Taurine modulates platelet aggregation in cats and humans¹⁻³

KC Hayes, A Pronczuk, AE Addesa, and ZF Stephan

ABSTRACT To explore the relationship between whole-body taurine status and function, the taurine concentration in plasma and platelets was measured and evaluated in terms of ex vivo collagen-induced platelet aggregation in taurine-depleted cats and taurine-supplemented humans. Taurine status exerted a significant effect on platelet aggregability. Platelets from taurine-depleted cats were twice as sensitive to aggregation as platelets from cats receiving taurine. On the other hand, platelets from humans with normal taurine status increased resistance to aggregation by 30–70% when supplemented with taurine at 400 or 1600 mg/d, respectively. Decreased platelet aggregability was associated with increased platelet taurine and glutathione concentrations and decreased thromboxane release on aggregation. These data indicate that taurine in vivo stabilizes platelets against aggregation such that during taurine depletion platelets become overly sensitive whereas during supplementation their tendency to aggregate is depressed. Am J Clin Nutr 1989;49:1211–6.

KEY WORDS Taurine, platelet aggregation, thromboxane, platelet glutathione

Introduction

Taurine is considered a modulator of neuromuscular excitation, stabilizing the reactivity to various stimuli presumably by regulating cellular calcium flux (1). One possibility is that it acts indirectly by modulating cellular peroxidation (2) to influence the generation of specific peroxides and their metabolites, eg, prostaglandins such as thromboxane (3), which might influence the intracellular Ca concentration and associated metabolic processes such as secretion or contraction (4). Although taurine is present in most tissues, it is especially concentrated in the nervous system and platelets (5, 6). Because of their accessibility and their accumulation of neurotransmitters (7), platelets and platelet taurine concentrations have been assessed as a model system in clinical diseases where synaptosomal defects in neural or muscular excitation were suspected (8–10). It was also demonstrated that the addition of taurine to platelets in vitro stabilizes them against a variety of aggregating agents via a mechanism involving Ca flux (11). Not to be overlooked is the fact that cats commonly are afflicted with a taurine-deficiency cardiomyopathy (12), which is often complicated by systemic arterial thromboembolism (13), the pathogenesis and etiology of which is unknown.

Generalized platelet aggregation (PA) depends in part on thromboxane formation and generation of intracellular oxygen radicals (peroxides) (14) and on a precise balance in reducing equivalents, including the available pool of glutathione (GSH). The latter tends to depress sensitivity to aggregating stimuli (15). Because we recently found that taurine supplementation of Hep G2 cells spared the intracellular pool of cysteine (16) and because cysteine is the precursor for both GSH and taurine biosynthesis, this aspect of taurine metabolism and function was investigated in platelets.

The questions we posed were whether whole body taurine status would be reflected in the concentrations of platelet taurine and glutathione and thereby influence PA and whether platelet taurine status and its impact on aggregation might provide a meaningful index of whole-body taurine status. The results from cats and humans indicate that platelet taurine concentration does reflect taurine intake and does influence PA and that this aggregation threshold is at least associated with platelet glutathione and the release of thromboxane.

Materials and methods

Cat study

To evaluate taurine status and platelet function in cats with taurine deficiency, two groups of six cats each were raised from

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the time of weaning on a casein-based purified diet (Table 1) either without taurine (test) or with a 500-µg/kg diet taurine supplement (control). Half the cats were male and half female with equal distribution between groups; all were aged 10–24 mo when tested. After an overnight fast each cat was sedated with 0.2 mL ketamine hydrochloride (Ketaset®) and 18 mL blood was drawn from the jugular vein into a plastic syringe containing 2 mL of 3.8% trisodium citrate. Platelet aggregability, plasma and platelet taurine, as well as platelet GSH and thromboxane production were measured as described below.

**Human study**

This study consisted of three trials, which utilized the same five normolipidemic, nonsmoking healthy males aged 20–47 y. The study was approved by the Brandeis University Human Use Committee and participants had given their signed consent. None were taking any chronic medication and they were asked to maintain their normal diets during the experiment and refrain from consuming any alcohol or drugs known to affect PA, eg, aspirin. In the first trial the normal basal PA threshold for each subject was determined four times during a 4–8 wk period. Citrated blood samples (one volume of 3.8% trisodium citrate per nine volumes of blood) were collected from the antecubital vein just before midday and ~4 h after a light breakfast (< 300 kcal). The basal PA threshold varied as much as 300% between individuals but was highly reproducible within individuals (CV < 12%). In the second part of the study the participants were supplemented with 400 mg taurine/d for 14 d during which time their PA, plasma taurine (platelet poor plasma-PPP), and platelet taurine concentrations were measured at 0, 1, 4, 8, 14 d. Additionally, on days 0 and 8 the platelet glutathione was monitored. After 1 mo without taurine supplementation the same individuals were supplemented with 1600 mg taurine/d for 8 d and selected variables were remeasured at 0 and 8 d. In this last trial in conjunction with aggregation, the thromboxane B2 (TXB₂) released from platelets upon stimulation with a fixed amount of collagen (0.93 µg/mL) was also measured.

