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Mechanisms underlying taurine-mediated alterations in membrane function

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Summary. Taurine mediates a plethora of membrane-linked effects in excitable tissues. To account for these multiple actions, four hypotheses have been proposed. One theory is based on the observation that taurine diminishes the inflammatory response of several cytotoxic oxidants. It is proposed that a reduction in the extent of membrane oxidative injury contributes to these cytoprotective actions. The second theory maintains that alterations in protein phosphorylation may underlie certain effects of taurine, particularly its effect on calcium transport. The third hypothesis assumes that the interaction of taurine with the neutral phospholipids leads to altered membrane calcium binding and function. The final theory ties the actions of taurine to inhibition of phospholipid N-methylation and the resulting changes in membrane composition and structure. While each of these hypotheses has merit, none of them can fully explain the membrane actions of taurine. Further studies are required to ascertain the importance of each theory.

Keywords: Amino acids – Taurine – Antioxidant – Protein phosphorylation – Phospholipid structure – Calcium transport – Phospholipid N-methyltransferase

Taurine is an ubiquitous sulfur containing compound, which plays a central role in the development and normal functioning of excitable tissue (Hayes, 1988; Huxtable, 1992; Lombardini, 1991; Oja and Kontro, 1983; Pasantes-Morales and Martin del Rio, 1990; Schaffer and Azuma, 1992; Sturman, 1993; Wright et al., 1986). Although often referred to as an amino acid, taurine differs from the classical amino acids by the location of the amino group on the β carbon rather than the α carbon and the substitution of a sulfonic acid moiety for the α carboxyl group (see Fig. 1). The latter change is considered

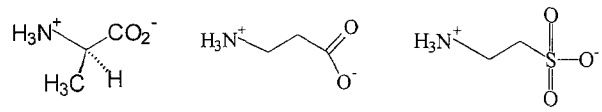

Fig. 1. The configuration of the α-amino acid, α-alanine, the β-amino acid, β-alanine, and the β-amino acid, taurine, Taurine differs from the classical α-amino acids by the location of the amino group on the β carbon and the presence of a sulfonic acid group in place of a carboxylic acid moiety

important because the sulfonic acid moiety is substantially more negative than the carboxylic acid group (−0.84 vs. −0.58 e, respectively). As a result, taurine readily forms salt bridges with cations rather than chelating them (Irving et al., 1980). Moreover, the sulfonic acid group significantly decreases the pK of the amino group. Thus, at physiological pH (7.27), approximately 2.8% of taurine remains in its deprotonated state while only 0.1% of β-alanine is deprotonated. According to Nakada et al. (1992) this difference is physiologically relevant because enough deprotonated taurine is present in the cell to function as an intracellular buffer. However, the Nakada conclusion is open to criticism because the contribution of taurine pales in comparison to the buffer capacity of inorganic phosphate and bicarbonate.

Although the physiological function of taurine has not been established, nutritional studies have uncovered a relationship between taurine deficiency and the development of retinal, neurological and cardiac dysfunction (Hayes et al., 1975; Imaki et al., 1986; Novotony et al., 1991; Palackal et al., 1986, 1993; Pion et al., 1987; Sturman et al., 1985; Vallecalle-Sandoval et al., 1991), immune deficiency (Schuller-Levis et al., 1990), elevated rates of abortions and stillbirths (Sturman, 1991; Sturman and Messing, 1991, 1992), reduced birth weights (Sturman and Messing, 1991) and impaired hindlimb development (Sturman et al., 1985). At the core of most of these effects are taurine-mediated changes in ion transport, oxidative stress, protein modification and/or hormonal and receptor responsiveness (Huxtable, 1992; Lombardini, 1991; Schaffer and Azuma, 1992; Sturman, 1993; Wright et al., 1986). Four hypotheses have been introduced as putative mechanisms for the membrane-actions of taurine. These include the antioxidant hypothesis, the protein phosphorylation hypothesis, the taurine-phospholipid binding hypothesis and the phospholipid N-methylation hypothesis. This review evaluates the validity of each of these theories. Two other often mentioned hypotheses have been formulated which tie the regulation of osmotic and sodium homeostasis to the transport of taurine. These latter theories have been recently reviewed and will not be addressed in this article (Chapman et al., 1993; Pasantes-Morales and Martin del Rio, 1990).

