# PRELIMINARY DATA ON TAURINE EFFECTS IN CUTANEOUS AND VISCERAL PAIN MODELS

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## REZUMAT

### Rezultatele preliminare ale efectelor taurinei în durerea cutanată şi visceralã

Taurina este un derivat al aminoacizilor care conţin sulf. Chimic este acid 2-aminoetansulfonic. Este sintetizată în organism în ficat, provenind din metionină şi cisteină, cu implicarea vitaminei B6. Date din literatură arată implicarea taurinei în numeroase procese biofizice, biochimice şi fiziologice din organismul uman, aceasta având rol de neurotransmiţător sau de neuromodulator.

**Scopul experimentului:** studierea efectelor taurinei pe modele de durere somatică şi visceralã şi asupra comportamentului spontan la şoareci.

**Material şi metodă:** Pentru experiment s-au utilizat şoareci albi Swiss (greutate 20-25g), cu repartiţie uniformă pe sexe, la care s-au administrat substanţele intraperitoneal în acelaşi volum:

| Grupul I: | Grupul II: |
|---|---|
| Lot 1. ser fiziologic 0,3ml | Lot 4. ser fiziologic 0,3ml |
| Lot 2. taurină 200mg/kg | Lot 5. taurină 200mg/kg |
| Lot 3. taurină 1000mg/kg | Lot 6. taurină 1000mg/kg |

Efectele taurinei au fost studiate la testele hot-plate şi tail-flick, testul Activity Cage şi la testul de contorsiune cu acid acetic 0,6%.

Analiza statistică s-a efectuat prin programul SPSS versiunea 9.0, cu ajutorul metodei ANOVA unifactorială, urmată de testul post-hoc Bonferonni.

**Rezultate:** La testele de durere somatică, taurina manifestă efecte antinociceptive doar la doza de 1000mg/kg şi numai după 60 minute după aplicare stimulului termic dureros. La testul de durere visceralã taurina administrată atât în doză de 200mg/kg cât şi de 1000mg/kg produce efecte antinociceptive semnificative. În condiţiile noastre experimentale, indiferent de doza administrată (200mg/kg şi 1000mg/kg) taurina nu influenţează comportamentul motor global al animalelor.

**Cuvinte cheie:** taurina, nocicepţie, hot-plate, tail-flick, activity cage.

## ABSTRACT

Taurine (2-aminoethane sulfonic acid) is a conditionally essential nutrient. Its synthesis in humans is from these two amino acids, primarily in the liver with assistance of vitamin B6 (pyridoxal-5' phosphate). Taurine, either directly or indirectly, is able to exert either a proven or highly probable role in a number of diverse conditions that affect nearly every major organ or body system and is responsible for a wide variety of critical body processes.

**The aim of our study** was the experimental research of the effects of taurine in some cutaneous, visceral and behavioral pain models.

**Material and method:** The experiment was carried out, with white mice (20-25g). Mice were distributed into 2 groups with 3 lots of 7 animals each, with equal repartition between sexes, treated intraperitoneally with the same volume of solution as follows:

| Group I: | Group II: |
|---|---|
| Lot 1. saline solution 0,3ml | Lot 4 saline solution 0,3ml |
| Lot 2. taurine 200mg/kbw | Lot 5. taurine 200mg/kbw |
| Lot 3. taurine 1000mg/kbw | Lot 6. taurine 1000mg/kbw |

The nociceptive cutaneous testing was performed using hot plate (Ugo Basile) and tail-flick (Ugo Basile) assays. Writhing test has used as a visceral pain test. The taurine psycho-motor abilities were tested in the Activity Cage device in order to investigate the both global motor behavior and the number of escape attempts.

**Conclussions:** Using tests for somatic we determined taurine at the dose at 1000mg/kbw could produce antinociceptive actions 60 minutes after the thermal noxious stimulation. In visceral pain model used, both doses of taurine (200mg/kbw and 1000mg/kbw) exhibited significant antinociceptive effects. Both doses of taurine administered in our experiment (200mg/kbw and 1000mg/kbw) do not influence the global motor behavior in mice.

**Key words:** taurine, nociception, hot-plate, tail-flick, activity cage.


## Introduction:

Taurine (2-aminoethane sulfonic acid) is a conditionally essential nutrient. As such, taurine is derived directly from the breakdown of food but the body can produce its own taurine from other pre-proteins (the amino acids methionine and cysteine). Its synthesis in humans is from these two amino acids, primarily in the liver with assistance of vitamin B6 (pyridoxal-5' phosphate).

Its role in the functions of the body has been long underestimated. Absence of taurine does not result in immediate deficiency and disease, but long-term deprivation can cause a multitude of health problems.

Taurine, either directly or indirectly, is able to exert either a proven or highly probable role in a number of diverse conditions that affect nearly every major organ or

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body system and is responsible for a wide variety of critical body processes. Most notably, it promotes bile secretion and hepatic processes, cellular functions in the brain and retina, and optimizes cardiac and circulatory performance.