**Platelet aggregation threshold and thromboxane production**

To obtain platelet-rich plasma (PRP) for aggregation studies, blood was centrifuged (model K, International Equipment Co, Needham Heights, MA) at room temperature at 200 x g for 10 min. The top 1–2 mL (PRP) was removed and the remaining blood was reincubated at 2000 x g for 10 additional minutes to obtain platelet-poor plasma (PPP). The platelet concentration was determined with a hemocytometer (Improved Neubauer, American Optical Co, Buffalo, NY) and the PRP was diluted with PPP to obtain standard PRP containing 2.5 × 10⁴ platelets/mL. Aggregation was performed with a model 400 aggregometer (Payton Scientific Inc, Buffalo, NY) by using 0.35 mL of standard PRP. After 5 min of incubation at 37 °C in 1.0 mL siliconized cuvettes, a metal siliconized stir bar was added followed by a measured quantity of purified collagen (equine tendon, Hormon-Chemie, Munchen GMBH). The aggregation induced was quantified turbidimetrically as the percent difference in light transmission between standard PRP and PPP during the subsequent 6 min (cat) or 4 min (human). Different times were chosen as endpoints of the aggregation threshold because of differences in platelet shape change and aggregation lag time evidenced by the aggregation curves generated in the two species (Fig 1). For each cat and human sample an aggregation threshold was determined by stimulating platelets with different concentrations of collagen (four to five levels) until 10% of the predetermined maximum aggregation was identified. The result was expressed as the amount (µg) of collagen required for a 10% maximum PA (max PA) response for 1 mL standard PRP containing a standard number of platelets (2.5 × 10⁴). The generation of the PA threshold is depicted for cats and humans in Figure 1. For measurement of TXB₂ production, 0.35-mL aliquot of this standard PRP was incubated with a constant amount of collagen reagent (0.93 µg/mL) for 4 min to induce a variable degree of aggregation between threshold and 100% PA. A 0.3-µL aliquot of this reacting solution was then rapidly transferred to a capped plastic vial containing 30 µL of 43 mmol aspirin/L and frozen in acetone and dry ice for 1 min before storage at −20 °C until TXB₂ was measured by radioimmunoassay (17). TXB₂ was expressed as nanograms released per 2.5 × 10⁴ platelets after maximal aggregation.

**Taurine analysis**

For determination of the platelet taurine concentration, a measured number of platelets was centrifuged from PRP in a plastic test tube. The plasma was removed and replaced by an equal volume of distilled water. The tube was vortexed, allowed to sit overnight at room temperature, and then frozen and thawed to lyse the platelets and release their contents. To precipitate protein, the platelet lysate (or plasma sample when it was being assayed) was treated with 0.1 volumes of 50% trichloroacetic acid (TCA). The supernatant was removed after centrifugation and analyzed for taurine by HPLC according to Stabler and Siegel (18). Briefly, a 250-µL aliquot of sample supernatant was passed over a cation/anion cleanup column (5 mm id) prepared by layering 20 mm Dowex 100–200 mesh (H⁺) over 20 mm Dowex 200–400 mesh (Cl⁻) and washed through

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**Table 1**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
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<tbody>
<tr>
<td></td>
<td>%</td>
</tr>
<tr>
<td>Casein</td>
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</tr>
<tr>
<td>Cystine</td>
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</tr>
<tr>
<td>Arginine</td>
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</tr>
<tr>
<td>Threonine</td>
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</tr>
<tr>
<td>Beef tallow</td>
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</tr>
<tr>
<td>Sucrose</td>
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</tr>
<tr>
<td>Dextrin</td>
<td>25.0</td>
</tr>
<tr>
<td>Ausman-Hayes salt mix†</td>
<td>6.4</td>
</tr>
<tr>
<td>Cellulose</td>
<td>2.4</td>
</tr>
<tr>
<td>Hayes cat vitamin mix‡</td>
<td>0.6</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.3</td>
</tr>
</tbody>
</table>

