Effects of Taurine on Depletion of Erythrocyte Membrane Na-K ATPase Activity Due to Ozone Exposure or Cholesterol Enrichment

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Summary The objective of this study was to investigate the inter-relationship between taurine and erythrocyte-membrane Na-K ATPase activity. A comparison was conducted to test whether taurine or uric acid (a water-soluble scavenger of free radicals) prevents or recovers the depletion in membrane ouabain-sensitive Na-K ATPase activity resulting from ozone exposure or cholesterol enrichment of the erythrocyte membrane. A depletion of 44% and 27% in ouabain-sensitive Na-K ATPase activity was respectively caused by ozone exposure and cholesterol enrichment. Taurine as well as uric acid partially prevented the activity loss from ozone exposure. In addition, taurine at high concentrations (from 1.5 to 4.5 mM) restored the depletion of erythrocyte-membrane Na-K ATPase activity due to ozone exposure and prevented the depletion of the enzyme activity due to cholesterol enrichment. In contrast, although the same high concentrations were used, uric acid failed to show either of the above effects. These results suggest that taurine acts (1.5–4.5 mM) polyvalently as not only an antioxidizing agent but also as a membrane stabilizer to maintain the functions of membrane Na-K ATPase, a membrane-bound protein.

Key Words taurine, lipid peroxidation, erythrocyte, ozone, cholesterol, uric acid, Na-K ATPase

Na-K ATPase, which is an accessory factor in the pathogenesis of cardiovascular diseases (1), plays an important role in cellular function. Many in vivo and in vitro studies have indicated that suppression of Na-K ATPase may result from lipid peroxidation caused by free radical chain reactions (2–4). Cholesterol, a necessary and abundant sterol in mammalian cell membranes, helps to determine membrane fluidity. However, the excess incorporation of membrane cholesterol has been
shown to result in the alteration of cholesterol-to-phospholipid molar ratios, which influences the biochemical properties of various integral membrane proteins (1). Enrichment of the membrane with cholesterol has been reported to inhibit the ATPase activity derived from membranes or membrane fragments of kidney, muscle, brain and erythrocyte (2, 5, 6). Although some people suggest that this inhibition is related to peroxidation (6), precise molecular mechanisms of the injury to Na-K ATPase are not clear.

In recent years, there has been excitement about a naturally occurring molecule present in most tissues of most species, 2-aminoethanesulfonic acid (taurine). Taurine, the most abundant free amino acid in mammals and, typically, present in the millimolar cellular concentration range, has a fundamental role in processes relating to the maintenance of cellular functions (7). Taurine has been shown to be an essential nutrient. A deficiency of taurine in the diet results in retinal and tapetal degeneration, cardiac abnormalities, a compromised immune function, and greatly increased reproductive wastage (8). Taurine has been shown to lower blood pressure in deoxycorticosterone acetate-salt induced hypertension and in the spontaneously hypertensive rat (9, 10). Furthermore, evidence for a role of taurine in membrane function and stabilization has greatly increased the potential importance of dietary taurine. It is said that most actions of taurine appear to be membrane-based (7) because it has detoxifying, antioxidant, and membrane-stabilizing properties apparently due to its molecular structure. The present study was, therefore, designed with the objectives of determining whether taurine prevents or restores the suppression of erythrocyte membrane Na-K ATPase activity resulting from in vitro ozone exposure or cholesterol enrichment of the membrane. Furthermore, the effects of taurine and an other antioxidizing agent, uric acid, were also compared in this study.

MATERIALS AND METHODS

All chemicals used were of the highest purity available. Ouabain, cholesterol, uric acid and taurine were obtained from Wako Pure Chemical Industries Ltd., Japan. L-α-Phosphatidylcholine dipalmitoyl (C16:0) and bovine serum albumin were purchased from Sigma Chemical Co., USA.

Erythrocyte preparation (11). Blood was drawn into each heparinized tube, from 15 healthy adults (male volunteers). The blood was centrifuged at 2,500 × g for 10 min at 4°C. The plasma and buffy coat were removed by aspiration, and the erythrocytes were washed three times with cold isotonic 10 mM Tris-MOPS buffer containing 75 mM MgCl₂·6H₂O, 85 mM sucrose and 10 mM glucose. For the following experiments, the cells were suspended in Hank’s balanced salt solution.

Preparation of cholesterol-rich sonicated emulsions (12). Sonicated mixtures were prepared from cholesterol, L-α-dipalmitoyl lecithin and albumin. Eighty milligrams of pure cholesterol and 40 mg of L-α-dipalmitoyl lecithin were added to 10 ml of 0.155 M NaCl in a fluted metal container surrounded by an ice slurry and
sonicated at 75 watts for 60 min in a metal container in ice (Tomy Seiko Co. Ltd.,
Tokyo). Human serum albumin (4 ml) was then added at a final concentration of
2%. The mixture was centrifuged at 21,800 × g for 30 min to sediment undispersed
lipids.