The antioxidant hypothesis

One of the major targets of oxidative stress is the unsaturated fatty acid moiety located in biological membranes. Since initiation of lipid peroxidation

causes extensive membrane disruption and cellular malfunction, tissue antioxidants capable of protecting against oxidative injury represent a key line of defense in combatting the adverse effects of biological oxidants. Several lines of evidence suggest that taurine functions as an important antioxidant *in vivo*. First chronic administration of taurine in the diet or water supply renders animals resistant to the development of bleomycin- or amiodarone-induced pulmonary fibrosis (Wang et al., 1991, 1992a,b), prevents acute inflammatory infiltration of bronchi following exposure of the taurine-treated animal to 30 ppm nitrogen dioxide (Gordon et al., 1986), and abolishes symptoms characteristic of focal segmental glomerulosclerosis following repeated administration of low dose injections of puromycin aminonucleoside (Trachtman et al., 1992). Second, doxorubicin-induced cardiotoxicity and carbon tetrachloride-induced hepatotoxicity are accentuated by drug-induced taurine depletion (Harada et al., 1990; Waterfield et al., 1993). Third, preincubation with buffer containing millimolar concentrations of taurine reduces the rate of lipid peroxidation in a wide range of cell lines exposed to cytotoxic oxidants (Alvarez and Storey, 1983; Banks et al., 1991; Pasantes-Morales and Cruz, 1984, 1985; Pasantes-Morales et al., 1985; Trachtman et al., 1993).

While some investigators have attributed these antioxidative effects of taurine to scavenger activity, Aruoma et al. (1988) have shown that taurine does not react rapidly with most biologically important oxidants, including the hydroxyl radical, hydrogen peroxide and superoxide anion. The only oxidant effectively scavenged by taurine is hypochlorous acid (Aruoma et al., 1988; Lin et al., 1988; Nakamori et al., 1993), a substance produced by neutrophils and monocytes and believed to play a role in cellular cytotoxicity and connective tissue damage (Weiss, 1989). Since the product of the taurine scavenging reaction, taurine monochloramine, is significantly less cytotoxic than hypochlorous acid, the ability of taurine to reduce hypochlorous acid levels has been touted as a mechanism underlying its cytoprotective activity (Nakamori et al., 1993; Wang et al., 1988). However, taurine monochloramine has recently been assigned a new role in the antioxidant hypothesis of taurine action. Based on studies in RAW 264.7 cells, Park et al. (1993) proposed that the major factor diminishing the inflammatory response of cytotoxic oxidants is the reduction in nitric oxide and tumor necrosis factor production by taurine monochloramine, with the scavenging of hypochlorous acid playing a minor role. Yet, the theory of Park et al. (1993) ignores some potentially toxic effects of taurine monochloramine, including its ability to directly react with thiols, proteins and lipids. Moreover, the toxicity of taurine monochloramine can be considerably enhanced by its conversion to taurine dichloramine (Cantin, 1994). Thus, while taurine can function as an effective antioxidant, its efficacy may be limited by the toxicity of the taurochloramines.

The protein phosphorylation hypothesis

Lombardini and coworkers (Li and Lombardini, 1991a,b; Lombardini, 1992a,b, 1993) have proposed that alterations in the phosphorylation of several retinal, brain and cardiac proteins may serve as the basis for many of the

actions of taurine. Particularly prominent is the effect of taurine on the phosphorylation of a 20 kDa protein found in the mitochondrial fraction of rat retina and another 20 kDa protein located in the P_2 synaptosomal fraction of rat cortex (Li and Lombardini, 1991a; Lombardini, 1992b). Although the function of the two 20 kDa proteins has not been determined, an inverse relationship exists between ATP-dependent calcium uptake and protein phosphorylation in the retina and rat cortex, implying that the phosphoproteins are regulators of the calcium transporter (Li and Lombardini, 1991a; Liebowitz et al., 1989). Nonetheless, differences in the isoelectric points and mode of phosphorylation of the two proteins indicate that the two proteins are distinct proteins (Lombardini, 1992a). While the cortex protein is a substrate for protein kinase C (Li and Lombardini, 1991b), the retinal protein is not phosphorylated by any of the classical protein kinases (cAMP, cGMP, calmodulin or protein kinase C) (Lombardini, 1992b).

