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SHORT COMMUNICATION

Evidence of a Direct Action of Taurine and Calcium on Biological Membranes

A COMBINED STUDY OF ^{31}P-NUCLEAR MAGNETIC RESONANCE AND ELECTRON SPIN RESONANCE

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ABSTRACT. To determine the actions of taurine and calcium on biological membranes, the effects of these compounds on the mobility of phospholipids of resealed and sonicated ghosts of human erythrocytes were investigated, using ^{31}P-NMR spectroscopy. In addition, the effects of taurine and calcium on lipid fluidity were investigated by ESR spectroscopy, using a spin-labeling method with 5-doxyl stearic acid. The mobility of the membranes decreased following treatment with 10 mM taurine, but coadministration of 2.5 mM calcium blocked this effect. The fluidity of the membranes was not changed following treatment with 10 mM taurine, but decreased following coadministration of 2.5 mM calcium. These actions of taurine and calcium on the dynamics of biological membranes might explain, in part, the observation that most of the pharmacological effects of taurine on mammalian organs occur in the presence of calcium ions. BIOCHEM PHARMACOL 52;1:173–176, 1996.

KEY WORDS. taurine; calcium; fluidity; erythrocyte ghost; ^{31}P-NMR; ESR

Taurine (2-aminoethanesulfonic acid), a sulfur-containing amino acid, is present in a number of tissues and has been used clinically as a therapeutic agent in cardiovascular diseases such as heart failure and arrythmia [1], and in liver diseases such as acute hepatitis and cholestasis [2]. There have been many reports indicating that the pharmacological effects of taurine closely relate to the modulation of the transport of calcium ions through cell membranes [3–5] or the binding of calcium ions to membranes [6, 7].

In the biomembrane, cholesterol and protein coexist in the phospholipid bilayer, and the phospholipids in membranes move from side to side, as described in the fluid mosaic model of Singer and Nicolson [8]. Since enzymes and calcium channels in membranes are surrounded by phospholipids, the functions of enzymes and calcium channels may be affected by changes in the dynamics of membrane phospholipids [9]. The mobility of ^{31}P at the polar head group of phospholipids can be analyzed using ^{31}P-NMR spectroscopy [10]. Furthermore, the spin-label method of ESR is a technique that permits the investigation of lipid fluidity at a fixed depth of the membrane phospholipid bilayer [11].

In this study using NMR and ESR, it was demonstrated that the direct effects of taurine on membranes were modified in the presence of calcium ions.

MATERIALS AND METHODS

Materials

Taurine was obtained from the Taisho Seiyaku Co. (Tokyo, Japan). 5-DSA§ was purchased from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). The other chemicals used were commercially available and of guaranteed grade.

Preparation of Erythrocyte Ghosts

The erythrocyte ghosts were prepared from stored human erythrocytes by the method of Dodge *et al.* [12]. The erythrocyte ghosts obtained were resealed in isotonic buffer (154 M NaCl containing 5 mM Tris–HCl buffer, pH 7.4) and then sonicated at 0°, 100 W for 90 sec using an ultrasound sonicator (Kubota, Insonator 200M, Japan). Following ultracentrifugation at 100,000 *g* for 60 min, the precipitates (vesicles) were suspended in the isotonic buffer mentioned above and incubated at 37° for 10 min after the addition of 10 mM taurine and/or 2.5 mM $CaCl_2$.

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§ *Abbreviations:* 5-DSA, 5-doxyl stearic acid; PC, phosphatidylcholine; SM, sphingomyelin; PE, phosphatidylethanolamine; PS, phsophatidylserine; and PI, phosphatidylinositol.

^{31}P-NMR Measurement

The vesicles treated with taurine and/or calcium were packed in glass NMR tubes and analyzed using a JNM-PFT-100 NMR spectrometer (JEOL, Japan). The measurement conditions were: static magnetic field, 24,000 G; observation frequency, 40.29 MHz; spectral range, 5 KHz; data points, 4096; repetition interval of 90° pulse, 0.55 sec; and temperature, 24°. Proton noise decoupling was used, and 85% H_3PO_4 was employed as an external standard for chemical shift. Magnetic lock was done using a glass capillary tube (1 mm ϕ) containing deuterium.

