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Biphasic effects of acetaldehyde-biogenic amine condensation products on membrane fluidity

Hironori Tsuchiya

Abstract

I have studied the effects of four acetaldehyde-biogenic amine condensation products on membrane fluidity of liposomes, consisting of 1-palmitoyl-2-oleoylphosphatidylcholine and cholesterol, by measuring fluorescence polarization using different probes.

The condensation products were 1-methyl-1,2,3,4-tetrahydro-β-carboline (MTBC), 6-hydroxy-1-methyl-1,2,3,4-tetrahydro-β-carboline (6-OH-MTBC), 3-carboxy-1-methyl-1,2,3,4-tetrahydro-β-carboline (3-C-MTBC) and 6,7-dihydroxy-1-methyl-1,2,3,4-tetrahydroisoquinoline (salsolinol). They changed the fluidity of the hydrophobic and hydrophilic regions of liposomal membranes at micromolar levels almost corresponding to their antibacterial and antiplatelet concentrations, but their membrane effects varied by structure, concentration and membrane lipid composition. MTBC and salsolinol showed biphasic effects on the inner layers of membranes to enhance the fluidity at 250–1000 μM and reduce the fluidity at 50–100 μM, whereas both of them fluidized the outer layers of the membranes. 3-C-MTBC concentration-dependently fluidized both layers of membranes. 6-OH-MTBC most weakly enhanced and reduced the fluidity of the outer and inner layers, respectively. The membrane effect of MTBC was the greatest of the four condensation products. MTBC (50–1000 nM) significantly reduced the fluidity by exclusively acting on the membrane core, but was less effective in fluidizing the membrane surface. However, the others were not active at low nanomolar levels.

The membrane effects may be partly responsible for the antibacterial and antiplatelet actions of the acetaldehyde-biogenic amine condensation products, although they do not appear to be simple membrane fluidizers.

Introduction

Acetaldehyde condenses with biogenic amines to produce a series of pharmacologically active compounds: 1-methyl-1,2,3,4-tetrahydro-β-carboline (MTBC) from tryptamine, 6-hydroxy-1-methyl-1,2,3,4-tetrahydro-β-carboline (6-OH-MTBC) from serotonin (5-HT), 3-carboxy-1-methyl-1,2,3,4-tetrahydro-β-carboline (3-C-MTBC) from tryptophan, and 6,7-dihydroxy-1-methyl-1,2,3,4-tetrahydroisoquinoline (salsolinol) from dopamine (Figure 1). These so-called Pictet-Spengler condensation products show neuropharmacological activities such as binding to different receptors, inhibition of neurotransmitter-relating enzymes, influence on the uptake and release of neurotransmitters etc. (Melchior & Collins 1982; Rommelspacher et al 1991). They are contained in many plants, foods and beverages, some of which have been used as traditional medicines (Allen & Holmstedt 1980; Tsuchiya et al 1996a, 1999a). In addition to the exogenous origin, it is possible that tetrahydro-β-carbolines and tetrahydroisoquinolines are produced

Department of Dental Pharmacology, Asahi University School of Dentistry, 1851 Hozumi, Hozumi-cho, Motosu-gun, Gifu 501-0296, Japan