Like rat cortex and retina, the heart contains a unique taurine-dependent phosphorylation pattern. Heart lacks the 20 kDa phosphoprotein, but contains several other proteins whose phosphorylation pattern is altered by taurine (Lombardini, 1992a; Schaffer et al., 1990). The protein kinase involved in the phosphorylation of taurine-sensitive phosphoproteins remains an area of some debate (Lombardini, 1992a; Schaffer et al., 1990). Also unclear is the function of the myocardial taurine-sensitive phosphoproteins.

Recently, Li and Lombardini (1991a) has raised the possibility that taurine may not directly inhibit a specific protein kinase. Rather, the reduction in tissue protein phosphorylation is secondary to taurine-mediated decreases in cytosolic calcium levels and inhibition of phosphoinositide turnover. This mechanism would explain the effects of taurine in rat cortex, where taurine is believed to alter protein kinase C activity. However, in the retina the activity of the taurine-sensitive protein kinase is not stimulated by phorbol esters, raising serious doubts about the generality of the mechanism. Yet, it is important to recognize that in the retina the taurine-sensitive protein kinase may be a specific protein kinase C isozyme, whose stimulation by the phorbol esters is masked by the presence of other protein kinase C isozymes. Moreover, protein kinase C activity of the retinal preparation may be less sensitive to the activating effects of the phorbol esters. It is known that diacylglycerol, the active phorbol esters and related compounds perturb the structure of the lipid bilayer, an effect necessary to achieve maximal activation of protein kinase C (DeBoeck et al., 1989; Goldberg et al., 1994). Thus, if taurine also alters the structure of the membrane, the response to the protein kinase C activator could be affected. In this regard, it is relevant that two of the theories discussed in this review depend upon taurine-mediated changes in membrane structure. In the taurine-phospholipid binding hypothesis, taurine binds directly to the phospholipid bilayer, inducing structural changes within the membrane. Similarly, membrane structural changes occur in the phospholipid N-methylation hypothesis because of the change in the phospholipid composition of the membrane. The phosphorylation hypothesis is also linked to the antioxidant hypothesis through an effect on protein kinase C. Taurine, or more specifically taurine monochloramine, can affect protein kinase C activity

by inhibiting the production of nitric oxide, an inactivator of protein kinase C (Gopalakrishna et al., 1993).

Since protein phosphorylation represents an extremely important regulatory mechanism, the protein phosphorylation theory has a definite appeal, particularly since it can be tied to most other theories of taurine action. Nonetheless, the significance of this theory awaits further information on the nature of the taurine-sensitive protein kinase(s) and the identity of the affected phosphoproteins.

The taurine-phospholipid binding hypothesis

Kulakowski et al. (1978) initially reported that at physiological concentrations of taurine, an interaction develops between taurine and biolgical membranes. These binding sites exhibit cooperativity and are highly selective, as evidenced by their binding of hypotaurine but not β-alanine (Kulakowski et al., 1978, 1981). Interestingly, the interaction is dramatically reduced by proteases and phospholipase C, suggesting that proteins and phospholipids are involved in the taurine-membrane interaction. In support of this notion, a protein with a Stokes radius of 4.4 nm has been isolated which contains the low affinity taurine binding sites and appears to contribute to taurine-mediated changes in membrane calcium binding (Chovan et al., 1980; Kulakowski et al., 1981). Although it has been proposed that the 4.4 nm protein is the taurine receptor, recent evidence suggests that taurine may be binding to the phospholipid moiety of the membrane protein rather than the polypeptide chain. First, taurine binding to synthetic phospholipid vesicles exhibits many of the characteristic features of taurine binding to rat heart sarcolemma (Kulakowski et al., 1978; Sebring and Huxtable, 1986). These include the amount of taurine bound, the affinity of taurine binding and the degree of binding cooperativity. Second, taurine stimulates calcium binding to both isolated cardiac sarcolemma and artificial phospholipid vesicles consisting of either phosphatidylserine or a mixture of phospholipids (Chovan et al., 1979; Sebring and Huxtable, 1986).

Figure 2 illustrates a likely complex between taurine and phosphatidylethanolamine. The major interactions involve electrostatic bonding between the phosphate group of phosphatidylethanolamine and the amino group of taurine, as well as the phosphatidylethanolamine amino group with the taurine sulfonic acid group.

