

Taurine and Isolated Mitochondria: A Concentration-Response Study

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Abstract

Taurine (TAU) is the most abundant free amino acid in the human body. High concentrations of this amino acid are found in tissues such as the skeletal muscle, brain, and kidney. Recently, a focus has emerged on the effects of TAU on cellular mitochondria. It has been found that TAU could positively affect this organelle by enhancing mitochondrial membrane potential, increasing ATP levels, and mitigating mitochondria-mediated ROS formation. The current study aimed to evaluate the effect of a wide range of TAU concentrations (0.01 mM-1000 mM) on mitochondrial function. Mice liver mitochondria were isolated and exposed to different concentrations of TAU (30 min). Several indices, including mitochondrial depolarization, dehydrogenases activity, permeabilization, and ATP content, were monitored. It was found that TAU supplementation significantly enhanced parameters such as mitochondrial ATP levels and mitochondrial membrane potential in comparison with the control group. Moreover, TAU prevented Ca²⁺-induced mitochondrial permeabilization. This amino acid revealed no significant adverse effect on isolated mitochondria even at very high and supra-physiological concentrations (e.g., 100, 250, and 500 mM). These data suggest TAU as an ideal and safe agent to protect mitochondria against toxic insults or regulating cellular function in different mitochondria-linked disorders.

Keywords: Amino acid, Cytoprotection, Mitochondrial cytopathies, Nutraceuticals, Oxidative stress.

1. Introduction

Taurine (TAU) is a β -amino acid abundantly found in the human body. Skeletal muscle, heart, brain, and kidneys contain a high TAU level (1). Several pharmacological properties have been attributed to TAU (2-5). TAU also exhibits cytoprotective effects against a wide range of xenobiotics as well as several human diseases (4-12). However, the mechanisms of cytoprotection pro-

vided by this amino acid are mostly unknown. Recently, a focus has emerged on the effects of TAU on cellular mitochondria (1, 13-15). Interestingly, it has been found that TAU also incorporates essential mitochondrial components, such as transfer RNA (tRNA) (15-17). tRNA plays a pivotal role in transferring amino acids to ribosomes and the synthesis of mitochondrial proteins (e.g., electron transport complexes) (4, 18). On the other hand, several diseases have been identified, which are directly associated with the lack of mitochondrial tRNA taurine modification (19, 20). Hence, this

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amino acid is a new candidate for drug design against several human diseases. Therefore, it is essential to evaluate the safety of TAU in biological systems, especially on cellular mitochondria as the principal target of action of this amino acid.

Taurine reaches high concentrations in some tissues, such as skeletal muscle. However, there is no investigation on the effects of different and mostly supraphysiological concentrations of TAU on cellular mitochondria as a vital target for this amino acid. The current study was designed to evaluate the effect of a wide range of TAU concentrations (0.01 mM-1000 mM) on isolated mitochondria. Several mitochondrial indices were monitored to evaluate if any deterioration in mitochondrial function occurs in the presence of TAU. The data could estimate the safety of this amino acid on cellular mitochondria, help to protect this organelle against toxic insults, and finally regulating and improving cellular function in different mitochondria-linked disorders.

2. Material and method

2.1. Chemicals

Trichloroacetic acid (TCA), sodium acetate, thiobarbituric acid (TBA), mannitol, phosphoric acid, sucrose, ethylene diamine tetraacetic acid (EDTA), calcium anhydride, 2 amino 2-hydroxymethyl-propane-1,3-diol-hydrochloride (Tris-HCl), and essentially fatty acid-free bovine serum albumin (BSA), were obtained from Merck (Darmstadt, Germany). Taurine (2-aminoethanesulfonic acid), rhodamine 123, and methyl tetrazolium (MTT) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Animals

Male C57BL6/J mice (n=60; 20-25 g weight) were obtained from Shiraz University of Medical Sciences, Shiraz, Iran. Animals were housed in plastic cages over wood-chip bedding. There was an environmental temperature of 23±1 °C and a 12L: 12D photoschedule along with a ≈40% of relative humidity. Animals were allowed free access to tap water and a regular chow diet (Behparvar®, Tehran, Iran). The experiments were done in compliance with the guidelines for care and use of experimental animals approved by the

ethics commission at Shiraz University of Medical Sciences (#1396-01-36-16627).