Hironori Tsuchiya

Correspondence: Email: hiro@dent.asahi-u.ac.jp

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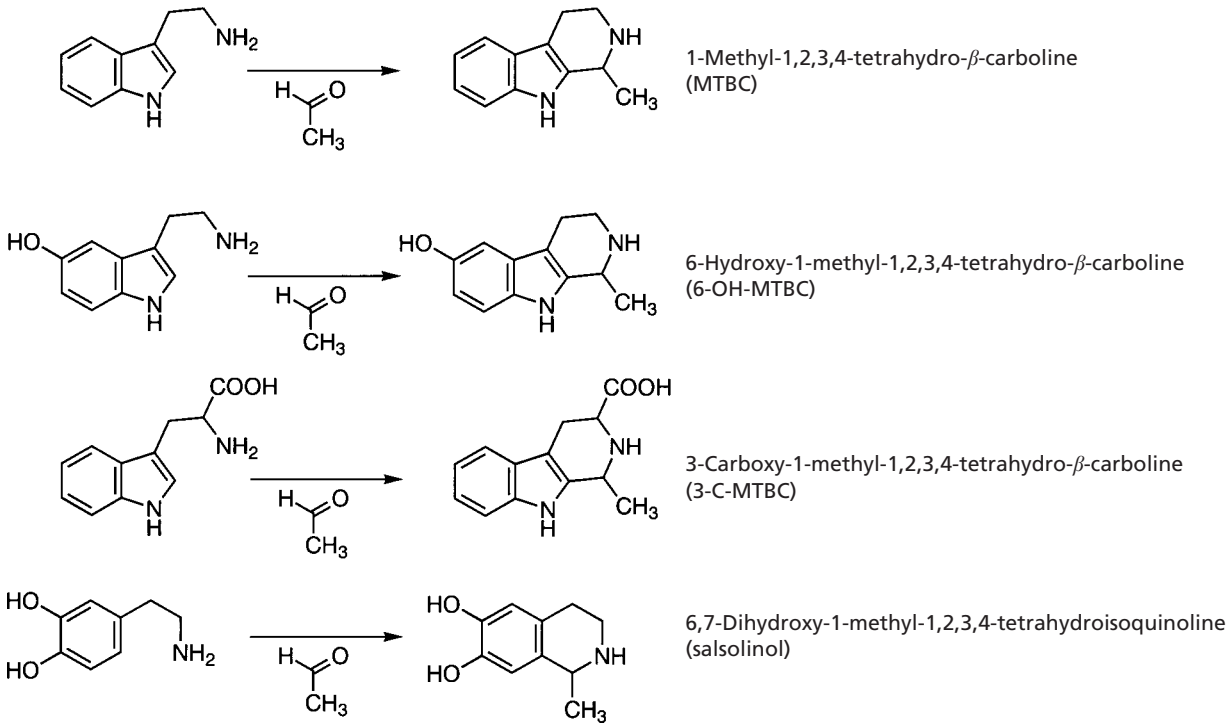

Figure 1 Condensation of acetaldehyde with biogenic amines.

in the human body by the endogenous condensation reaction, therefore they have been referred to hypothetically as the aetiological factors responsible for certain neuropsychiatric disorders including alcoholism (Collins 1988).

The acetaldehyde-biogenic amine condensation products have been revealed also to exert non-neuronal effects. MTBC, 6-OH-MTBC, 3-C-MTBC, salsolinol and their derivatives inhibit the growth of various bacteria (Prinsep et al 1991; Ahmad et al 1992) and the platelet aggregation induced by collagen, adrenaline, platelet aggregation factor, arachidonic acid, ADP and thrombin (Given & Longenecker 1987; Tsuchiya et al 1999b). The diversity of susceptible bacterial species and aggregation inducers suggests a non-specific pharmacological mechanism underlying the antibacterial and antiplatelet effects. Several antibacterial and antiplatelet agents alter the properties of bacterial cellular membranes and platelet plasma membranes (Katsu et al 1986; Kitagawa et al 1990). The acetaldehyde-biogenic amine condensation products have been speculated to influence membrane fluidity (Peura et al 1980). In this study, liposomal membranes were treated with MTBC, 6-OH-MTBC, 3-C-MTBC or salsolinol and the membrane effects were evaluated comparatively by measuring fluorescence polarization.