* Taurine-supplemented diet contained 0.5 g taurine per kilogram diet.
† Contains (g/kg): calcium carbonate, 290; potassium phosphate (dibasic), 328; calcium phosphate (dibasic), 72; magnesium sulfate, 99; sodium chloride, 162; magnesium oxide, 32; ferric citrate, 13; potassium iodide, 0.08; manganese sulfate, 1.2; zinc chloride, 0.92; copper sulfate, 0.3; chromium acetate, 0.04; sodium selenite, 0.0044; sodium fluoride, 0.023.
‡ Contains (g/kg): dextrin, 944; inositol, 10; alpha-tocopheryl acetate (500 IU/g), 20; niacinamide, 8; calcium pantothenate, 5; vitamin A acetate (500 000 IU/g), 5; riboflavin, 1.6; thiamin HCl, 0.8; pyridoxine, 0.8; folic acid, 0.8; menadione, 0.1; cholecalciferol (400 000 IU/g), 0.625; biotin, 0.04; cyanocobalamin, 0.003.
TAURINE AND PLATELET AGGREGATION

![Graph](image)

**Results**

**Cat study**

The generation of the platelet aggregation curve and threshold for a typical cat is depicted in Figure 1. When the PA threshold was determined, taurine-supplemented cats revealed thresholds that were significantly higher (140%) than taurine-deficient cats (Table 2). Thus, taurine depletion rendered cat platelets decidedly more susceptible to collagen-induced aggregation. The decrease in aggregation threshold was associated with lower concentrations of plasma and platelet taurine as well as platelet glutathione, all of which were significantly lower in taurine-deficient cats (Table 2).

**Human study**

The platelets from taurine-supplemented humans responded to taurine by increasing their threshold to collagen-induced aggregation (Fig 2). Furthermore, when a constant level of collagen (0.93 µg/mL) was used to stimulate aggregation, the taurine-supplemented platelets underwent less aggregation and released less TXB₂ (Fig 2). The degree of resistance to aggregation expressed by

**TABLE 2**

Relationship between platelet sensitivity to ex vivo aggregation and plasma and platelet concentrations of taurine or GSH in taurine-deficient or supplemented cats*

<table>
<thead>
<tr>
<th>Diet</th>
<th>Plasma taurine (µmol/L)</th>
<th>Platelet aggregation threshold (µg collagen/mL PRP)</th>
<th>Platelet taurine (µmol/10⁶ platelets)</th>
<th>Platelet glutathione (µmol/10⁶ platelets)</th>
</tr>
</thead>
<tbody>
<tr>
<td>With taurine</td>
<td>74 ± 9</td>
<td>2.12 ± 0.21</td>
<td>4.18 ± 0.56</td>
<td>0.97 ± 0.10</td>
</tr>
<tr>
<td>Without taurine</td>
<td>18 ± 3*</td>
<td>0.88 ± 0.22</td>
<td>2.00 ± 0.28*</td>
<td>0.63 ± 0.03*</td>
</tr>
</tbody>
</table>

* X ± SEM; n = 6 for each group. Values with superscripts differ significantly (p < 0.05 or greater) from control values based on paired t test.
† PRP adjusted with PPP to 250 000 platelets/µL.

with 2 mL distilled water. A 0.5-mL aliquot of filtrate was placed in a test tube and 0.25 mL sodium borate buffer (pH 8.3) and 0.25 mL fluorescamine solution (0.3 mg fluorescamine/mL acetonitrile) were added. After 1–2 min was allowed for full conjugation before 100 µL of the mix was injected into an HPLC system composed of a Millipore-Waters Radial Compression Separation System with a Nova-Pak C₁₈ cartridge column (Waters Associates, Milford, MA). The mobile phase consisted of 80 parts sodium phosphate buffer (15 mmol/L, pH 6.0) and 20 parts acetonitrile, and was pumped at 1 mL/min by using a Rainin Rabbit HP® (Rainin Instrument Co, Inc, Woburn, MA) solvent delivery system. The effluent was monitored with a fluorometer (SLM Aminco Fluoro-Monitor II®, SLM Instruments, Inc, Urbana, IL) with 395 nm excitation and 455 nm emission filters and recorded with a CR3A Integrator® (Shimadzu Sci, Instruments, Inc, Columbia, MD).