ESR Measurement

The vesicles treated with taurine and/or calcium were further spin-labeled by incubating with 5-DSA for 15 min in isotonic buffer. The precipitates obtained after centrifugation were transferred to a capillary tube for analysis with an ESR spectrometer (FE2XG, JEOL, Japan). The measurement conditions were: microwave power, 4 mW; sweep width, 50 G; sweep time, 2 min; modulation amplitude, 2.5 G; time constant, 0.03 sec; and temperature, 24°. The order parameter (S) was calculated according to the equation of Gaffney [11] as follows: $S = (T_{\parallel} - T_{\perp} - C)/(T_{\parallel} + 2T_{\perp} + 2C) \times 1.723$, $C = 1.4G - 0.053\ (T_{\parallel} - T_{\perp})$, where $T_{\parallel}$ is the outer hyperfine splitting when the external magnetic field is applied along the main axis of the probe molecule, and $T_{\perp}$ is the inner hyperfine splitting that corresponds to the membrane area where the static magnetic field is perpendicular to the main axis.

Statistical Analysis

Results are expressed as the means ± SEM of N experiments. Statistical analysis was performed by ANOVA. Differences were accepted as statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

The vesicles of resealed and sonicated erythrocyte ghosts revealed a ^{31}P-NMR spectrum that showed two absorption lines (Fig. 1). The line in the higher magnetic field (+10.4 ppm) comes from the phospholipid bilayer arranged in order, and the line in the lower magnetic field (+0.6 ppm) comes from the phospholipids in the hexagonal phase or the phases where isotropic motion occurs [13]. The absorption lines were assigned by the chemical shift of pure phospholipids found in human erythrocyte membrane as follows: the absorption line in the higher field was due to PC (+10.9 ppm) and SM (+12.1 ppm), and the absorption line in the lower field was due to PE (0 ppm) and PS (+0.3 ppm). The area under the absorption lines was in proportion to the numbers of these phospholipids. In ^{31}P-NMR linewidth data, the broadening of linewidth indicates a decrease in mobility, and the narrowing of linewidth indicates an increase in mobility.

Treatment with taurine broadened the linewidth of the spectrum in the higher magnetic field (Figs. 1 and 2), which indicated that taurine decreased the mobility of PC and SM, particularly where the phospholipids are arranged in order. On the other hand, calcium at 2.5 mM had no effect on mobility. However, the broadening of the absorption line was observed in the lower magnetic field at a calcium concentration of more than 5 mM (data not shown). This finding agrees with the previous observation that calcium at high concentrations directly facilitates the formation of the hexagonal phase in the ghost membrane [13]. It is also noteworthy that a change in the linewidth of taurine-treated ghosts was not observed in the presence of calcium.

Huxtable [14] has proposed that taurine acts as a membrane expander and alters the ion exchange qualities of the membrane owing to its dipolar effect, and that taurine