2.3. Mice liver mitochondria isolation

Mitochondria were isolated from mice liver based on previously reported procedures based on differential centrifugation (21-23). First, the liver was washed and minced in an ice-cold (4 °C) isolation buffer medium (220 mM sucrose, 0.5 mM EGTA, 75 mM mannitol, 2 mM HEPES, 0.1% BSA, and pH=7.4). The minced liver tissue was transported into a fresh isolation buffer at a 10:1 buffer to tissue ratio (v: w). Samples were homogenized using a PYREX® Potter-Elvehjem tissue homogenizer. As mentioned, cellular mitochondria were isolated based on the method of differential centrifugation (21, 24). First, intact cells and nuclei were pelleted (1000 g, 20 min, 4 °C); second; the supernatant was collected and centrifuged (10,000 g, 20 min 4 °C). The recent step was repeated at least three times to increase mitochondrial yield and purity. Finally, mitochondrial pellets were re-suspended in the incubation buffer medium (10: 1 v: w buffer: mitochondrial pellet) containing 225 mM sucrose, 75 mM mannitol, 2 mM HEPES, and 0.5 mM EGTA, pH = 7.4, except for the mitochondrial preparations used to assess mitochondrial depolarization and mitochondrial permeabilization, which were suspended in mitochondrial depolarization assay buffer (225 mM Sucrose, 2 mM MgCl₂, 10 mM KCl, 75 mM Mannitol, 5 mM KH₂PO₄, 50 μM EGTA, and 10 mM HEPES, pH=7.2) and swelling buffer (250 mM sucrose, 2 mM HEPES, 0.5 mM KH₂PO₄, 5 mM Sodium succinate; pH=7.4) (21, 24). Samples, protein concentrations were assessed by the Bradford method using BSA as a standard (25)

2.4. Mitochondrial depolarization assay

Rhodamine 123 was used as a cationic fluorescent probe to estimate mitochondrial depolarization (24, 26-28). Rhodamine 123, accumulates in the matrix of functional mitochondria. When mitochondria are depolarized and damaged, the extent of rhodamine 123 in the medium is high (21, 29-31). Briefly, 1 mL of the mitochondrial fractions (1 mg protein/mL in the depolarization assay buffer) were incubated with 10 μL of rhoda-

mine 123 (10 μM Final concentration; 15 minutes; in the dark; with continuous shaking) (32). Afterward, samples were centrifuged (16,000 g, 2 min, 4 $^{\circ}\text{C}$) and the fluorescence intensity of the supernatant was assessed (FLUOstar Omega[®] multifunctional microplate reader, BMG Labtech[®], Germany, λ excitation=485 nm and λ emission=525 nm) (21, 33).

2.5. Mitochondrial permeabilization

The light scattering method was used to estimate the mitochondrial permeabilization (24, 26, 28, 34). Briefly, isolated mitochondria (0.5 mg protein/mL) were suspended in the pre-warmed (37 $^{\circ}\text{C}$) swelling buffer (2 mM HEPES, 250 mM sucrose, 4.2 mM sodium succinate, 0.5 mM KH₂PO₄; pH=7.4). The mitochondrial permeability transition was induced by adding calcium (Ca^{2+} 100 μM) and assessed by monitoring the absorbance changes at $\lambda=540$ nm (EPOCH plate reader, Bio-Tek[®] Instruments, Highland Park, USA) (21, 33). A decrease in the absorbance implies an increase in mitochondrial volume and organelle swelling (13, 21, 33).

2.6. Mitochondrial dehydrogenases activity

A colorimetric method using methyl tetrazolium (MTT) was used to estimate mitochondrial dehydrogenase activity (35-39). Briefly, mitochondrial suspension in a buffer containing 1 mM EDTA, 10 mM Tris-HCl, and 320 mM sucrose, pH=7.4, was treated with 40 μL of MTT (0.4% w:

v in buffer) and incubated for 15 minutes (37 $^{\circ}\text{C}$, in the dark). Then, samples were centrifuged (12,000 g, 5 min), and the purple formazan crystals pellets were dissolved in DMSO (1 ml). Samples were centrifuged again (12000 g, 5 min), and 100 μl of the supernatant was added to 96 well-plate and the optical density (OD) at $\lambda=570$ nm was assessed with an EPOCH plate reader (BioTek[®] Instruments, Highland Park, USA) (40, 41).