Incubation of erythrocytes with cholesterol. Cholesterol-rich sonicated mixture
was added to 2.0 mM and incubated with a 4-ml suspension of erythrocytes
containing either taurine or uric acid at various concentrations at 37°C for 12 h.
After the cells were washed with 10 mM Tris-MOPS (pH 7.4) containing 75 mM
MgCl₂·6H₂O, 85 mM sucrose, the preparation of membrane fragments was carried
out according to the procedure of Dodge et al. (11).

Ozone treatment. Ozone was generated by passing an oxygen stream (50
ml/min) through a high-voltage electric ozonizer (Sibata Corporation, Tokyo)
equipped with an ozone determinator (TOXITEC, Gastec Corporation, Tokyo)
which indicated the level of ozone production. The delivery was modulated with a
balanced bottle. For treatment of each sample, ozone was passed into samples (5
ml/tube) at 0.5 ppm for 5 min. Caprylic alcohol (5 µl per tube) was also added as
an antifoaming agent.

Preventive experiment. After incubation of the erythrocytes with uric acid or
taurine for 30 min with various concentrations at 37°C, the above ozone treatment
or cholesterol enrichment was carried out. The final concentration ranges of uric
acid and taurine were 0.06 to 1.0 mM and 0.06 to 0.24 mM, respectively.

Recovery experiment. Either taurine or uric acid at 37°C for 40 min was
incubated from 0.3 mM to 4.5 mM, respectively, with a 1-ml suspension of erythro-
cyte-membrane fragment (3.0 mg protein/ml) which had been treated with ozone
exposure or cholesterol enrichment, and the membrane Na-K ATPase activity
assay was performed (13).

Analysis. The ouabain-sensitive Na-K ATPase assay was carried out accord-
ing to Chan et al's method (2). The measurement of cholesterol and lipid phospho-
rus was done according to Zlatkis et al. (14) and Bartlett (15), respectively. The
membrane proteins were determined by Lowry et al's method (16) with bovine
serum albumin (BSA) as a standard. Statistical significance was determined by
two-way analysis of variance (F-test) or Student's t-test for paired/unpaired data
where appropriate.

RESULTS

The effects of taurine and uric acid as antioxidizing agents were investigated.
Figures 1 and 2 show that erythrocyte membrane Na-K ATPase activity was
abolished by ozone exposure (0.5 ppm over 4 min). When taurine or uric acid were
added to the erythrocyte suspension prior to ozone exposure, taurine as well as uric
acid significantly prevented a loss of erythrocyte-membrane Na-K ATPase activity.
The maximal preventive effects were achieved by taurine at 0.12 mM (from 56%
without taurine to 74% with), and the uric acid at 0.5 mM (from 56% without uric
Fig. 1. Effect of taurine on inactivation of erythrocyte-membrane ouabain-sensitive Na-K ATPase by ozone exposure.

Fig. 2. Effect of uric acid on inactivation of erythrocyte-membrane ouabain-sensitive Na-K ATPase by ozone exposure (Mean±SD).

The effects of taurine and uric acid in similar concentrations on the cholesterol enrichment are illustrated in Fig. 3. Taurine (0.06 to 0.24 mM) or uric acid (0.06 to 0.83% with).

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Fig. 3. Effect of taurine and uric acid on loss of Na-K ATPase activity induced by cholesterol enrichment in erythrocyte membranes.

to 0.24 mM) were incubated with erythrocyte suspensions before cholesterol enrichment. After incubation with cholesterol for 12 h, erythrocyte membrane ouabain-sensitive Na-K ATPase activity was decreased by about 27% compared with the control. Under this circumstance, incorporation of cholesterol was observed from 0.6 to 0.98 mg/mg protein of the erythrocyte membrane and the ratio of cholesterol to phospholipid (C/P) was increased from 0.50 to 1.63. The results (Fig. 3) demonstrated that either taurine or uric acid did not prevent depletion of the membrane Na-K ATPase activity.

Taurine at high concentrations (from 1.5 to 4.5 mM) were incubated with erythrocyte membranes before cholesterol enrichment in order to determine whether it prevented the inhibition of Na-K ATPase by cholesterol. The results show (Fig. 3) that the depletion of erythrocyte membrane Na-K ATPase activity due to cholesterol enrichment was prevented (74 to 87%). In contrast, uric acid at high concentrations (from 1.5 to 4.5 mM) failed to protect ATPase activity.