Materials and Methods

Chemicals

MTBC, 6-OH-MTBC and 3-C-MTBC were synthesized as reported previously (Brossi et al 1973; Hayashi et al 1990; Tsuchiya et al 1995). Salsolinol was purchased from Aldrich (Milwaukee, WI). 1-Palmitoyl-2-oleoylphosphatidylcholine (POPC), *N*-phenyl-1-naphthylamine (PNA), 1-anilinonaphthalene-8-sulfonic acid (ANS), 1,6-diphenyl-1,3,5-hexatriene (DPH) and 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene (TMA-DPH) were obtained from Funakoshi (Tokyo, Japan), and cholesterol from Wako (Osaka, Japan). The solutions of POPC and cholesterol (2.8 μmol mL^{-1} of each) were prepared by dissolving them in chloroform of spectroscopic grade (Nacalai Tesque, Kyoto, Japan). The fluorescence probes PNA (1.0 μmol mL^{-1}), ANS (1.0 μmol mL^{-1}) and DPH (0.5 μmol mL^{-1}) were dissolved in acetone of liquid chromatographic grade (Kishida, Osaka Japan), and TMA-DPH (0.5 μmol mL^{-1}) in a mixture of acetone and water (1:1, v/v). Dimethylsulfoxide (DMSO) of spectroscopic grade (Kishida) was used for dissolving the tested chemicals. All other chemicals were of the highest analytical grade available. Water was re-distilled by an all-glass ap-

paratus after purifying by a Milli-RO water purification system (Nihon Millipore, Tokyo, Japan).

Preparation of liposomes

POPC (14.0 μmol) in chloroform or POPC (8.4 μmol) and cholesterol (5.6 μmol) in chloroform was dried and suspended in 100 mL 10 mM Tris–HCl buffer (pH 7.4) containing (mM): KCl 100, dithiothreitol 0.1, EDTA 0.1 and sodium azide 7.5. The suspension was sonicated by a Sonifier 200 (Branson, Danbury, CT) equipped with a titanium microtip at 50 °C for 10 min, and then centrifuged at 100 000 g for 30 min to remove titanium particles and any multilamellar vesicles remaining at the end of sonication. The obtained supernatant was used as the source of liposomes with a bilayer structure, which consisted of POPC, and 40 mol % cholesterol and 60 mol % POPC (Peura et al 1982; Tsuchiya 1999).

Effects on membrane fluidity

MTBC and salsolinol were dissolved in DMSO, and 6-OH-MTBC and 3-C-MTBC in a mixture of DMSO and water (1 : 1, v/v). They were added to the liposomal suspensions to give the final concentrations ranging from 50 nM to 1000 μM, and then incubated at 37°C for 30 min. The DMSO concentration was adjusted to be less than 0.5 % (v/v) of the total volume. This concentration of DMSO, which did not show any influence on membrane fluidity, was added for control samples. PNA, ANS, DPH or TMA-DPH was added to the liposomal suspensions so that each probe concentration was 0.625 or 1.25 nmol mL^{-1} and the acetone concentration was less than 0.125 % (v/v) of the total volume. PNA and ANS were used as indicators for the fluidity changes in hydrophobic and hydrophilic regions of membrane bilayers, respectively (Jacobson & Papahadjopoulos 1976; Shinitzky & Barenholz 1978).

After incubation at 37°C for either 20 min (for TMA-DPH) or 60 min (for PNA, ANS and DPH), fluorescence polarization was measured as reported previously (Prendergast et al 1981; Tsuchiya 1999). The polarization values were calculated by the formula of Katsu et al (1986). Compared with control values, a decrease or an increase of polarization meant enhancement or reduction of membrane fluidity, respectively.

Data analysis

All results are expressed as mean $\pm$ s.e.m. (n = 7–8 for each experiment). The data were analysed by one-way analysis of variance for repeated determinations and paired t-test where appropriate. A value of $P < 0.05$ was considered significant.