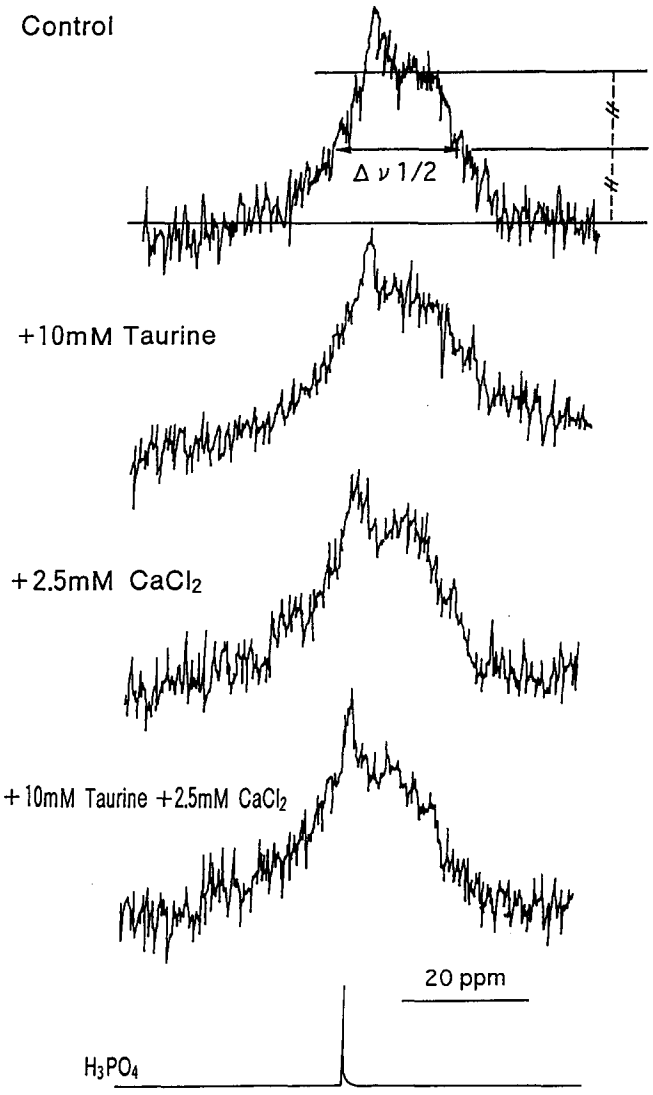

FIG. 1. ^{31}P-NMR spectra of human erythrocyte ghosts treated with taurine and/or calcium. $\Delta\nu$½ indicates the linewidth of half-height.

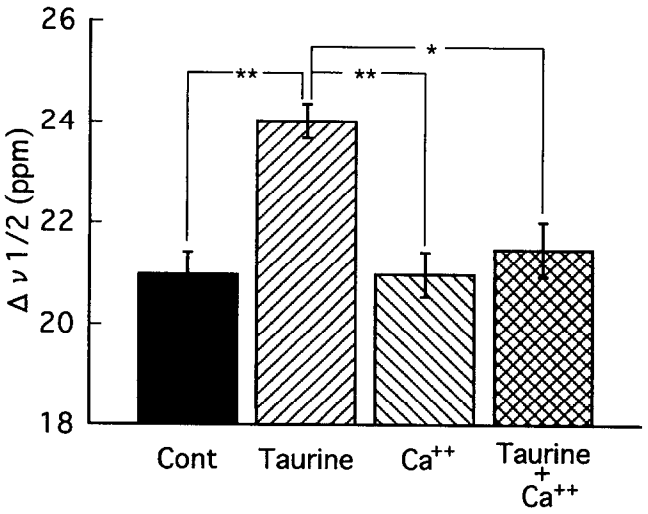

FIG. 2. Effects of taurine and/or calcium on linewidths of ^{31}P-NMR spectra of human erythrocyte ghosts. The linewidth at half-height ($\Delta\nu 1/2$) was measured at each ^{31}P-NMR spectrum, as shown in Fig. 1. Values are means ± SEM, N = 4 each. Key: (*) $P < 0.05$, and (**) $P < 0.01$.

might "dissolve" in the phospholipid moiety of membranes by virtue of ion–ion interaction with the zwitterionic charged portion of the phospholipids, that is, the structural resemblance of taurine to the bipolar ion group of phospholipids. Shindo and Huxtable [15] have also studied taurine action on artificial membrane vesicles composed of PC and PS with Eu^{3+} as a paramagnetic analog of Ca^{2+} by using ^{31}P-NMR, which indicates that taurine acts as an inhibitor of Eu^{3+} binding to PC and PS membrane. Furthermore, it has been revealed previously that taurine binds substantially to the neutral phospholipid, PC [16]; that calcium binds substantially to the acidic phospholipids, PS and PI [17]; and that the binding of taurine to neutral phospholipids modifies the binding of calcium to adjacent acidic phospholipids, and vice versa [18]. These findings may explain the result found in our present study, that there is an interaction of taurine with calcium on the mobility of the membranes.

Figure 3 shows the ESR spectrum of the ghosts labeled with 5-DSA. The values for outer ($2T_{\parallel}$) and inner ($2T_{\perp}$) hyperfine splitting of 5-DSA were calculated. In the ESR spin-labeling method, an increase in the order parameter (S) indicates a decrease in fluidity, and a decrease in S indicates an increase in fluidity.

