The clear, pinkish supernatant containing soluble AChE was concentrated using a 50-ml Amicon ultrafiltration cell, and dialyzed against *Torpedo* physiological saline buffer (TPS buffer). TPS buffer was made of 5 m*M* NaP_i, pH 7.0 containing 250 m*M* NaCl, 4 m*M* KCl, 3 m*M* $CaCl_2$ and 2 m*M* $MgCl_2$. The dialysate containing soluble AChE was stored under liquid nitrogen for later use. All manipulations were performed at 4°C.

2.4 Spectrophotometric assay of AChE activity

Using a Lambda 4B UV/visible spectrophotometer (Perkin-Elmer), the activity of AChE (EC 3.1.1.7) in soluble enzyme and AChR-enriched membrane vesicles was assayed according to a modified Ellman et al. procedure [17], while using acetylthiocholine (ATC) as the substrate. The enzyme activity was measured by following the increase of yellow color produced from thiocholine when it reacted with dithiobisnitrobenzoate ion. The rate of color production was measured spectrophotometrically at 412 nm. The reaction mixture, in a final volume of 3 ml, contained 20 μl of 75 m*M* ATC, 20 μl of 10 m*M* 5,5′-dithiobis(2-nitrobenzoic acid), 2.95 ml of 0.1 *M* potassium phosphate buffer (pH 8.0) and 10 μl of enzyme solution. The hydrolysis reaction of ATC was initiated by adding the enzyme solution to the sample cuvette. Ten seconds after mixing, the increase in the absorbance of the sample at 412 nm was monitored to obtain a linear plot of the absorbance versus time for 30 s. The specific activity of AChE obtained on the basis of the rate of hydrolysis (μmole of product/(min mg of protein) or units/mg of protein) was calculated on the basis of an extinction coefficient of the yellow anion (13.6 mM^{-1} cm^{-1}) [17].

2.5 Microcalorimetry studies of ACh hydrolysis reaction catalyzed by AChE

A twin-reaction cell batch microcalorimeter was used to examine the heat of reaction associated with the hydrolysis of acetylcholine (ACh) catalyzed by AChE. Its instrumentation has been described previously [18–20]. It was employed in our previous studies of protein interactions [18], membrane fluidity [19] and energetics of mem-

brane transport [20]. Both sample and reference cells were divided into two compartments with capacities of about 1.2 ml. For a typical run, the two compartments of the sample cell were loaded with 0.3 ml of 5 m*M* ACh and 0.3 ml of AChR-containing membrane vesicles or soluble AChE in buffer solution, respectively. The two compartments of the reference cell contained 0.3 ml of an appropriate concentration of ACh and the same volume of buffer solution, respectively. The buffer solution was TPS buffer (see above). The cells were then allowed to thermally equilibrate at room temperature for 20–30 min before mixing. The mixing thermogram was recorded on a Leeds and Northrup Speedomax XL (North Woods, PA) recorder. The area under the thermogram was measured with a sonic digitizer (Science Accessories Corp., Southport, CT) and was converted into thermal units.

2.6 Ethanol effects

To study the effect of ethanol on AChE, the ionic strength of the sample solution was kept constant over various concentrations of ethanol and the dilution effect was considered. In microcalorimetric measurements, ethanol was added to both the sample and reference cells.

3. Results

3.1 AChE activity in AChR-enriched membrane vesicles

AChE activity in AChR-enriched membrane vesicles is shown in Fig. 1 curve (a). A linear increase in the absorbance at 412 nm as a function of time was found, after mixing membrane vesicles with the substrate, acetylthiocholine iodide (ATC). The increase in the absorbance as the reaction proceeded arose from the hydrolysis of ATC catalyzed by AChE. In contrast, if membrane vesicles were pre-equilibrated with diisopropyl fluorophosphate (DFP) for about 30 min before mixing with ATC, no change in the absorbance was observed (curve b). The absence of ACh hydrolysis reaction in curve (b) was due to the inhibition of AChE activity by DFP.