While the molecular models indicate the feasability of taurine forming a stable ionic linkage with the head group of the neutral phospholipids, questions remain regarding the significance of the interaction. Huxtable (1987) has proposed that most actions of taurine can be attributed to this interaction. According to his hypothesis, the charge redistribution caused by the ionic linkage mediates membrane conformational changes, opening up new calcium binding sites and increasing the affinity of available sites for calcium. These changes are envisioned to impact other membrane functions, such as transport, receptor activity, membrane stability, protein phosphorylation and

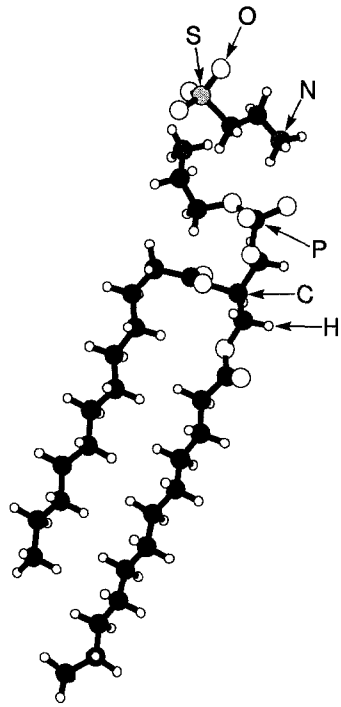

Fig. 2. The electrostatic interaction between taurine and phosphatidylethanolamine. The amino and sulfonic acid groups of taurine complex with the phosphate and amino groups of phosphatidylethanolamine, respectively. Carbon, hydrogen, oxygen, nitrogen, sulfur and phosphorus are represented by balls designated C, H, O, N, S and P, respectively

activity of membrane bound enzymes. Yet, an analysis of the calcium binding data raises major concerns regarding the physiological significance of taurine-mediated changes in calcium binding. From the studies of Chovan et al. (1980), the maximal number of high affinity calcium binding sites (B_{max}) of rat heart sarcolemma is only 0.7 mmol Ca^{2+}/mol phospholipid in the absence of taurine and 1.2 mmol Ca^{2+}/mol phospholipid in the presence of 10 mM taurine. At a medium calcium concentration of 1 μM in the presence of low Na^+-high K^+, conditions found intracellularly during systole, taurine increases total cardiac membrane calcium binding from 0.14 mmol Ca^{2+}/mol phospholipid to 0.37 mmol Ca^{2+}/mol phospholipid, an elevation comparable to taurine-mediated increases in calcium binding to artificial phosphatidylserine vesicles (Sebring and Huxtable, 1985, 1986). Thus, under conditions mimicking the intracellular milieu, taurine elevates total membrane calcium binding by only 0.2 moles of calcium for every 1000 moles of phospholipid. These data clearly reveal that taurine promotes the interaction of calcium with membrane phospholipids; however, the number of phospholipid molecules affected by taurine represents only a small fraction of the total phospholipid composition of the membrane bilayer. Because of the minute number of phospholipid molecules involved in the taurine-mediated perturbation, serious questions have been raised regarding the importance of the alteration in calcium binding. Insight into the significance of the taurine effect

can be obtained from examination of the types of membrane changes triggered by cations. In mixed phosphatidylethanolamine and phosphatidylcholine artificial membranes, elevations in medium calcium to the millimolar range promotes the separation of the phospholipids into two phases, a phosphatidylserine cochleate structure and a phosphatidylethanolamine hexagonal phase (Hui et al., 1983). Calcium also sequesters phosphatidylserine in mixed phosphatidylserine-phosphatidylcholine bilayers, forming phosphatidylserine cochleate structures and phosphatidylcholine bilayers (Silvius and Gagne, 1984). Another reported effect of calcium is an alteration in the head group conformation of the phospholipid (Brown and Selig, 1977). Although these polymorphic structural changes, involving transitions between bilayers and hexagonal structures, depend upon a number of factors, such as temperature, mole percentage of phosphatidylserine and the nature of the fatty acyl side chain (Cullis et al., 1986; Kirk et al., 1984; Siegel, 1986a, 1986b; Silvius and Gagne, 1984; Tilcock et al., 1984), they can only be detected at fairly high phospholipid:calcium molar ratios (0.1–1.0). These data reveal that when large amounts of calcium interact with the phosphate moiety of the phospholipid, the hydration of the phospholipid head group and the electrostatic repulsion between head group charges are altered, permitting the appropriate phase transition to take place. Since taurine only facilitates the binding of calcium to less than 0.05% of the phospholipid moieties, no major change in charge distribution or hydration of the phospholipid head group will occur. Thus, taurine is incapable of mediating polymorphic structural changes within the membrane. This conclusion is supported by electron spin resonance studies by Chovan et al. (1979), who found no evidence for changes in the structure of the lipid bilayer of rat heart sarcolemma following exposure to taurine.