**GSH analysis**

Platelet glutathione concentration was measured by a specific fluorometric assay (19). Platelets from 5 µL of PRP were counted, lysed, and homogenized in 3 mL of 0.1 mmol HCOOH/L. The homogenate was centrifuged at 20 000 × g for 20 min. An aliquot (100 µL) of the supernatant was mixed with an equal volume of buffered formaldehyde to reduce the fluorogenicity of interfering substances and reacted with o-phthalaldehyde in the presence of 0.1 mmol sodium phosphate/L:5 mmol EDTA buffer/L (pH 8.0). The fluorescence was measured after 45 min in a model 430 spectrophotofluorometer (Turner Associates, Palo Alto, CA) set at 345 nm (excitation) and 425 nm (emission).

**References**

- 1. Taurine deficiency and platelet dysfunction: a review
- 2. The role of taurine in platelet aggregation
- 3. Taurine and platelet aggregation in cats
- 4. Taurine supplementation and platelet function
- 5. Taurine and platelet sensitivity to ex vivo aggregation
- 6. Taurine and platelet concentrations of taurine or GSH in taurine-deficient or supplemented cats

**Figure 1**

Schematic representation of typical platelet aggregation (PA) curves for collagen-induced aggregation in cats (Panel A) and humans (Panel B). Note longer aggregation lagtime in cats and their failure to demonstrate the platelet shape change (sub-base-line dip) observed in humans. Platelet aggregation threshold was identified in each case as micrograms collagen needed to elicit 10% maximal aggregation (100% PA was induced by excess collagen) by a set time (6 min for cats and 4 min for humans).
platelets directly reflected the level of supplementation, i.e., 8 d of 400-mg supplementation increased resistance an average of 25% whereas 1600 mg/d caused a 72% elevation in the aggregation threshold (Table 3). These changes were paralleled by increases of 55 and 115% in platelet taurine concentration, respectively. In contrast to the incremental response in platelet taurine with taurine supplementation, the plasma taurine concentration increased ~40% with the 400-mg/d supplement but was not elevated further by the four-fold increase to 1600 mg/d. Similar to cats, taurine supplementation increased the GSH concentration in platelets (Table 3).

As seen in Figure 3 the response to taurine supplementation by human platelets was rapid. Both an increase in platelet taurine concentration and resistance to aggregation were demonstrated in 24 h. Although only one-half the final increase in platelet taurine concentration had occurred in 24 h, 75% of the increase in the aggregation threshold was evident. By 4 d the plasma and platelet taurine concentrations had peaked whereas the PA threshold continued to increase modestly (25%) during the remaining 10 d.

When all the values from the five subjects (at 400 and 1600 mg/d for day 8) were tested for a correlation between the percent increase in platelet taurine concentration vs the percent increase in PA threshold, the correlation was positive and relatively good ($r = 0.71, p < 0.005$, Fig 4). A similar correlation between plasma taurine and platelet aggregation revealed a rather poor relationship ($r = 0.33, p < 0.64$).

Discussion

So far as we know no clinical evidence exists describing an association between platelet aggregation and taurine deficiency in man or cats, but before these experiments little reason existed to explore this possibility. An exception might be argued on the basis of the highly documented susceptibility of cats to a taurine-deficiency syndrome (20) including retinal degeneration, cardiomyopathy, growth and reproductive failure, fetal malfomations, cerebellar spasticity with kyphosis, and neutrophil dysfunction. In this context is the observation that the cardiomyopathy in cats is frequently associated with a high incidence of thromboembolism, presumably related to the mural thrombi formed in the left side of the failing heart (13). On the basis of this report, cardiomyopathy and thromboembolism are quite possibly linked to a common etiology, namely taurine depletion.

The rationale for taurine depletion in cats has been addressed (20) but current consensus would suggest that it reflects inadequate taurine synthesis and exceptional competition for the available cysteine pool by competing biosynthetic pathways. Because of this imbalance taurine is considered an essential amino acid for cats (21) and conditionally essential for humans under adverse circumstances that impair the transulfuration pathway for synthesis of taurine from methionine (22). The biochemistry and physiology of taurine have been widely reviewed (2, 23, 24).

The present data reveal several points concerning taurine and platelet function. First, the taurine status of an organism definitely influences platelet aggregation induced ex vivo by collagen. This was demonstrated under two circumstances, i.e., the platelets from taurine-deficient cats proved more sensitive to aggregation than those from taurine-supplemented cats whereas platelets from human subjects with presumably normal taurine status were made more resistant to aggregation by dietary taurine supplementation. Second, within limits, the degree of increased platelet resistance was proportional to the level of supplementation and the taurine concentration in platelets. These data support the previous obser-
viation on the stabilizing effect of taurine added to platelets during aggregation in vitro (11) and extend that observation in vivo to raise important questions concerning the clinical implications of taurine status on thromboembolic events. Third, plasma taurine status did not prove to be as good a predictor of platelet function as the platelet taurine concentration. This presumably reflects the fact that several tissues, including platelets, can accumulate taurine against a concentration gradient and thus achieve increasingly high intracellular concentrations of taurine upon supplementation (25) whereas the upper plasma concentration for taurine is presumably limited by kidney tubular reabsorption at high plasma concentrations (26).