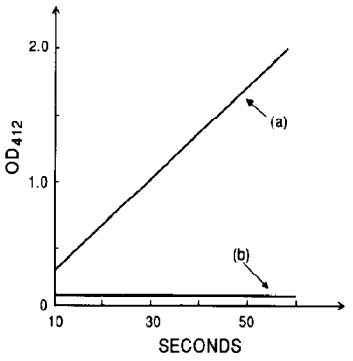

Fig. 1. Enzyme activity assay profile of AChE bound to AChR-enriched membrane vesicles in TPS buffer (see text). The substrate is acetylthiocholine (ATC): curve (a) in the absence of an inhibitor, and curve (b) in the presence of the inhibitor, diisopropyl fluorophosphate (DFP). The concentrations of membrane vesicles, ATC and DFP are 9.13 μg of proteins/ml, 0.50 m*M* and 0.1 m*M*, respectively.

3.2 Heat of ACh hydrolysis reaction

The hydrolysis reaction of acetylcholine (ACh) was performed with microcalorimetric measure-

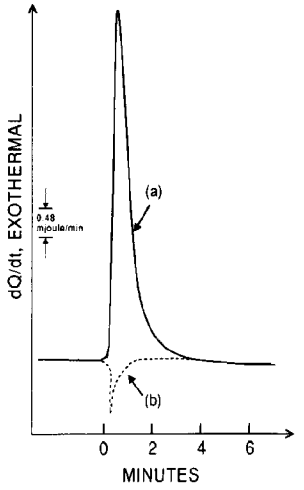

Fig. 2. Microcalorimetric measurements of the mixing of AChR-enriched membrane vesicles with acetylcholine: curve (a) in the absence of an inhibitor. The amount of heat observed = −5.24 mJ; curve (b) in the presence of the inhibitor (DFP). The amount of heat observed = 0.05 mJ. The concentrations of ACh, membrane vesicles and DFP are 4 m*M*, 1.35 mg of protein/ml and 0.1 m*M*, respectively.

ments. Fig. 2 curve (a) shows a typical thermogram involving the mixing of AChR-enriched membrane vesicles with ACh. The mixing reaction involving soluble AChE (final concentration = 1.82 mg/ml) was essentially as rapid as shown in Fig. 2 curve (a). The thermogram is a profile of dQ/dt versus t, where Q is the heat released in the mixing reaction and t is the time. The area under the thermogram represents the observed Q. The observed sharp exothermal peak was due to the heat released in the hydrolysis of ACh (see below for a quantitative analysis). This peak essentially disappeared if membrane vesicles were equilibrated with the inhibitor DFP for about 30 min prior to the mixing experiment (curve (b) in Fig. 2). The figure shows that DFP inhibits the enzyme activity of ACHE, resulting in the elimination of AChE-catalyzed hydrolysis reaction.

3.3 AChE activity in AChR-enriched membrane vesicles in response to ethanol

Figure 3 shows that, below [EtOH] of 35 mM, the AChE activity in AChR-enriched membrane vesicles increases with increasing [EtOH]. At [EtOH] > 35 mM, the activity was found to decrease monotonically. This biphasic behavior is consistent with our previous observation on membrane-bound AChE from bovine erythrocytes. The activity of a membrane-bound enzyme is usually influenced by its association with membrane lipids [21]. A lower concentration of ethanol can cause an increase in membrane fluidity of membrane-bound AChE in AChR-enriched membrane vesicles and consequently an increase in the AChE activity. However, as [EtOH] continues to increase, ethanol can disrupt the protein–lipid interaction needed to maintain the conformation of membrane-bound AChE, thus causing a decrease in the enzyme activity.

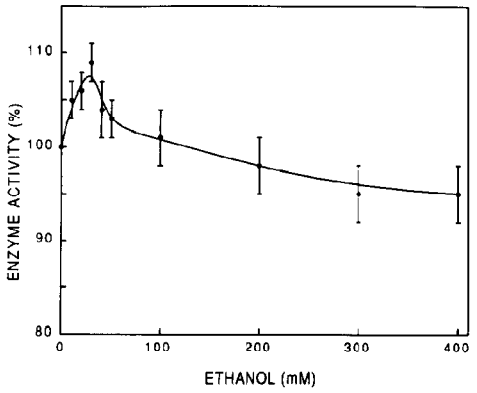

Fig. 3. The dependence of the relative enzyme activity of AChE in AChR-enriched membrane vesicles on the concentration of ethanol. The AChE activity in the absence of ethanol is taken as 100%. The concentrations of membrane vesicles and ATC are 4.57 μg of protein/ml and 0.50 mM, respectively.