In addition to polymorphic phase transitions, biological membranes can undergo transitions between a gel (crystalline) state and a liquid crystalline (fluid) state. Since numerous membrane enzymes and transporters are sensitive to changes in lipid structure, these fluidity phase transitions lead to alterations in the properties of the affected proteins. One such transporter is the ATP-dependent calcium transporter of rat retina, which undergoes a sudden change in the activation energy of calcium transport at a specific temperature referred to as the transition temperature. It has been proposed that at the transition temperature the retinal membrane undergoes a fluidity phase transition between its crystalline and liquid crystalline states (Lombardini, 1985). Taurine alters the transition temperature and the activation energy of the high temperature calcium transport reaction (Lombardini, 1985). While Lombardini (1985) concluded that taurine modifies the characteristics of the fluidity phase transition, the data are also consistent with other interpretations, such as a taurine-mediated change in the conformation of the transporter, localized structural changes or modulation of the ATP-dependent reaction by taurine. To distinguish between these mechanisms, further studies using physical techniques will be required. The power of these physical techniques in examining taurine-mediated changes in membrane structure has been previously demonstrated. Chovan et al. (1979) used a

paramagnetic spin label probe embedded into the phospholipid environment of isolated rat heart sarcolemma to monitor changes in membrane fluidity by electron spin resonance. The rotational correlation time of the probe was measured at various temperatures in the presence and absence of taurine. Chovan et al. (1979) concluded that taurine caused no change in membrane fluidity, as evidenced by the Arrhenius plot of the logarithm of the rotational correlation time versus reciprocal temperature. Since taurine mediated changes in calcium binding under similar conditions, it was logical to assume that neither taurine nor the rise in membrane calcium binding affected the fluidity of the membrane. Conversely, membrane fluidity changes were not responsible for taurine-mediated stimulation of calcium binding.

While extensive screening of negative charges on the outer surface of the membrane can promote polymorphic and fluidity phase transitions, moderate screening decreases the magnitude of the negative potential of the membrane, creating a positive surface potential and shifting the conductance-voltage curve of the cell along the voltage axis (Ekerdt and Papahadjopoulos, 1982; McLaughlin et al., 1978, 1981). It may also provide a pool of calcium which can enter the cell during electrical stimulation (Langer, 1978). These effects have major biological implications, but are restricted to perturbation of the outer membrane leaflet. Since taurine is concentrated within the cell, it presumably cannot bind to the cytoplasmic surface of the membrane and therefore is incapable of influencing divalent cation mediated events on the outer surface of the membrane.

Thus, if the calcium perturbation theory is physiologically relevant, two conditions must be satisfied. First, physiological levels of taurine must be capable of altering the interaction of calcium with the inner membrane leaflet. Second, calcium binding to the inner membrane leaflet must modulate a physiologically important event. When considering point 1, it is important to recognize that in most cells an asymmetric distribution of phospholipids exists, with phosphatidylserine and phosphatidylethanolamine preferentially occupying the inner leaflet and the outer leaflet containing most of the phosphatidylcholine and sphingomyelin (Post et al., 1988; Schroit and Zwaal, 1991). Since taurine can bind to both phosphatidylserine and phosphatidylethanolamine vesicles and increase calcium binding to phosphatidylserine vesicles, there seems little doubt that taurine will affect calcium binding to the inner leaflet of the cell membrane. Less certain is the significance of this altered calcium binding. According to Langer and coworkers (Langer and Rich, 1993; Post et al., 1993) a Na^+-Ca^{2+} exchange-dependent calcium compartment is located on the inner leaflet of the sarcolemma. This compartment is in equilibrium with a component of the sarcoplasmic reticulum and functions to increase the local calcium concentration. Since the dissociation constant of the Na^+-Ca^{2+} exchanger is greater than 6 μM while peak cytoplasmic calcium concentration is less than 1 μM, the Na^+-Ca^{2+} exchange-dependent calcium compartment would serve as a concentrated source of calcium, insuring maximal functioning of the exchanger. This would facilitate the extrusion of calcium from the cell via the exchanger, a process necessary for maintaining normal calcium homeostasis. Thus, it follows that if taurine's