Treatment with taurine or calcium alone had no definite effect on the fluidity of membranes, whereas treatment with taurine in the presence of calcium decreased S (increased the fluidity) significantly (Fig. 4). These findings clearly suggest that an interaction of taurine with calcium exists on the lipid fluidity of the membranes. There have been a few reports concerning the effects of taurine on membrane fluidity. In our previous ESR study using hepatic microsomal membranes [19], lipid fluidity was increased slightly by the treatment of taurine. Chovan *et al.* [6] did not find any changes in ESR spectra of rat heart sarcolemma following

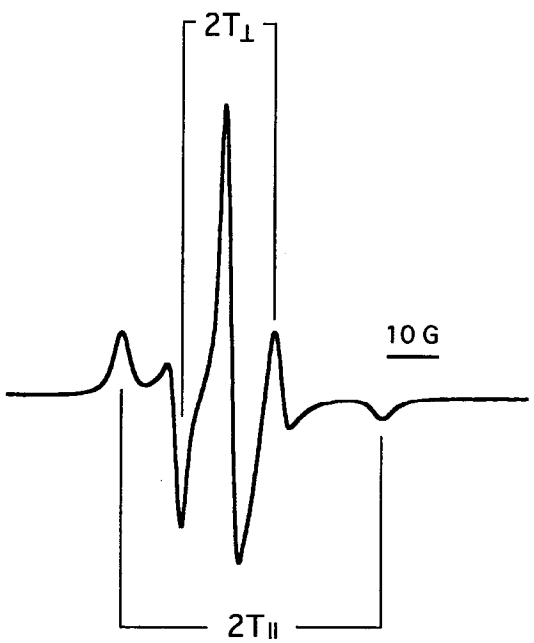

FIG. 3. ESR spectrum of human erythrocyte ghosts labeled with 5-DSA. The outer ($T_{\parallel}$) and inner ($T_{\perp}$) hyperfine splittings of the spectra were determined.

treatment with taurine. However, the calcium contents of samples were not determined in these experiments. On the other hand, it has been suggested that fluidity is influenced during the influx of calcium through the membranes [20]. Although it is possible that taurine may modify the transport of calcium, the exact mechanism of the interaction between taurine and calcium on fluidity remains to be elucidated.

The result of the present study revealed that taurine alone decreased the mobility of the polar head group of phospholipids but had no effect on lipid fluidity. However, the coadministration of calcium eliminated the effect of

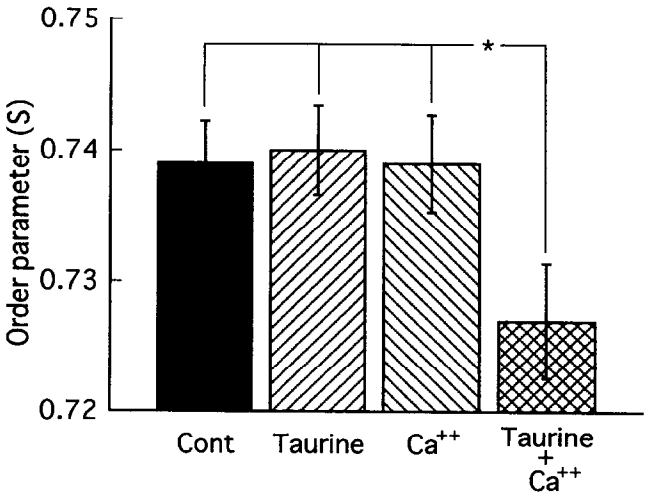

FIG. 4. Effects of taurine and/or calcium on the order parameter (S) of 5-DSA in human erythrocyte ghosts. Values are means ± SEM, N = 4 each. Key: (*) $P < 0.05$.

taurine on mobility and increased fluidity. Although the present data positively support the hypothesis of Huxtable [14, 18], it is not clear whether there is a relationship between the mobility estimated by ^{31}P-NMR and the fluidity estimated by ESR, and how the changes in the dynamics of membranes influence the taurine/calcium transport/binding of the cell membranes. Furthermore, it is possible that taurine affects the membranes by interaction with specific proteins [21].

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