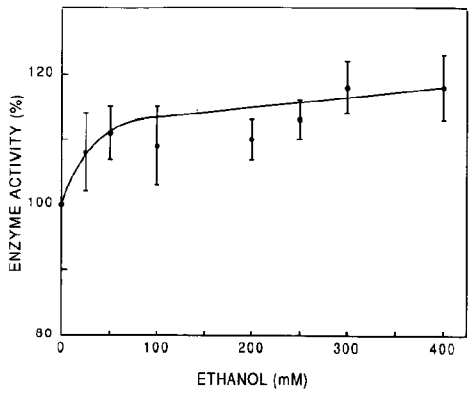

Fig. 4. The effect of ethanol on the enzyme activity of soluble AChE. The AChE activity in the absence of ethanol is taken as 100%. The concentrations of AChE and ATC are 2.43 μg/ml and 0.50 mM, respectively.

Comparative studies were also performed on the effects of ethanol on membrane-bound AChE in AChR-enriched membrane vesicles with those on soluble AChE. Fig. 4 shows that, In contrast to membrane-bound AChE, the enzyme activity of soluble AChE increases up to 18% at [EtOH] = 25 to 400 mM, and exhibits no biphasic characteristic. An increase in the enzyme activity in the presence of ethanol was also observed for soluble AChE isolated from electric eel [11], honeybee head [22], and cockroach [23].

Table 1 lists the enzyme activity data for AChE isolated from various sources and their response to ethanol. The table shows activity profiles distinguished between membrane-bound AChE and soluble AChE. For membrane-bound AChE, the enzyme activity increases with the addition of a lower [EtOH], but decreases at a higher [EtOH].

Table 1

Comparison of the effects of ethanol on the enzyme activity of AChE isolated from various sources

[Ethanol] (m*M*)	Activity of soluble AChE		Activity of membrane-bound AChE	
	Torpedo californica	Electric eel [a]	AChR-enriched membrane vesicles from *Torpedo californica*	Bovine erythrocytes [a]
0 [b]	100	100	100	100
10		103	105±2	100
20			106±2	104
25	108±6	104		
30			109±2	103
40			104±3	
50	111±4		103±2	103
100	109±6	106	101±3	98
200	110±3		98±3	92
250	113±3			
300	118±4	108	95±3	
400	118±5	108	95±3	81

[a] Data taken from Shin et al. [11].

[b] At [EtOH] = 0, activity is taken as 100% (as the control). The activity of soluble AChE and membrane-bound AChE (from *Torpedo californica*) in each measurement contains 900 and 700 units, respectively.

In contrast, the enzyme activity of soluble AChE gradually increases with increasing [EtOH] up to 400 m*M*.

3.4 The heat of ACh hydrolysis reaction catalyzed by AChE in AChR-enriched membrane vesicles

Solution microcalorimetry was employed to quantitatively examine the heat change in the hydrolysis of ACh catalyzed by membrane-bound AChE in AChR-enriched membrane vesicles. Control experiments involving the mixing between membrane vesicles and buffer solution revealed that the observed heat was small and negligible (0.04 mJ; figure not shown). In a typical measurement involving the mixing of membrane vesicles (1.35 mg of proteins/ml) with ACh (4 m*M*) as shown in Fig. 2 curve (a), the observed heat (ΔQ) was −5.24 mJ. The value of ΔQ is dependent on the ACh concentration and reaches a plateau at [ACh] = 5 m*M*. The above mixing experiment was also performed in the presence of DFP to inhibit the hydrolysis reaction (Fig. 2 curve b). A value of only 0.05 mJ was obtained. This value is equivalent to that in the control experiment, suggesting that the observed heat change in Fig. 2 curve (a) is derived from the heat of hydrolysis reaction. Taking into consideration of the amount of ACh hydrolyzed ($3.0\ 10^{-6}$ mol), the enthalpy change (ΔH) in the ACh hydrolysis reaction can be calculated as -1.63 ± 0.21 kJ/mol (average value).