Results

At high concentrations (50–1000 μM), the four condensation products acted on the hydrophobic and hydrophilic regions of POPC liposomal membranes and changed fluorescence polarization differentially among their structures, concentrations and the used probes. For PNA polarization (Figure 2A), MTBC (50–100 μM) and salsolinol (50–500 μM) increased the polarization, but conversely above these concentrations polarization decreased. Such biphasic effects indicated that MTBC and salsolinol reduced the fluidity of the inner layers of the membranes at lower concentrations, while fluidizing the inner layers of the membranes at higher concentrations. 6-OH-MTBC reduced membrane fluidity and 3-C-MTBC enhanced with an increase in concentration. For ANS polarization (Figure 2B), MTBC, 6-OH-MTBC and 3-C-MTBC decreased the polarization over micromolar concentrations, indicating fluidization of the outer layers of the membranes. Salsolinol reduced membrane fluidity at 50–100 μM and enhanced it at 250–1000 μM. Using identical concentrations, MTBC changed PNA and ANS polarization more than salsolinol, and its membrane effect was the greatest of the tetrahydro-β-carbolines.

The condensation products at 50–1000 μM also acted on both membrane regions of liposomes composed of 40 mol% cholesterol and 60 mol % POPC. For PNA polarization (Figure 3A), MTBC and 3-C-MTBC decreased the polarization with an increase in concentration, indicating that they fluidized the inner layers of the membranes. However, 6-OH-MTBC reduced membrane fluidity. Salsolinol showed a biphasic effect on the inner layers of the membranes that reduced the fluidity at 50–100 μM and enhanced at 250–1000 μM. For ANS polarization (Figure 3B), all the condensation products concentration-dependently fluidized the outer layers of membranes. At identical concentrations, of the four condensation products MTBC acted most intensively on liposomes containing 40 mol % cholesterol and 60 mol % POPC, as shown by the largest changes in PNA and ANS polarization. The membrane effects were greatest in the order MTBC, 3-C-MTBC, salsolinol and 6-OH-MTBC, similarly to that observed in POPC liposomes.

At low concentrations (50–1000 nM), MTBC changed fluorescence polarization, especially PNA polarization,

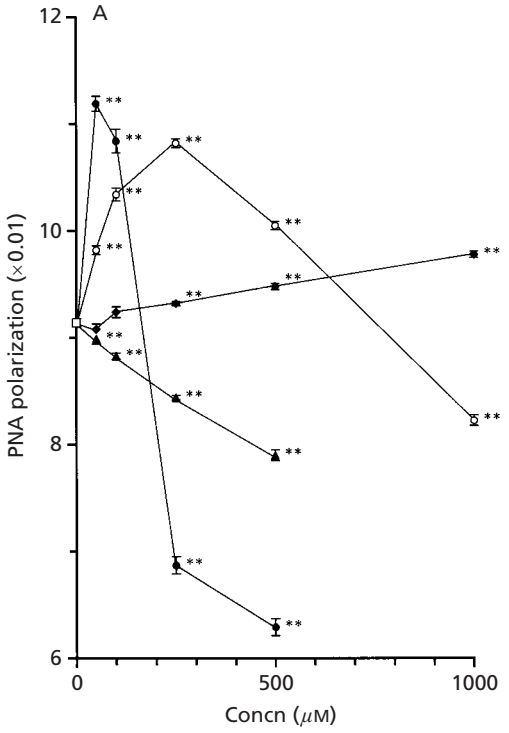

Figure 2 Effects of acetaldehyde-biogenic amine condensation products on PNA polarization (A) and ANS polarization (B) of POPC liposomes. Control (□), MTBC (●), 6-OH-MTBC (◆), 3-C-MTBC (▲) and salsolinol (○). Data are mean ± s.e.m. (n = 8). $^{**}P < 0.01$ compared with control.