effects on phospholipid calcium binding are physiologically important, taurine should alter calcium efflux via the Na^+-Ca^{2+} exchanger. Although there have been several suggestions that taurine might prevent calcium overload in heart failure by promoting calcium efflux via the exchanger (Ihara et al., 1992; Takihara et al., 1985), no direct evidence is available to support this view. In fact, the only study designed to directly examine the influence of taurine on Na^+-Ca^{2+} exchanger activity of isolated rat heart sarcolemma has proven negative (Schaffer et al., 1992). While the taurine-mediated alteration in calcium binding to the inner membrane leaflet is probably not physiologically important, the binding of taurine to the phospholipids of the inner surface of the membrane may mediate important calcium-independent effects. Further studies are required to identify which actions of taurine, if any, are caused by this interaction.

Phospholipid N-methylation hypothesis

The conversion of phosphatidylethanolamine to phosphatidylcholine is catalyzed by the enzyme phospholipid N-methyltransferase. This three step methylation reaction does not alter the charge of the phospholipid, but significantly increases the size of the phospholipid head group, thereby altering the molecular shape of the phospholipid. In solution, individual molecules of phosphatidylethanolamine assume a cone shape while phosphatidylcholine belongs to the group of phospholipids which become cylindrical (Fig. 3). To enhance their stability, individual phospholipids with the cylindrical shape join together to form a bilayer (Cullis et al., 1986). By contrast, the phospholipids with a cone shape preferentially organize into a hexagonal H_{II} structure (Cullis et al., 1986).

Biological membranes contain a variety of lipids, some which prefer the bilayer structure and others which are hexagonal formers. According to the fluid mosaic model of membrane structure, membrane phospholipids are arranged as a fluid semipermeable bilayer. However, the membrane is a

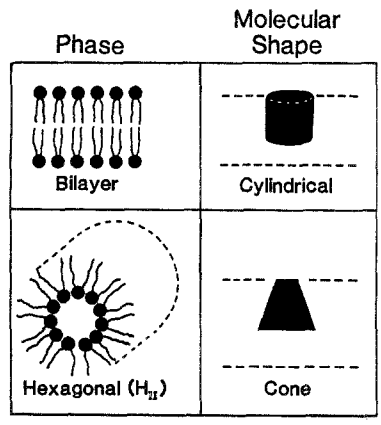

Fig. 3. Schematic representation of polymorphic phases and molecular shapes of phosphatidylethanolamine (hexagonal former) and phosphatidylcholine (bilayer former)

dynamic entity and can undergo transient departures from the bilayer structure. These phase transitions are promoted by hexagonal formers and play a crucial role in membrane function, influencing such biochemical events as fusion, transport and enzyme activity (Epand, 1990).

Another important characteristic of many membranes is phospholipid asymmetry. While phosphatidylcholine is located on the outer membrane leaflet, phosphatidylserine and phosphatidylethanolamine are the main components of the cytoplasmic leaflet (Post et al., 1988; Schroit and Zwaal, 1991). This asymmetric distribution is largely maintained by an ATP-dependent translocase (Calvez et al., 1988). However, phospholipid N-methylation also appears to contribute to phospholipid distribution by promoting the transfer of newly synthesized phosphatidylcholine to the outer membrane leaflet (Crews, 1985). Disruption of the asymmetric pattern is thought to contribute to a destabilization of the phospholipid bilayer (Musters et al., 1993).