While the hydrolysis reaction at equilibrium should be essentially complete, inhibition of the enzyme action could prevent this from occurring [24]. At a higher substrate concentration, the hydrolysis reaction velocity (as measured by the rate of production of thiocholine) can be reduced due to an inhibition by the excess substrate. It has been demonstrated that, in the range of [ACh] = 0.3 to 3.0 μmol, the reaction is accompanied with insignificant enzyme inhibition [25]. The present experiments were carried out within this substrate concentration range. An essential completion of the hydrolysis reaction under our experimental conditions was further supported by the reasonable agreement of our determined value for ΔH with the literature data, using soluble AChE (see Section 3.5 and Table 2).

Table 2

The enthalpy change (ΔH) in acetylcholine hydrolysis reaction catalyzed by AChE from various sources and different media at 25°C

[Ethanol] (m*M*)	ΔH (J/mol)		
	Membrane-bound AChE in AChR-enriched membrane vesicles from *Torpedo californica* [a]	Soluble AChE from *Torpedo californica* [a]	Soluble AChE from *Electrophorus electricus*
0	586	−544	−448 [b]
≤300	586	−544	

[a] The buffer is *Torpedo* physiological saline buffer 5 m*M* NaP_i pH 7.0 containing 250 m*M* NaCl, 4 m*M* KCl, 3 m*M* $CaCl_2$ and 2 m*M* $MgCl_2$. The mixing solution in a microcalorimetric measurement contains ACh (≤3 μmol) and AChR-enriched membrane vesicles (0.41 mg of proteins) or soluble AChE (0.54 mg). Each value is an average of 3 to 5 measurements with an experimental error of 9–12%.

[b] 0.1 *M* phosphate buffer pH 7.2. Data taken from Ref. [25].

The ACh hydrolysis reaction at neutral pH is

$$CH_3COOCH_2CH_2N^+(CH_3)_3 + H_2O \rightarrow CH_3COO^- + HOCH_2CH_2N^+(CH_3)_3 + H^+ \quad (1)$$

where one proton is liberated. In phosphate buffer, the released H^+ in the ACh hydrolysis reaction can be uptaken by phosphate ion. This heat of protonation of phosphate should be corrected for in order to obtain the actual heat of hydrolysis [25,26]. In TPS buffer (see above), the ionic strength (μ) is about 0.27. At 25°C, the enthalpy change in the second ionization of phosphoric acid has been reported as 987 cal/mol for $\mu = 0$ and -200 cal/mol for $\mu = 0.70$ [26]. Interpolation of these data gives a value of 530 cal/mol for $\mu = 0.27$. That is, the enthalpy change in the protonation reaction is -530 cal/mol or -2218 Joule/mol. After correction for this buffer protonation heat, the determined ΔH in ACh hydrolysis reaction is 586 J/mol $[-1632-(-2218)]$ as shown in Table 2.

3.5 The heat of ACh hydrolysis reaction catalyzed by soluble AChE

To examine the contribution of membrane lipids to ACh hydrolysis reaction, the enthalpy (ΔH) of ACh hydrolysis reaction catalyzed by soluble AChE was also determined as -2.76 ± 0.25 kJ/mol in TPS buffer, pH 7.0 (figure not shown). After correction for buffer protonization heat, the value became -544 Joule/mol, as compared to 586 J/mol obtained above for membrane-bound AChE in AChR-enriched membrane vesicles. The discrepancy between these two values is due to the presence of membranes (see the discussion in Section 4). It is interesting to note that our determined figure of -544 J/mol for ΔH catalyzed by soluble AChE isolated from *Torpedo californica* is comparable with -448 J/mol reported for that catalyzed by soluble AChE prepared from *Electrophorus electricus* [25] (Table 1), demonstrating the reliability of our data.