2.7. Mitochondrial ATP

A luciferase–luciferin-based kit from Promega[™] (ENLITEN[®]) was used to assess mitochondrial ATP content (42). Buffer solutions and samples and were prepared based on the ENLITEN[®] kit pieces of advice. Briefly, one milliliter of mitochondria preparation (10 mg protein/ml) was treated with TCA (0.1%) and incubated on ice for 5 minutes. Finally, 100 μL of the supernatant was treated with 100 μL of the kit content, and the luminescence intensity was measured at $\lambda=560$ nm (FLUOstar Omega[®] multifunctional microplate reader). For the standardization of data, samples protein concentrations were determined by the Bradford method (43).

2.8. Statistical analysis

Data are represented as the mean \pm SD. Data comparison was made by the one-way analysis of variance (ANOVA) with Tukey's multiple comparisons as the post hoc test. Differences were considered statistically significant when $P<0.05$.

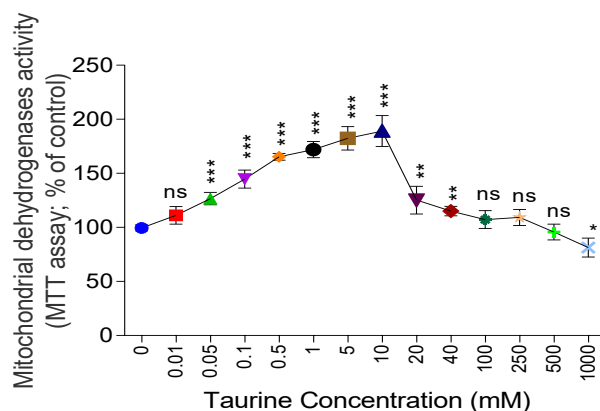


Figure 1. Mice liver mitochondrial dehydrogenases activity in a medium containing increasing concentrations of taurine. Data are represented as mean \pm SD (n=7). Asterisks indicate significantly different as compared with the control group (0 mM taurine) (* $P<0.05$, ** $P<0.01$, *** $P<0.001$).ns: not significant as compared with the control group (0 mM taurine).

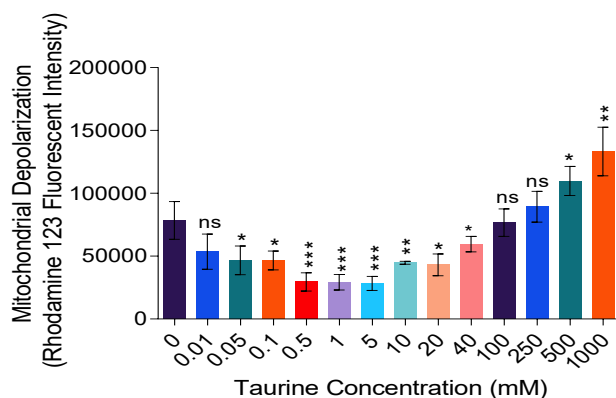


Figure 2. Mitochondrial depolarization in the presence of different taurine concentrations. Data are represented as mean \pm SD (n=7). Asterisks indicate significantly different as compared with the control group (0 mM taurine) (*P<0.05, **P<0.01, ***P<0.001). ns: not significant as compared with the control group (0 mM taurine).

3. Results

Mitochondrial dehydrogenases activity was evaluated in a medium containing increasing concentrations of TAU (Figure 1). It was found that concentrations between 0.05 to 40 mM of this amino acid significantly increased mitochondrial dehydrogenases activity. On the other hand, even very high concentrations of TAU (100, 250, and 500 mM) had no adverse effects on mitochondrial dehydrogenases activity in comparison with the

control group (0 mM TAU) (Figure 1). Finally, it was found that 1000 mM of TAU adversely affected mitochondrial dehydrogenases activity and significantly decreased this parameter (Figure 1).

