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Taurine: a preventive agent of the acute ethanol depletive action on the isolated human amniotic membrane

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Summary. The preventive effect of taurine towards the acute ethanol reduction action was studied on the ionic transfer through the isolated human amniotic membrane. Taurine increased 3 components of the ionic transfer expressed by the conductance measurements (Na^+ and K^+ paracellular conductances through the intercellular spaces and coupling cell factor between 2 adjacent epithelial cells, expressed by a voltage ratio). These components were decreased by ethanol. Electrophysiological studies (conductance and voltage measurements) indicated that the addition of taurine (0.1–1 mM) before ethanol (0.4 g/l) hindered the decrease action of ethanol on the Na^+ and K^+ paracellular conductances and on the coupling cell factor. These data indicated a common target between taurine and ethanol: the membraneous phospholipids, particularly the distribution of the external fixed charges. The preventive action of taurine *versus* ethanol, on the human amniotic membrane, was exerted on the polar groups of phospholipids, hindering the incorporation of ethanol molecules.

Keywords: Amino acids – Amniotic membrane – Ethanol – Potassium – Sodium – Taurine – Transfer

Introduction

Taurine, 2-aminoethanesulfonic acid, is a β amino acid which is present in many tissues of man and other animal species in millimolar concentrations. Humans have a limited capacity for taurine biosynthesis (Jacobsen et al., 1964) and are generally dependent on dietary supplementation (Sturman and Gaull, 1985). Accordingly, taurine may be considered as a conditionally essential amino acid during gestation, and an exogenous source which would be particularly important during periods of rapid growth such as fetal development.

Moreover during gestation, the transfer between mother and fetus is regulated by the placenta and also, by the amniotic membrane. Ionic transfer

through the human amniotic membrane, a leaky epithelium, is regulated by transcellular and shunt paracellular pathways (Guiet-Bara and Bara, 1984). Previous *in vitro* studies (Bara et al., 1990) have indicated that taurine supplementation, in physiological medium, increases ionic transfer through Na and K channels, electrical coupling cell factor between two adjacent cells obtained from recorded voltage ratio, Na and K paracellular pathways in the intercellular spaces.

Alcohol ingestion, during gestation, can have a deleterious effect on fetal development in both humans and animals. Chronic *in utero* esposure to ethanol can produce malformations, growth deficiency and central nervous abnormalities. The cluster of particular facial features, growth deficiency and mental retardation observed in children of some chronic alcoholic mothers has been referred to as the fetal alcohol syndrome (Jones and Smith, 1975). For example, the impairment of placental amino acid transport is one of the causes of the intrauterine growth retardation (Asai et al., 1985).

Previous *in vitro* studies (Guiet-Bara et al., 1985) have indicated that the ion transfer through the paraplacental amniotic membrane is considerably reduced after ethanol addition to the physiological survival fluids. Moreover, ethanol interacts particularly with coupling cell factor, ATPase component, Na/H antiport and Na^+, K^+ paracellular components (Guiet-Bara, 1990).

The relations between alcoholism and taurine are well established: the stress of the alcoholic weaning increases the loss of taurine (Durlach, 1988). Furthermore, taurine is considered to be an antagonist of ethanol (Mac Broom et al., 1986, Streissguth et al., 1980; Watanabe et al., 1985) and levels of taurine are increased by acute intraperitoneal administration of ethanol in rat brain nucleus accumbens (Dahchour et al., 1994). On the amniotic membrane, a relationship has been observed between taurine and ethanol: taurine exerts a preventive action *versus* decreasing effect of ethanol on the total ionic transfer (Guiet-Bara et al., 1987, 1988).

The aim of this work is to identify the cellular and paracellular targets of the preventive effect of taurine *versus* ethanol on the isolated human amniotic membrane.

Material and methods

Tissue sampling

Strips of human amnion (6–8 pieces from each placenta), isolated from the placental zone of the amniotic sac, were obtained after 8 normal deliveries at term and transferred into Hanks' solution at 37 ± 1°C and pH 7.4. Each sample of amnion (1 cm^2) was set up horizontally between two Lucite chambers according to Bara, Guiet-Bara and Durlach's device (1985).

Electrophysioplogical measurements

The study had been realized on the common ionic transfer components increased by taurine and decreased by ethanol: Na^+ and K^+ paracellular pathways and coupling cell factor.