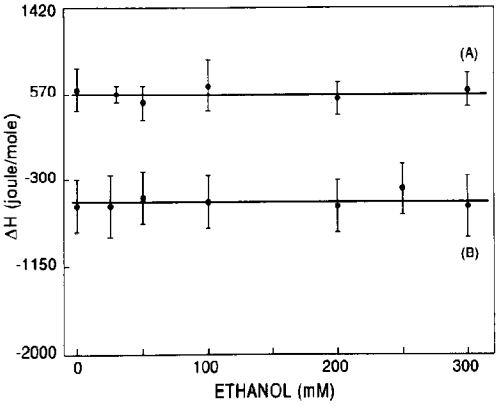

Fig. 5. The action of ethanol on the enthalpy change (ΔH) of ACh hydrolysis reaction catalyzed by: curve (A) AChE in AChR-enriched membrane vesicles (1.35 mg of proteins/ml); and curve (B) isolated soluble AChE (1.82 mg/ml). The concentration of ACh is 4 m*M*.

3.6 Effects of ethanol on the heat of ACh hydrolysis reaction catalyzed by membrane-bound and soluble AChE

Unlike the enzyme activity of AChE which is affected by ethanol (Figs. 3 and 4), Fig. 5 curve (A) shows that the enthalpy change (ΔH) in the ACh hydrolysis reaction catalyzed by membrane-bound AChE in AChR-enriched membrane vesicles is essentially unchanged in the presence of [EtOH] from 25 to 300 m*M*. The shape of the thermogram in the mixing reaction is also unaffected by ethanol (not shown here). The observed ΔH of the hydrolysis reaction should not be affected by the presence of a low concentration of ethanol, unless a secondary reaction such as a membrane structural or conformational change accompanies the hydrolysis reaction. A similar observation of no effect on ΔH by ethanol was found for soluble AChE (Fig. 5 curve B).

4. Discussion

In extension of our previous work on the structural stability of AChR-enriched membrane vesicles [11], the present study investigated the action of ethanol on membrane-bound AChE in these

membrane vesicles. The results presented above show that ethanol increases the activity of membrane-bound AChE at [EtOH] < 35 mM and decreases it at [EtOH] > 100 mM. In contrast, ethanol (35–400 mM) increases the activity of soluble AChE. Comparison of these results (Figs. 3 and 4) reveals a critical role of ethanol–membrane interactions. This observed biphasic nature of ethanol action on membrane-bound AChE in AChR membrane vesicles is an interesting phenomenon which may be of importance in understanding physiological and biochemical effects of alcohol. The result is in consistence with the reported ethanol effect on (Na^+–K^+) [10] and membrane-bound AChE prepared from bovine erythrocytes [6].

A catalyst can change the Gibbs free energy of activation (ΔG^*), but not the Gibbs free energy of the reaction ($\Delta G°$) at any temperature or the entropy change (ΔS) ($= -(\partial \Delta G/\partial T)_p$). Consequently, ΔH (calculated from $\Delta G + T\,\Delta S$) will not be affected by the enzyme. However, Table 2 or Fig. 5 shows that the value of ΔH in ACh hydrolysis reaction is +586 J/mol with membrane-bound AChE in AChR-enriched membrane vesicles, as compared to −544 J/mol with soluble enzyme under identical experimental conditions. These results suggest that an additional process must be occurring in association with membrane-bound AChE. The presence of membranes in the medium could alter the solvent property so as to affect the observed ΔH. More importantly, it could contribute to the stability of membrane-bound AChE. A structural perturbation on AChR-enriched membrane vesicles would be required in the interaction of membrane-bound AChE with ACh in the process of ACh hydrolysis reaction. Such a structural perturbation could well be this additional process.

In the hydrolysis reaction of ACh catalyzed by AChE, the products are choline and acetic acid. The enzyme, AChE, is present at a concentration much lower than that of the substrate. An important aspect derived from the law of enzyme reactions is that an effector (inhibitor or activator) of an enzyme influences the forward and backward reaction rate by the same factor—the ratio has to remain the same to keep the equilibrium constant unchanged [27]. Accordingly, ethanol is not expected to change the equilibrium constant or $\Delta G°$ of ACh hydrolysis reaction.