The effects of taurine or uric acid on the depletion of erythrocyte membrane Na-K ATPase activity after exposure to ozone or cholesterol enrichment are shown in Table 1. Taurine at 1.5–4.5 mM restored the depletion of membrane Na-K ATPase activity to 62.5–71.1%, compared with the control (55.6%) of ozone exposure, and to 78.6–88.7%, compared with the control (73.2%) of cholesterol enrichment, respectively.

Uric acid at 0.3–4.5 mM, however, did not show any restorative actions for ozone exposure (from 57.7 to 52.9%) or for cholesterol enrichment (from 73.5 to 70.2%).
Table 1. Recovery due to taurine and uric acid on depletion of Na-K ATPase activity caused by ozone exposure and cholesterol enrichment in membranes.

| Chemical          | After ozone exposure | % Control Na-K ATPase activity | After cholesterol enrichment | % Control Na-K ATPase activity |
|-------------------|----------------------|-------------------------------|-----------------------------|--------------------------------|
| None              | 0.187 ± 0.03         | 100                           | 0.178 ± 0.0                 | 100                            |
| Ozone             | 0.104 ± 0.02         | 55.6                          | —                           | —                              |
| Cholesterol       | —                    | —                             | 0.130 ± 0.02                | 73.2                           |
| Taurine 0.3 mM    | 0.108 ± 0.02         | 57.7                          | 0.135 ± 0.02                | 75.8                           |
| Taurine 1.5 mM    | 0.117 ± 0.01         | 62.5                          | 0.140 ± 0.02                | 78.6                           |
| Taurine 4.5 mM    | 0.133 ± 0.02         | 71.1                          | 0.158 ± 0.01                | 88.7                           |
| Uric acid 0.3 mM  | 0.108 ± 0.01         | 57.7                          | 0.131 ± 0.01                | 73.5                           |
| Uric acid 1.5 mM  | 0.103 ± 0.01         | 55.0                          | 0.131 ± 0.02                | 73.5                           |
| Uric acid 4.5 mM  | 0.099 ± 0.03         | 52.9                          | 0.125 ± 0.01                | 70.2                           |

DISCUSSION

Unlike other amino acids, taurine, (2-aminoethanesulfonic acid NH₂-CH₂-CH₂-SO₃H), is abundant in free form. This chemical property may be critical for its biological function, that is, protecting membranes by attenuating toxic compounds and oxidants (17).

In the present study, taurine at lower concentrations, approximating its physiological concentration in human serum, show the same preventive action as uric acid did, i.e., preventing depletion of the erythrocyte membrane Na-K ATPase activity from ozone exposure. Uric acid as a scavenger may play a role as an antioxidizing agent in plasma by maintaining Fe²⁺ in the reduced state (18,19). It has also been suggested that the sulfonic acid group of taurine could bind or complex to free metal ion species (Fe²⁺, Cu²⁺) or to oxidant metalloproteins, and react with by-products of oxidation reactions, thereby preventing a direct attack by oxidants on cell membranes (20).

On the other hand, taurine at high concentrations, but not uric acid, restored depletion of the erythrocyte membrane Na-K ATPase activity after ozone exposure or cholesterol enrichment. Cholesterol was reported to increase lipid peroxidation in cell membranes and to decrease membrane Na-K ATPase activity after a high cholesterol diet in experiments using feeding rats (6). *In vitro* experiments (12), including our results, have shown that the direct enrichment of cholesterol in membranes also depletes membrane Na-K ATPase activity. However, it is questionable whether *in vivo* experiments can be used to explain *in vivo* phenomena. Our results demonstrate that uric acid, even at high concentrations, does not prevent the activity loss from the *in vitro* cholesterol enrichment. This study implies that
taurine may act by a different mechanism than uric acid. Some researches have reported that cholesterol, when incorporated into membranes in excess, can substantially alter the physical state of the bilayer lipid as well as the biochemical properties of various integral membrane proteins (21) and a normal environment of phospholipids is important for maintaining Na-K ATPase activity. Taurine is capable of slowing the phospholipase C-induced reduction in ATPase (13). Two relevant aspects of the (zwitterionic) structure of taurine are an electronic similarity to the neutral membrane phospholipids, phosphatidylcholine and phosphatidylethanolamine (7). Taurine by intercalating into plasma membranes, could thereby stabilize the lipid component and prevent lipid peroxidation (22). These observations suggest that taurine may not only prevent lipid peroxidation, as does uric acid, but it may also stabilize the environment of the membrane for maintaining Na-K ATPase activity.

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