The cellular conductance (Gc) in the maternal-to-fetal and in the fetal-to-maternal directions was measured by injecting a constant current, square-wave pulse through a double-barelled microelectrode and analyzing the recorded transient voltage.

The coupling cell factor was estimated by the ratio V2/V1: recorded transient voltage in cell 1 (V1) and in cell 2 (V2) after current injection in cell 1.

The paracellular conductance was estimated by: Gp = Gt − Gc. Gt, transamniotic conductance, was measured by observing the transepithelial potential difference when a direct current (100 μA) was passed across the whole tissue. The potential was recorded with two agar-agar salt bridges placed 1.3–1.5 mm from each side of the tissue, while an electrical current was passed across the tissue by means of Ag/AgCl electrodes and agar-agar salt bridges and was measured on a Schlumberger electrometer. Na and K paracellular conductances were analyzed using specific inhibitors, respectively triaminopyrimidinium (10 mM) (Moreno, 1974) and protamines (100 mg/litre) (Palade, 1987; Bara and Guiet-Bara, 1994).

Solution and chemicals

The Hanks' solution used contained (mM/l): NaCl 150, KCl 6, $MgSO_4$ 0.5, $MgCl_2$ 0.5, $CaCl_2$ 1, glucose 5.5 and was buffered to pH 7.4 with 1 mM NaH_2PO_4-KH_2PO_4-$NaHCO_3$.

Taurine (Sigma) (0.1, 0.5 and 1 mM) and ethanol (0.4 g/l) were added either on the maternal side (MS) or on the fetal side (FS).

Measurement of the preventive action of taurine "versus" ethanol

The ionic conductance was firstly measured in the Hanks' solution only. Then 0.4 g/l ethanol was added in the medium and the percentage of conductance decrease was calculated (control value). After rinsing and a new measure of the conductance in Hanks' solution only, 0.1, 0.5 or 1 mM of taurine was added on the FS or on the MS, before the addition of ethanol at a concentration equal to 0.4 g/l.

The percentage of conductance decrease was calculated from the relation:

$$G_0 - G_1 \times 100/G_0$$

With G_0: conductance in the Hanks' solution only

G_1: conductance in the Hanks' solution + Taurine + ethanol (in the case of the control, G_1 = conductance in the Hanks' solution + ethanol only).

In the case of the coupling cell factor, the protocol of measurement was the same, the conductance value G was replaced by V2/V1.

Statistical analysis

All the data presented were provided as means ± S.D. The data statistical processing was made with the one-way analysis of variance test (ANOVA) and the Tukey-Kramer multiple comparisons test (p being the significance level).

Results

1. Preventive action of taurine on K^+ paracellular pathway

In Hanks' solution only, ethanol (0.4 g/l) decreased significantly ($p < 0.001$) the K^+ paracellular conductance (GpK) on the FS (36.3 ± 3.8 %) and on the MS (48.1 ± 4.5%). When taurine (Fig. 1) was added on the MS or on the FS of the amniotic membrane, before ethanol, the percentage of GpK decrease

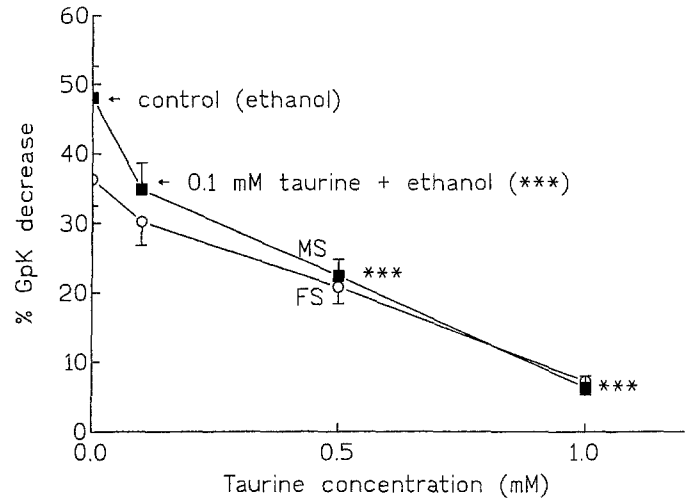

Fig. 1. Effect of increasing taurine concentration on the percentage of paracellular potassium conductance (*GpK*) decrease (taurine was added before ethanol) (control: % GpK decrease with ethanol only, point 1: % GpK decrease with 0.1 mM taurine + ethanol) (*MS* maternal side, *FS* fetal side) (***: $p < 0.001$)