Figure 5 reveals that ethanol at 25–300 mM affects neither the value of ΔH of ACh hydrolysis reaction catalyzed by membrane-bound AChE nor that catalyzed by soluble AChE. The finding that the observed ΔH in the presence of membranes is not affected by ethanol suggests that no secondary reaction such as a membrane structural or conformational change accompanies the hydrolysis reaction. This result implies that the interaction of ethanol with membrane vesicles is nonspecific (such as increasing lipid solubility), which does not contribute to the observed enthalpy change in the hydrolysis reaction.

References

1 C.K. Erickson and D.T. Graham, J. Pharmacol. Exp. Ther. 185 (1973) 583.
2 J.W. Clark, H. Kalant and F.J. Carmichael, Can. J. Physiol. Pharmacol. 55 (1977) 758.
3 B. Tabakoff, P.L. Hoffman and S. Liljequist, Enzyme 37 (1987) 70.
4 J.O. Owasoyo and C.A. Iranmain, Toxicol. Lett. 9 (1981) 267.
5 C.-H. Chen, in: Focus on biochemistry and physiology of substance abuse, vol. 1 ed. R.R. Watson (CRC Press, Boca Raton, FL, 1989) Chap. 1.
6 G.M. Baker and C.-H. Chen, Biochem. Biophys. Acta. 992 (1989) 333.
7 S. Kelly-Murphy, A.J. Waing and H. Rottenberg, Lab. Invest. 50 (1984) 174.
8 T.C.K. Chan, D.V. Gordin and M.C. Sutter, Drug Alcohol Dependence 12 (1983) 249.
9 D.R. Wing, D.J. Harvey and S.J. Belcher, Biochem. Pharmacol. 23 (1984) 1626.
10 A.Y. Sun and R.N. Seaman, Neurochem. Res. 5 (1980) 537.
11 S. Shin, P. Wu and C.-H. Chen, Int. J. Biochem. 23 (1991) 169.
12 K.W. Miller, L.L. Firestone and S.A. Forman, Ann. N.Y. Acad. Sci. 492 (1987) 71.
13 T. Heidemann and J.P. Changeux, Annu. Rev. Biochem. 47 (1978) 317.
14 T.J. Anderson, D.R. Doerge and M.G. McNamee, Arch. Biochem. Biophys. 194 (1979) 468.
15 A. Sobel, M. Weber and J-P. Changeux, Eur. J. Biochem. 80 (1977) 215.
16 S.L. Lee, S. Heinemann and P. Taylor, J. Biol. Chem. 257 (1982) 12283.

17 G.L. Ellman, K.D. Courtney, V. Audres Jr. and R.M. Featherstone, Biochem. Pharmacol. 7 (1961) 88.
18 C.-H. Chen, J. Phys. Chem. 84 (1980) 2050.
19 C.-H. Chen, D.S. Berns and A.S. Berns, Biophys. J. 36 (1981) 359.
20 L.-I.S. Chen and C.-H. Chen, Arch. Biochem. Biophys. 246 (1986) 515.
21 L. Gordon and P.W. Mobley, in: Membrane fluidity in biology, vol. 4, eds. R.C. Aloia and J.M. Boggs (Academic Press, New York, NY, 1985) p. 1.
22 D.K. Lewis, Nature 213 (1967) 205.
23 E.H. Colhoum, Nature 189 (1961) 309.
24 J.R. Pieklik and R.W. Guynn, J. Biol. Chem. 250 (1975) 4445.
25 Y.T. Das, H.D. Brown and S.K. Chahopadhyay, Biophys. Chem. 23 (1985) 105.
26 R.M. Izatt and J.J. Christensen, in: Handbook of biochemistry and molecular biology (CRC Press, Cleveland, OH) p. 151.
27 J.T. Edsall and H. Gutfreund, Biothermodynamics (Wiley, New York, NY, 1983) p. 145.