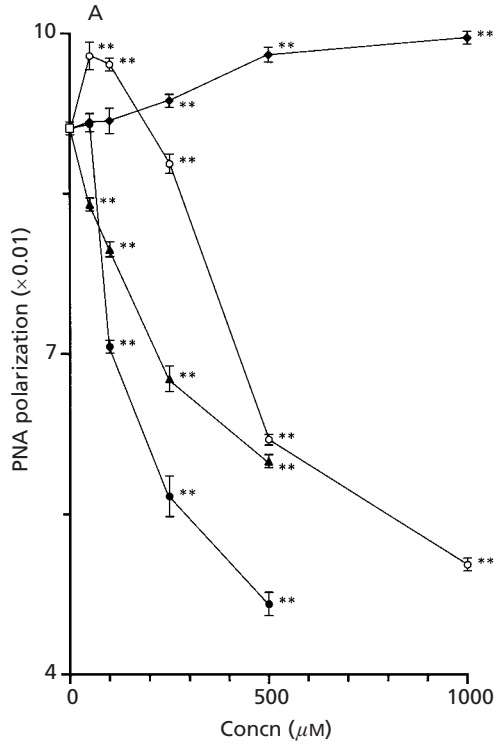

Figure 3 Effects of acetaldehyde-biogenic amine condensation products on PNA polarization (A) and ANS polarization (B) of 40 mol% cholesterol and 60 mol% POPC liposomes. Control (□), MTBC (●), 6-OH-MTBC (◆), 3-C-MTBC (▲) and salsolinol (○). Data are mean ± s.e.m. (n = 8). $^{**}P < 0.01$ compared with control.

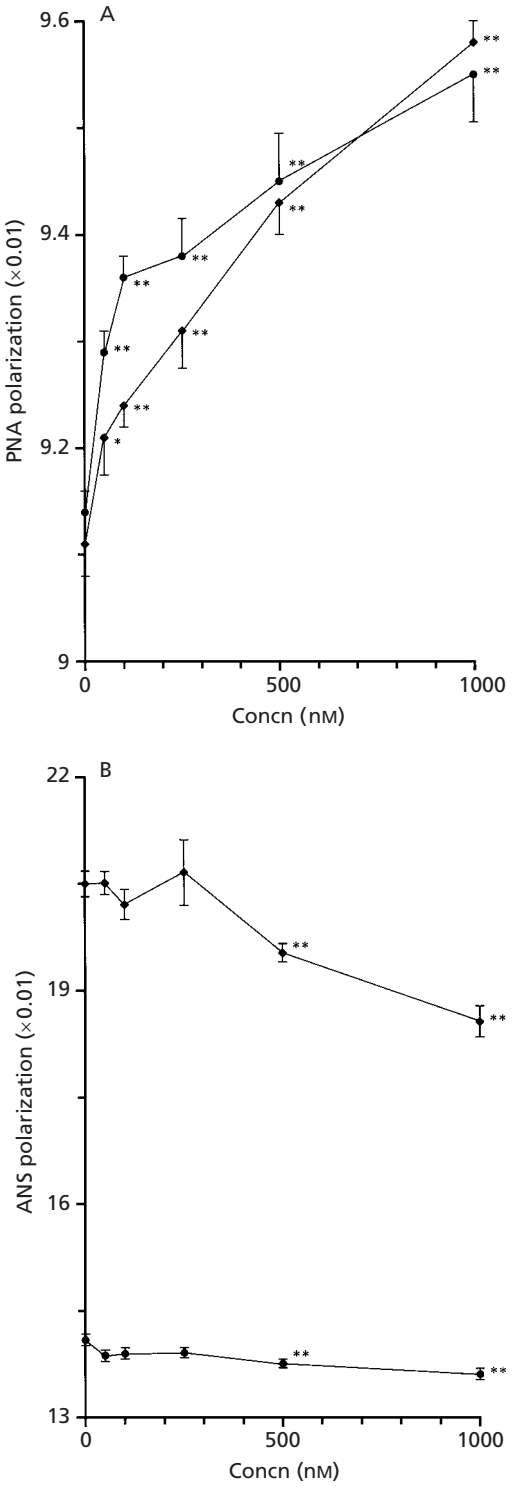

Figure 4 Effects of low concentration MTBC on PNA polarization (A) and ANS polarization (B). POPC liposomes (●) and 40 mol% cholesterol and 60 mol% POPC liposomes (◆). Data are mean ± s.e.m. (n = 8). $*P < 0.05$ and $**P < 0.01$ compared with control.

of POPC liposomes and 40 mol% cholesterol-containing POPC liposomes, although the other condensation products were inactive. The increase of PNA polarization shows that MTBC acted on the inner layers of both liposomal membranes and reduced the fluidity at low nanomolar levels (Figure 4A). MTBC weakly fluidized the outer layers of the membranes to slightly decrease ANS polarization only at 500–1000 nM (Figure 4B).