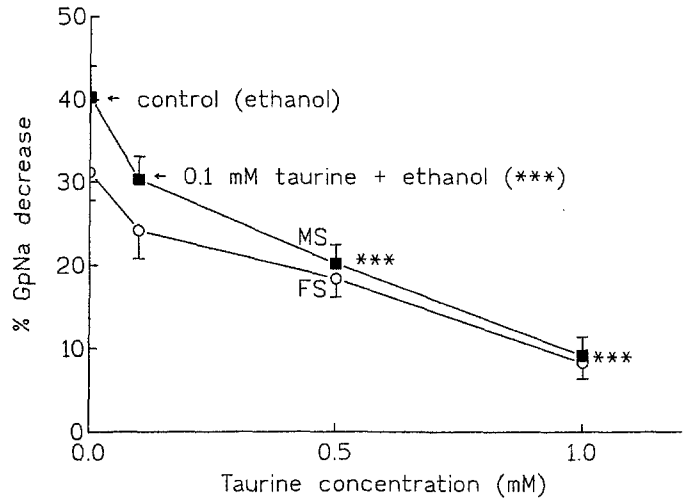

Fig. 2. Effect of increasing taurine concentration on the percentage of paracellular sodium conductance (*GpNa*) decrease (taurine was added before ethanol) (control: % GpNa decrease with ethanol only, point 1: % GpNa decrease with 0.1 mM taurine + ethanol) (***: $p < 0.001$)

was reduced significantly ($p < 0.001$). When 1 mM taurine was added, ethanol had scarcely no effect and the action was more important on the MS than on the FS.

2. *Preventive action of taurine on Na^+ paracellular pathway*

In Hanks' solution only, ethanol (0.4 g/l) decreased significantly ($p < 0.001$) the Na^+ paracellular conductance (GpNa) on the FS (31.2 ± 3.4%) and on the MS (40.3 ± 3.8%). When taurine (Fig. 2) was added on the MS or on the FS

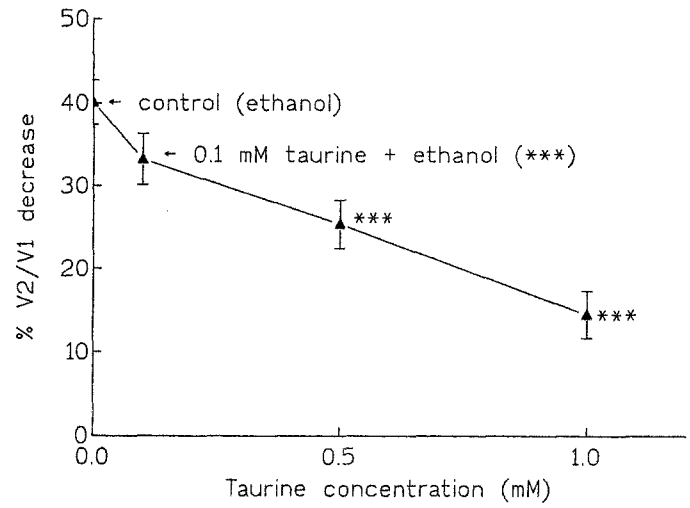

Fig. 3. Effect of increasing taurine concentration on the percentage of coupling cell factor (*V2/V1*) decrease (taurine was added before ethanol) (control: % V2/V1 decrease with ethanol only, point 1: % V2/V1 decrease with 0.1 mM taurine + ethanol) (***: $p < 0.001$)

of the amniotic membrane, before ethanol, the percentage of GpNa decrease was reduced significantly ($p < 0.001$). The preventive effect of taurine was more important on the MS than on the FS.

3. Preventive action of taurine on the coupling cell factor (V2/V1)

In Hanks' solution only, ethanol (0.4 g/l) decreased significantly ($p < 0.001$) the V2/V1 value (40.1 ± 2.7%). The addition of taurine in the external medium, induced a significant reduction ($p < 0.001$) of the V2/V1 percentage decrease, but the action of taurine was not absolute *versus* ethanol (Fig. 3).