The conversion of phosphatidylethanolamine, a hexagonal former preferentially situated on the cytoplasmic leaflet, to phosphatidylcholine, a bilayer former occupying the outer membrane leaflet, can have a profound influence on the structure and function of the membrane. In the heart, this is reflected by changes in calcium transporter activity. Panagia and coworkers (Ganguly et al., 1985; Panagia et al., 1986, 1987) have shown that phosphatidylethanolamine N-methylation leads to a decrease in sarcolemmal Na^+-Ca^{2+} exchanger activity and an activation of sarcoplasmic reticular and sarcolemmal calcium pump activity. A promotion of calcium leakage from isolated junctional sarcoplasmic reticulum following phosphatidylethanolamine N-methylation has also been reported (Punna et al., 1994).

In the heart, taurine inhibits each step in the conversion of phosphatidylethanolamine to phosphatidylcholine (Hamaguchi et al., 1991; Punna et al., 1994), thereby altering membrane structure and providing an explanation for many of the actions of taurine. One such taurine effect is the mediation of a positive inotropic effect in hearts made hypodynamic by either reductions in perfusate calcium, exposure to the calcium antagonist verapamil or promotion of phospholipid N-methylation (Chovan et al., 1980; Hamaguchi et al., 1991). This effect has been attributed to taurine-mediated alterations in calcium transport. In the case of phospholipid N-methylation, treatment of the hearts with 10 mM taurine prevents the decrease in Na^+-Ca^{2+} exchanger activity thought to cause the fall in contractile function (Hamaguchi et al., 1991). Another effect of taurine linked to phospholipid N-methylation is the prevention of tissue calcium overload (Schaffer and Azuma, 1992). The transporter responsible for extruding most of the calcium from the cell is the Na^+-Ca^{2+} exchanger. Taurine can prevent a drop in Na^+-Ca^{2+} exchanger activity by inhibiting phospholipid N-methylation (Hamaguchi et al., 1991). Taurine also promotes insulin-enhanced glucose transport (Lampson et al., 1983). It has been shown that bilayer stabilizers inhibit this process (Epand, 1990). Thus, taurine could potentiate the effects of insulin by inhibiting the conversion of a hexagonal former (phosphatidylethanolamine) to a bilayer former (phosphatidylcholine). Another effect of taurine attributed to altered phospholipid N-methylation is membrane stabilization (Huxtable and Bressler, 1973). In a

recent study, Punna et al. (1994) found that promotion of phospholipid N-methylation by exposure of the isolated heart to the methylating agent, L-methionine, increased calcium leakage of the junctional sarcoplasmic reticulum. Although the basis for this effect remains to be determined, a plausible explanation for the observation is that membrane structure is altered by the conversion of phosphatidylethanolamine to phosphatidylcholine. First, phosphatidylethanolamine content of the cytoplasmic leaflet will decrease and phosphatidylcholine content of the outer membrane leaflet will increase. Second, a change in membrane spacing secondary to the change in the size of the phospholipid head group will occur; bilayers containing phospholipids with smaller head groups, such as phosphatidylethanolamine, are more tightly packed than bilayers containing phospholipids with larger head groups, i.e., phosphatidylcholine.

The phospholipid N-methylation hypothesis assumes importance only in experimental settings in which phosphatidylethanolamine N-methylation is allowed to proceed. While most organelles and isolated membranes contain the enzyme, phospholipid N-methyltransferase, many *in vitro* studies are designed either intentionally or unintentionally to exclude the methylation reaction by carrying out the experimental protocol in the absence of a methylating agent. Thus, effects of taurine in those studies cannot be attributed to the original phospholipid N-methylation hypothesis. Because of this limitation, the phospholipid N-methylation hypothesis has been modified, taking into consideration the potential mechanism underlying the inhibition of phospholipid N-methyltransferase activity by taurine (Schaffer and Azuma, 1992). Although the kinetics of phospholipid N-methyltransferase is extremely complex, the data suggest that taurine interferes with the proper binding of phosphatidylethanolamine to the enzyme. This may involve direct competition between taurine and the phosphoethanolamine head group for the active site, an idea consistent with the structural similarity between taurine and the head group. Alternatively, the interaction of taurine with phosphatidylethanolamine (Fig. 2) may influence the binding characteristics of the phospholipid at the active site. One or both of these mechanisms may contribute to changes in the binding motif of phosphatidylethanolamine, not only to phospholipid N-methyltransferase, but also to other proteins and enzymes which depend upon phosphatidylethanolamine for activity. This general formulation of the phospholipid N-methyltransferase hypothesis is flexible enough to account for most actions of taurine; nonetheless, its validity still remains to be tested.

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