Although DPH and TMA-DPH have the same fluorescence characters, DPH penetrates into the hydrocarbon region of membrane lipids to indicate a fluidity change of the membrane core and TMA-DPH is anchored at the membrane surface (Prendergast et al 1981). Comparison of polarization changes between DPH and TMA-DPH would indicate the relative acting site in membranes. The ratios of changes in DPH polarization to changes in TMA-DPH polarization were obtained at the concentrations which significantly changed PNA and ANS polarization of POPC and 40 mol% cholesterol-containing POPC liposomes (Table 1). MTBC, 6-OH-MTBC, 3-C-MTBC and salsolinol (250–1000 μM) showed ratios of less than 1.00, indicating that they acted relatively on the membrane surface at high concentration. In 40 mol% cholesterol and 60 mol% POPC liposomes, salsolinol was more effective in changing the fluidity of the membrane surface than MTBC. 6-OH-MTBC, 3-C-MTBC and MTBC acted on the membrane surface in increasing order of intensity. In contrast, MTBC concentrations of 500 nM and less (data not shown) showed ratios larger than 1.00 for both liposomes. At low nanomolar levels, MTBC acted on the membrane core rather than the membrane surface to reduce the fluidity there.

Discussion

Agents fluidizing membranes interfere with bacterial growth and platelet aggregation (Katsu et al 1986; Kitagawa et al 1990). Since the acetaldehyde-biogenic amine condensation products show antibacterial and antiplatelet activities (Given & Longenecker 1987; Prinsep et al 1991; Ahmad et al 1992; Tsuchiya et al 1999b), it is postulated that they may be potent membrane fluidizers.

Peura et al (1982) measured changes in PNA and ANS polarization of liposomes consisting of 1,2-dipalmitoylphosphatidylcholine with varying temperature. They found that MTBC lowered the phase transition temperature of liposomal membranes at 100–

Table 1 Changes in DPH and TMA-DPH polarization by acetaldehyde-biogenic amine condensation products.

Product (concn)	Ratio of DPH polarization change to TMA-DPH polarization change	
	POPC liposome	40 mol% cholesterol and 60 mol% POPC liposome
MTBC (250 μM)	0.178 ± 0.001	0.694 ± 0.002
MTBC (500 nM)	1.135 ± 0.107	2.649 ± 0.424
6-OH-MTBC (1000 μM)	0.631 ± 0.020	0.212 ± 0.013
3-C-MTBC (250 μM)	0.110 ± 0.025	0.291 ± 0.014
Salsolinol (500 μM)	0.376 ± 0.002	0.352 ± 0.007

Values are mean ± s.e.m., n = 7.

1000 μM by fluidizing both the inner and outer layers of the membranes. At almost the same concentration range, MTBC induced membrane fluidization of POPC and 40 mol% cholesterol-containing POPC liposomes in this study. 6-OH-MTBC, 3-C-MTBC and salsolinol also influenced membrane fluidity differentially. The effects of MTBC and salsolinol were found to be biphasic so that they acted on the inner layers of membranes as either a fluidity-reducer or a fluidity-enhancer depending on concentration. All the condensation products enhanced the fluidity of the outer layers of membranes, except for salsolinol at low concentrations on POPC liposomes. The acetaldehyde-biogenic amine condensation products do not appear to be simply non-specific membrane fluidizers like alcohols and anaesthetics. The mechanism for the biphasic membrane effects of MTBC and salsolinol is unclear, while several membrane-acting agents are known to influence membrane fluidity in a biphasic manner as well as did MTBC and salsolinol (Meddings et al 1991; Gallova et al 1995).