Discussion

In the isolated human amnion, taurine, at a concentration ranged between 0.1 and 1 mM, increases the membrane conductance, interacting particularly with the paracellular ionic pathway, increasing the Na^+ and the K^+ paracellular conductance. Moreover, taurine stimulates the ionic transfer between two adjacent epithelial amniotic cells. It has been previously shown that taurine increases membrane conductance in the locust neural somata (Whitton et al., 1994) and that taurine exerts a dual effect on Na^+ current in embryonic chick ventricular myocytes: stimulation at lower concentration (1 mM) and inhibition at higher concentration (10–20 mM) (Satoh and Sperelakis, 1992). In the same concentration range, our studies indicate an increase of the Na^+ conductance after taurine supplementation. This effect is due to a direct action on the anionic fixed charges on the epithelial cell membranes (Bara et al., 1990). In fact, taurine, an ubiquitous sulphonic amino acid, has been described as a regulator of membrane activity in both normal and pathological states in nerve and muscle. The zwitterionic structure of taurine alters the cation affinity of the phospholipid membranes, thus determining conformational

changes (Lombardini, 1985). For example, taurine can react with membraneous phosphoethanolamine. It may also interact with specific proteins or glycoproteins (Lehmann and Hamberger, 1984; Sebring and Huxtable, 1985; Huxtable, 1992).

Electrophysiological evidence suggests that taurine has activity at proteinaceous receptor sites for inhibitory amino acids. Much of the evidence equally supports an indirect modification of receptor site activity (Huxtable, 1992). Moreover, taurine stimulates Cl^- conductance in rat skeletal muscle fibers (Pierno et al., 1994) and hyperpolarizes the membranes. This action could involve both direct effects on the Cl^- channel or indirect effects on the membrane environment of the channel (Huxtable, 1992). In the membrane of the epithelial amniotic cells, direct effects of taurine on membraneous phospholipids may be considered and consequently other lipid-dependent phenomena may be oberved. For example, the modification of distribution and regulation of fixed charges on the cell membrane delimiting the intercellular spaces may be elicited. Such actions implicate a more important transfer of Na^+ and K^+ ions.

Ethanol reduces the ionic transfer through the amniotic membrane, particularly the Na^+ and K^+ paracellular transfer and the coupling factor between two adjacent cells. This action, which is similar on the two faces of the amnion, can be explained by the small size of the molecule and the weak properties of dissociation and polarization of ethanol (Guiet-Bara et al., 1985). Biophysical studies, such as those with electron spin resonance and fluorescence polarization, show that ethanol and aliphatic alcohols perturb the fine structures of the membrane (Sun, 1979), affecting the structural arrangement in the hydrophobic region of the membrane lipid bilayer (Lyon et al., 1981). *In vitro*, ethanol alters the structural arrangements of the lipids in biomembranes, rigidifies and renders the membrane unusually tight by increasing the number of bindings between phospholipid headgroups and hindering substrate and ion entry in cell organelles (Ahluwalia et al., 1992). Elgavish and Elgavish (1985) show that ethanol exerts its biological effects by interacting with the phospholipid portion of biomembranes rather than by an interaction with a specific receptor molecule (Goldstein and Chin, 1981; Seeman, 1972). This interaction of alcohol with the membrane-lipids involves the incorporation of the alcohol molecules into the membrane as evident from the actual expansion of the membrane (Leao and Van Uden, 1984). Several studies have shown that the acute effect of ethanol results in increased fluidity of all studied biological membranes (Seeman, 1972). On the other hand, during experimental chronic ethanol exposure, this effect is balanced by changes in the membrane which reduce fluidity, thereby making the membrane resistant (Beaugé et al., 1984; Lieber, 1991) and consequently decrease the membrane conductance. In our studies, an ethanol-phospholipid interaction (particularly with the fixed charges of the phospholipids) may be considered and the new organization of the charges hinders the transfer of Na^+ and K^+ ions. In our model, the effect of ethanol may be explained by the fact that ethanol molecules screen the external fixed sites responsible for the ionic monovalent transfer (Guiet-Bara et al., 1988). The addition of taurine before ethanol hinders the effect of

ethanol, certainly on the external fixed sites. These data indicate a possible common target between taurine and ethanol: the membrane phospholipids (particularly the distribution of the external fixed charges responsible in major part of the ionic transfer in the intercellular spaces between adjacent epithelial cells (Bara and Guiet-Bara, 1983)). The preventive action of taurine *versus* ethanol, on the human amnion, may be exerted on the polar groups of phospholipids, hindering the incorporation of ethanol molecules, which in absence from taurine, reduce the exchange surface of the intercellular spaces. This effect would be peculiar to cation transfer (Na^+ and K^+), modifying the external anionic charges distribution. This effect is confirmed by the fact that ultrastructural and stereological studies (Guiet-Bara and Bara, 1993) indicate that taurine restores the size of intercellular spaces previously reduced by ethanol. In the human amnion, a leaky membrane, the preventive effect of taurine *versus* ethanol is exerted in the paracellular pathway, particularly on the sodium and potassium components, but also on the coupling factor between two adjacent epithelial cells located in desmosomes and gap-junctions (Bartels and Wang, 1983; Guiet-Bara and Bara, 1983).