The comparative aspect of this study suggests that factors other than plasma taurine or dietary taurine determine the platelet taurine concentration. For example the taurine concentration in platelets from taurine-depleted cats was still ~50% greater than the basal concentration for normal human platelets even though the plasma concentration in depleted cats was less than half the normal value for human plasma. Nonetheless, it is noteworthy that the inherent taurine concentration in platelets did seem to influence the basal PA threshold. Thus the cat, even when taurine-depleted, maintained a higher platelet taurine concentration and generally demonstrated substantially greater resistance to aggregation than did humans, even when the latter were taurine supplemented. Presumably the relationship between taurine and PA is linked to modulation of the calcium ion concentration (11). Varying the citrate concentration during platelet collection (ie, the degree of Ca ion removal) in itself substantially alters the PA threshold under our conditions (data not shown). Another caveat in this species comparison is the difference in lag times and shape of the aggregation curves, which required a 2-m discrepancy in the threshold reading and thus renders the interspecies comparison (assay) less than perfect.

Part of the observed difference in PA thresholds between cats and humans may be attributed to the relationship between concentrations of platelet taurine and GSH and the ability of the latter to depress platelet sensitivity to aggregation (15, 25). This concept is supported by two observations. First, taurine-adequate cats, which were more resistant to PA than humans, maintained higher

<table>
<thead>
<tr>
<th>Dietary taurine</th>
<th>Plasma taurine</th>
<th>PA threshold</th>
<th>Platelet taurine</th>
<th>Platelet glutathione</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\mu$mol/L</td>
<td>$\mu$g collagen/mL PRP†</td>
<td>$\mu$mol/10^9 platelets</td>
<td></td>
</tr>
<tr>
<td>Basal diet</td>
<td>50 ± 2a</td>
<td>0.32 ± 0.09a</td>
<td>1.31 ± 0.06a*</td>
<td>0.56 ± 0.04a*</td>
</tr>
<tr>
<td>+400 mg supplement</td>
<td>71 ± 3b</td>
<td>0.40 ± 0.06b</td>
<td>2.10 ± 0.08b*</td>
<td>0.75 ± 0.05b*</td>
</tr>
<tr>
<td>+1600 mg supplement</td>
<td>74 ± 4b</td>
<td>0.55 ± 0.16b</td>
<td>2.80 ± 0.10b*</td>
<td>NA</td>
</tr>
</tbody>
</table>

* $x \pm$ SEM; $n = 5$. Values with different superscripts differ significantly ($p < 0.05$ or greater); significance based on paired $t$ test.
† PRP adjusted with PPP to 250 000 platelet/$\mu$L.
GSH and taurine concentrations in their platelets than their human counterpart. Second, an increase in platelet taurine in humans was associated with an increase in platelet GSH.

The mechanism of taurine function in platelet aggregation is not resolved by these data, but one possibility that merits further study is that taurine served to enhance the redox potential of the platelet by increasing its GSH concentration. Glutathione has been demonstrated to decrease the sensitivity of normal human platelets to collagen-induced aggregation (15) whereas low GSH concentration in platelets from diabetics is associated with hyperaggregability (27). In our study when TXB₂ was measured before and after 1600 mg/d the decreased sensitivity to aggregation associated with taurine supplementation was reflected by decreased release of TXB₂. However, it is unclear from these data whether the lower TXB₂ released was the cause or result of decreased aggregation. Others have noted the inverse relationship between TXB₂ production and platelet GSH (27), which they attributed to a decline in hydroperoxides due to the reducing equivalents contributed by GSH. Because arachidonic acid-derived hydroperoxides are involved in the regulation of cyclooxygenase (14) and the production of TXA₂, their modulation by GSH might be expected to alter platelet TXA₂ generation and the associated Ca release from dense tubules responsible for platelet aggregation (4). The antioxidant potential of taurine has been reviewed recently (2) but the possibility that taurine may spare GSH and thus influence TXA₂ production has not been mentioned previously.

In summary these data suggest that platelet taurine status is more than plasma taurine affects platelet aggregation and that this taurine action may be linked to a sparing effect on the platelet pool of glutathione.

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References