The membrane effect of MTBC was the greatest of the tetrahydro-β-carbolines and greater than that of salsolinol. MTBC acted on the membrane core of 40 mol% cholesterol and 60 mol% POPC liposomes more effectively than 6-OH-MTBC, 3-C-MTBC and salsolinol. These differences are interpreted by the presence of additional polar groups in 6-OH-MTBC (6-hydroxyl), 3-C-MTBC (3-carboxyl) and salsolinol (6,7-dihydroxyl) which suppress the interaction with membrane lipids and the penetration into hydrophobic regions by increasing relative hydrophilicity compared with MTBC.

Bacterial membrane phospholipids typically consist of 1-saturated and 2-unsaturated fatty acids, and so the fluidity change found in POPC liposomes is suitable to address the mode of an antibacterial action. Tetrahydro-β-carbolines and their derivatives show the minimum growth inhibitory concentrations of 400–500 μM or more against various bacteria (Ahmad et al 1992). In this concentration range, MTBC and 3-C-MTBC fluidized the inner and outer layers of POPC liposomal membranes, and 6-OH-MTBC enhanced the fluidity of the outer layers of membranes. Tetrahydro-β-carbolines are considered to inhibit the bacterial growth by fluidizing cellular membranes to damage the membrane function.

The antiplatelet mechanism for tetrahydro-β-carbolines and salsolinol would be addressed by their membrane effects on 40 mol% cholesterol and 60 mol% POPC liposomes because platelet plasma membranes contain cholesterol as a lipid component in addition to phospholipids consisting of 1-saturated and 2-unsaturated fatty acids. All the condensation products fluidized the outer layers of membranes over micromolar concentrations. MTBC, 3-C-MTBC and salsolinol also fluidized the inner layers of membranes at 50 μM or more. Tetrahydro-β-carbolines and tetrahydroisoquinolines inhibit platelet aggregation induced by a variety of agents, and the concentrations to produce 50% inhibition are estimated as 25–350 μM for MTBC and 170–300 μM for salsolinol (Youdim & Oppenheim 1981; Given & Longenecker 1987; Tsuchiya et al 1999b). Therefore, membrane fluidization may be partly responsible for the antiplatelet actions of the acetaldehyde-biogenic amine condensation products. However, the 50% inhibition concentrations of MTBC and other condensation products remarkably vary with the type of aggregation inducer (Given & Longenecker 1987; Tsuchiya et al 1999b). Such variation suggests their specific interactions with platelet receptors and enzymes in addition to the membrane effects (Youdim & Oppenheim 1981; Given & Longenecker 1983).

The acetaldehyde-biogenic amine condensation products are naturally present in the human body, and their physiological and pathological significance as mammalian alkaloids has been speculated (Melchior & Collins 1982; Rommelspacher et al 1991). Although 6-OH-MTBC, 3-C-MTBC and salsolinol changed membrane fluidity, they needed non-physiological micromolar levels that were much higher than their in-vivo concentrations. It is unlikely that their membrane effects play any role physiologically. At low nanomolar levels, however, MTBC acted exceptionally on the membrane core and reduced the fluidity. While the natural concentration of MTBC in human tissues and body fluids is presumed to be no more than picomolar to nanomolar

levels (Tsuchiya et al 1995, 1996a), lipophilic MTBC effectively penetrates into the cellular membrane lipids and is possibly concentrated there. The in-vivo concentration of MTBC is significantly elevated after alcohol consumption and in alcoholics by the ingestion of MTBC contained in alcoholic beverages and the potent endogenous production of MTBC (Peura et al 1980; Collins 1988; Tsuchiya et al 1996b). An increase of MTBC associated with alcohol intake may influence the effects of membrane-acting agents such as anaesthetics through the interaction on membrane fluidity.

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