Moreover, it has been shown (Guiet-Bara et al., 1988) that γ-aminobutyric acid (GABA) and homotaurine have a preventive effect versus alcohol on the human amniotic total ionic conductance. In the same way (Guiet-Bara et al., 1995), a taurine derivative (Ca-Nacetyltaurinate) exerts a protective role *versus* ethanol on the same membrane. Also, structural analogues of taurine may protect the human amniotic membrane against the toxic effects of ethanol.

Thus, these data suggest a possible direct evidence that taurine alters the natural distribution of the external fixed charges in the amniotic epithelial cell membranes and exerts a preventive action against the deleterious effect of ethanol.

References

Ahluwalia B, Smith D, Adeyiga O, Akbasak B, Rajguru S (1992) Ethanol decreases progesterone synthesis in human placental cells. Alcohol 9: 395–401

Asai M, Narita O, Kashiwamata S (1985) Effects of acetaldehyde and/or ethanol on neutral amino acid transport systems in microvillous brush border vesicles prepared from human placenta. Experientia 41: 1566–1568

Bara M, Guiet-Bara A (1983) Evidence of charges on human epithelial amniotic cell membranes. In: Lambert PP, Bergmann P, Beauwens R (eds) Pathogenicity of cationic proteins. Raven Press, New York, pp 341–342

Bara M, Guiet-Bara A (1994) Inhibitor effects on the ionic exchanges through the human amniotic epithelial cell membranes. Cell Mol Biol 40: 1165–1171

Bara M, Guiet-Bara A, Durlach J (1985) Monovalent cations transfer through isolated human amnion: a new pharmacological model. Methods Find Exp Clin Pharmacol 7: 209–216

Bara M, Guiet-Bara A, Durlach J (1990) Comparative study of effects of magnesium and taurine on electrical parameters of natural and artificial membranes. VII. Effects on cellular and paracellular ionic transfer through isolated human amnion. Magnesium Res 3: 249–254

Bartels H, Wang T (1983) Intercellular junctions in the human fetal membranes. A freeze-fracture study. Anat Embryol 166: 103–120

Beaugé F, Fleuret-Balter C, Nordmann J, Nordmann R (1984) Brain sensitivity to ethanol during development of functional tolerance to ethanol in rats. Alcohol Clin Exp Res 8: 167–171

Dahchour A, Quertemont E, Dewitte P (1994) Acute ethanol increases taurine but neither glutamate nor GABA in the nucleus accumbens rats: a microdialysis study. Alcohol Alcohol 29: 485–487

Durlach J (1988) Magnesium in clinical practice. J. Libbey, London, p 360

Elgavish A, Elgavish GA (1985) *In vitro* ethanol effects on the transport properties of isolated renal brush-border membrane vesicle. J Membr Biol 88: 123–130

Goldstein DB, Chin JH (1981) Interaction of ethanol with biological membranes. Fed Proc 40: 2073–2076

Guiet-Bara A (1990) Transfert des ions à travers l' amnios humain isolé. Caractérisation des voies de passage paracellulaire et transcellulaire. Effect des modifications de l' environnement maternel. Thèse de Doctorat d' Etat ès-Sciences Naturelles, Université Pierre et Marie Curie, Paris, France

Guiet-Bara A, Bara M (1983) La membrane amniotique humaine: structure et perméabilité. I. Structure et composition chimique de l' amnios. Composition du liquide amniotique. Ann Biol 22: 315–332

Guiet-Bara A, Bara M (1984) Cellular and shunt conductance of human isolated human amnion. I. Effect of ouabain, DNP, amiloride and fructose. Bioelectr Bioenerg 13: 39–47

Guiet-Bara A, Bara M (1993) Ethanol effect on the ionic transfer through isolated human amnion. II. Cellular targets of the *in vitro* acute ethanol action and of the antagonism between magnesium, taurine and ethanol. Cell Mol Biol 39: 715–722

Guiet-Bara A, Bara M, Durlach J (1985) Comparative study of the effects of two tocolytic agents (magnesium sulfate and alcohol) on the ionic transfer through the isolated human amnion. Eur J Obstet Gynecol Reprod Biol 20: 297–304