Concentrations between 0.05 mM-20 mM of TAU positively enhanced the mitochondrial capability to capture rhodamine 123 (Mitochondrial polarization) (Figure 2). On the other hand, concentrations of TAU between 100-250 mM had no significant difference with the control group (Figure 2). It was found that 500- and 1000-mM TAU

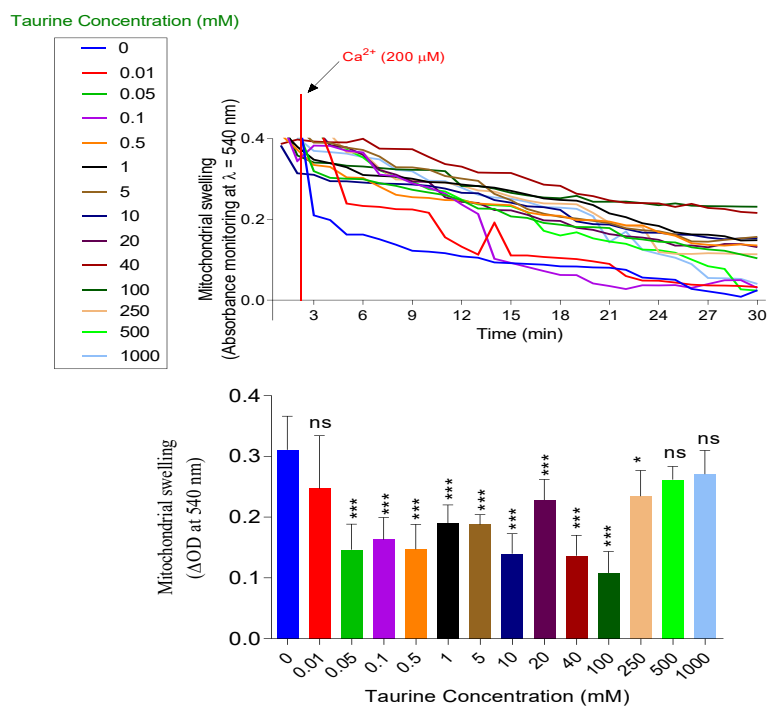


Figure 3. Calcium (Ca^{2+})-induced mitochondrial swelling in the taurine-containing medium. Data are represented as mean \pm SD (n=7). Asterisks indicate significantly different as compared with the control group (0 mM taurine) (*P<0.05, **P<0.01, ***P<0.001). ns: not significant as compared with the control group (0 mM taurine).

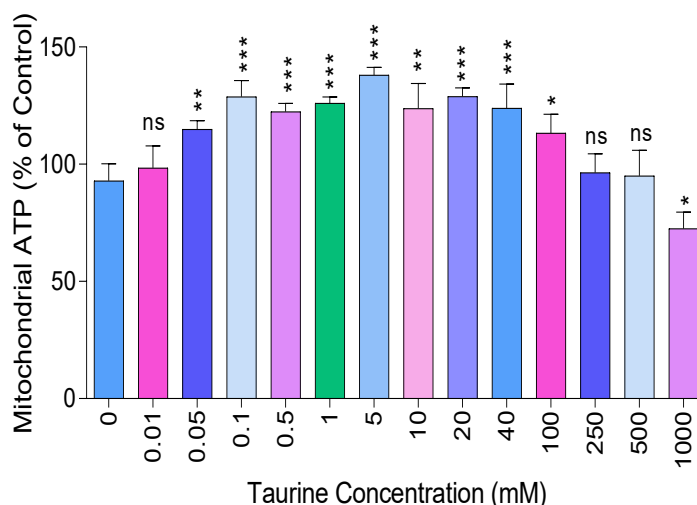


Figure 4. Mitochondrial ATP level in the presence of taurine. Data are represented as mean±SD (n=7). Asterisks indicate significantly different as compared with the control group (0 mM taurine) (*P<0.05, **P<0.01, ***P<0.001). ns: not significant as compared with the control group (0 mM taurine). significantly depolarized isolated liver mitochondria (Figure 2).

TAU (0.05-250 mM) administration significantly decreased Ca²⁺-induced mitochondrial permeabilization and swelling (Figure 3). On the other hand, the concentrations of 0.05, 500, and 1000 mM of TAU had no significant effect on mitochondrial permeabilization in the current study (Figure 3).