Guiet-Bara A, Bara M, Durlach J (1987) Comparative study of the effects of magnesium and taurine on electrical parameters of natural and artificial membranes. VI. Study of the antagonism between ethanol, magnesium and taurine on the human amnion. Mg Bull 9: 36–39

Guiet-Bara A, Bara M, Durlach J, Pechery C (1988) Ethanol effect on the ionic transfer through isolated human amnion. I. Preventive and antagonistic actions of some nutrients and of their synthetic congeners. Alcohol 5: 63–71

Guiet-Bara A, Bara M, Durlach J, Pechery C (1995) Comparative studies of Ca N-acetylhomotaurinate and Ca N-acetylaturinate. II. Preventive and antagonistic actions of the acute ethanol depletive effect on the ionic transfer through the isolated human amnion. Methods Find Exp Clin Pharmacol 17 (in press)

Huxtable RJ (1992) Physiological actions of taurine. Physiol Rev 72: 101–163

Jacobsen JG, Thomas LL, Smith Jr LH (1964) Properties and distribution of mammalian L-cysteine sulfinate carboxy-lyases. Biochim Biophys Acta 85: 103–116

Jones KL, Smith WD (1975) The fetal alcohol syndrome. Teratology 12: 1–12

Leao C, Van Uden N (1984) Effects of ethanol and other alkanols on passive proton influx in the yeast saccharomyces cerevisiae. Biochim Biophys Acta 774: 43–48

Lehmann A, Hamberger A (1984) Inhibition of cholinergic response by taurine in frog skeletal muscle. J Pharm Pharmacol 36: 59–61

Lieber CS (1991) Alcohol, liver and nutrition. J Am Coll Nutr 10: 602–632

Lombardini JB (1985) Taurine effects on the transition temperature in Arrhenius plots of ATP-dependent calcium ion uptake in rat retinal membrane preparations. Biochem Pharmacol 34: 3741–3745

Lyon RC, McComb JA, Schreurs J, Goldstein DB (1981) A relationship between alcohol intoxication and the disordering of brain membranes by a serie of short-chain alcohols. J Pharmacol Exp Ther 218: 669–675

Mac Broom MJ, Elkawad AD, Dlouha H (1986) Taurine and ethanol induced sleeping time in mice: route and time course effects. Gen Pharmacol 17: 97–100

Moreno JH (1974) Blockage of cation permeability across the tight junctions in gallbladder and other leaky epithelia. Nature 251: 150–151

Palade P (1987) Drug induced Ca release from isolated sarcoplasmic reticulum. III. Block of Ca-induced Ca release by organic polyamines. J Biol Chem 262: 6149–6154

Pierno S, Tricarico D, Deluca A, Campagna F, Carotti A, Casini G, Camerion DC (1994) Effects of taurine analogues on chloride channel conductance of rat skeletal muscle fibers – A structure-activity relationship investigation. Naunyn Schmiedebergs Arch Pharmacol 349: 416–421

Satoh H, Sperelakis N (1992) Taurine inhibition of fast Na^+ current in embryonic chick ventricular myocytes. Eur J Pharmacol 218: 83–89

Sebring L, Huxtable RJ (1985) Taurine modulation of calcium binding to cardiac sarcolemma. J Pharmacol Exp Ther 222: 445–451

Seeman P (1972) The membrane actions of anesthetics and tranquilizers. Pharmacol Rev 24: 583–655

Streissguth AP, Landesman-Dwyer S, Martin JC, Smith DW (1980) Teratogenic effects of alcohol in humans and laboratory animals. Science 209: 353–356

Sturman JA, Gaull GE (1985) Taurine in the brain and liver of the developing human and monkey. J Neurochem 25: 831–835

Sun AY (1979) Biochemical and biophysical approaches in the study of ethanol-membrane interaction. In: Majchrowics E, Noble EP (eds) Biochemistry and pharmacology of ethanol. Plenum Press, New York, pp 81–100

Watanabe A, Nobara N, Nagashima H (1985) Lowering of liver acetaldehyde but not ethanol concentrations by pretreatment with taurine in ethanol loaded rats. Experientia 41: 1421–1422

Whitton PS, Nicholson RA, Strang RHC (1994) Electrophysiological responses of isolated Locust (Schistocerca Gragaria) somata to taurine and GABA application. J Insect Physiol 40: 195–199

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