The effects of TAU on mitochondrial ATP production were also evaluated (Figure 4). It was found that TAU (0.05-100 mM) significantly enhanced mitochondrial ATP levels (Figure 4). On the other hand, the effects of 250 and 500 mM of TAU was not significantly different as compared with the control group (0 mM TAU) (Figure 4). It was also found that 1000 mM TAU could significantly deteriorate mitochondrial ATP synthesis (Figure 4).

4. Discussion

Since its introduction in 1827, numerous investigations have been done on the physiological and pharmacological properties of taurine (TAU) (44). Interestingly, the Springer publishing company publishes a book series on the investigations about this amino acid (e.g., <https://www.springer.com/gp/book/9789811380228>). The capacity of the human liver for TAU synthesis is low (1). Therefore, body TAU is mainly provided

by dietary sources. TAU concentration reaches to high levels in tissues such as cardiac and skeletal muscle (e.g., 6.5 mmol/g wet weight) (45, 46).

A wide range of therapeutic effects has been attributed to this semi-amino acid (5, 47). The effects of TAU on several disorders such as cardiovascular complications, metabolic diseases, hepatic function, CNS disorders, immune system dysregulations, inflammatory disorders, and renal impairment have been repeatedly investigated (48).

Several physiological roles, including the adjustment of cellular osmotic pressure, regulation of cytoplasmic Ca²⁺ homeostasis, enzyme activity, cell signaling, as well as stabilizing lipid membranes, have been proposed for TAU (49, 50). On the other hand, some mechanisms have been proposed for the cytoprotection provided by TAU. Regulation of cellular Ca²⁺ homeostasis is a pivotal mechanism for TAU (51, 52). It has been documented that TAU effectively decreased the release of Ca²⁺ from the endoplasmic reticulum (ER) as its primary cellular source and prevent ER stress (53-56). On the other hand, another exciting mechanism for TAU in cellular Ca²⁺ homeostasis is mediated through the effects of this amino acid on mitochondria (57, 58). TAU is actively transported to the cytoplasm and finally to the mitochondrial matrix, which is also a transporter-mediated phenomenon (1, 18, 59). Some investigations also

mentioned TAU synthesis in the mitochondrial matrix (60). As previous studies mentioned, cellular mitochondria act as safety valves for regulating cytoplasmic calcium (57, 58). Interestingly, it has been found that TAU could enhance mitochondrial capability for Ca²⁺ sequestration (57, 58). In the current study, we found that a wide range of TAU concentrations could effectively encounter Ca²⁺-induced mitochondrial permeabilization and swelling (Figure 3). These data could indicate that TAU, even at extremely high concentrations, is a safe agent against disorders where ER stress and cytoplasmic Ca²⁺ dysregulation is involved. It is noteworthy to mention that the adverse effects of high TAU concentrations (e.g., 1000 mM) might be associated with the dysregulation of the mitochondrial microenvironment (e.g., impairment of osmotic pressure of the mitochondria medium).

Another interesting finding of TAU is its incorporation in the basic mitochondrial structures (4, 18). Transferring RNA (tRNA) are proteins responsible for transferring amino acids to the ribosome. Hence, these molecules play a pivotal role in protein synthesis. Interestingly, it has been found that TAU plays a fundamental role in the formation of a functional tRNA (4, 16, 18). Several mitochondrial cytopathies and diseases have been identified in the association of defected TAU modification of mitochondrial tRNA (17, 18, 61, 62).

Disturbed mitochondrial protein synthesis

5. References

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induced by defected tRNA could lead to severe complications. For example, the synthesis of vital proteins such as mitochondrial respiratory complexes is impaired. These events could finally lead to mitochondria-facilitated ROS formation and severe defects in ATP synthesis. Low ATP levels and cellular energy crisis is dangerous in organs with high energy demand (e.g., Heart and brain).

Recently, TAU is under clinical trials for several diseases associated with mitochondrial disorders (19, 20) and stroke-like episodes of these diseases (19, 20). All these data could mention the importance of investigating the effects of different TAU concentrations (especially supraphysiological levels) on its primary target organelle (mitochondria) to ensure its safety and obtaining the most effective concentration(s) for application in the clinical settings. Conclusively, such investigations might help to the development of new therapeutic strategies against mitochondria-linked human diseases.

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Conflict of Interest

None declared.

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