The main interest has been into the importance of taurine as neurotransmitter, in which role it functions with glycine and gamma-aminobutyric acid, two neuroinhibitory transmitters. (Whitton P.S. et al., 1988) (Liu Q.R. et al., 1992)

The aim of our study was the experimental research of the effects of taurine in some cutaneous, visceral and behavioral pain models. We wanted to establish the activity profile of taurine on cutaneous, on a general accepted visceral pain model, and in behavioral experimental model, after intraperitoneal administration.

## Material and method:

The experiment was carried out, with white mice (20-25g). Standard laboratory food and tap water were freely available, except during the time of the experiment.

Mice were distributed into 2 groups with 3 lots of 7 animals each, with equal repartition between sexes, treated intraperitoneally with the same volume of solution as follows:

| Group I: | Group II: |
|---|---|
| Lot 1. saline solution 0,3ml | Lot 4 saline solution 0,3ml |
| Lot 2. taurine 200mg/kbw | Lot 5. taurine 200mg/kbw |
| Lot 3. taurine 1000mg/kbw | Lot 6. taurine 1000mg/kbw |

The nociceptive cutaneous testing was performed using hot plate (Ugo Basile) and tail-flick (Ugo Basile) assays. These experimental models consist of mice paws and respectively tail noxious thermal stimulation, followed in both situations by counting the response latency period.

Writhing test has proven predictive value as a screening tool for analgesic actions. This model of visceral pain consists of chemical peritoneal irritation induced by diluted acetic acid (0.6%). Pain responses were scored by counting the number of stretches or writhes per animal, every 5 minutes, during 30 minutes period after intraperitoneal injection of diluted acetic acid. Stretches or writhes (arching of the back, development of tension in the abdominal muscles, elongation of the body and extension of the forelimbs) are viscero-somatic reflex responses to noxious colorectal irritation. Hand-operated counters and stopwatches were employed to score writhing frequency of the mice placed in glass cages. (Tarţău L, Mungiu O.C., 2004)

The taurine psycho-motor abilities were tested in the Activity Cage device in order to investigate the both global motor behavior and the number of escape attempts. Mice were placed on the cage device and each movement produced a signal caused by variation in inductance and capacity of the apparatus resonance circuit. These signals were automatically converted to numbers. The horizontal and vertical mice movements were automatic counted for 2 minutes period intervals.

The experiment was performed according to the guidelines of the IASP Committee for Research and Ethical Issues. (Zimmermann, 1983) In particular, the duration of the experiments was kept as short as possible and the number of mice. For ethical reasons, all the animals were sacrificed at the end of the experiment.

Test latencies obtained were analyzed with SPSS for Windows version 9.0.

All values corresponding for every time period were processed using ANOVA one-way analysis followed by Bonfferoni post-hoc test. Results were expressed as arithmetic mean±SD and represented in the following graphs. P- values less then 0.05 are considered statistically significant comparing with those of control groups.

The drugs used were: taurine (Sigma, USA), acetic acid (Reactivul, Bucureşti), saline solution (Sicomed, Romania). All drugs were dissolved in saline, prepared immediately before use and administered intraperitoneally in the same volume of solution.

## Results:

1. The tail-flick test results were presented in the graph number 1.

Statistical analysis of the results obtained in tail-flick test show that:

- intraperitoneal administration of taurine 200mg/kg

**Table nr. I** **Tail-flick Latency (Seconds)**

| Determination Time | | N | Mean | Std. Deviation | Std. Error | Minimum | Maximum | P Bonferroni |
|---|---|---|---|---|---|---|---|---|
| 0 minutes | Control | 7 | 6,7429 | ,80178 | ,30305 | 5,60 | 7,70 | |
| | taurine200mg/kg | 7 | 6,3143 | ,36253 | ,13702 | 6,00 | 6,90 | 0,514 |
| | taurine1000mg/kg | 7 | 5,9429 | ,41975 | ,15865 | 5,20 | 6,40 | 0,048* |
| 15 minutes | Control | 7 | 5,7857 | ,52418 | ,19812 | 5,20 | 6,80 | |
| | taurine200mg/kg | 7 | 5,6429 | 1,14871 | ,43417 | 3,50 | 6,90 | 1,000 |
| | taurine1000mg/kg | 7 | 6,4714 | ,37733 | ,14262 | 5,80 | 6,80 | 0,327 |
| 30 minutes | Control | 7 | 6,0286 | ,77398 | ,29254 | 5,00 | 6,90 | |
| | taurine200mg/kg | 7 | 5,3286 | 1,32252 | ,49986 | 3,50 | 6,70 | 0,526 |
| | taurine1000mg/kg | 7 | 7,4286 | ,48550 | ,18350 | 6,90 | 8,40 | 0,034* |
| 60 minutes | Control | 7 | 6,0857 | ,91911 | ,34739 | 4,60 | 7,30 | |
| | taurine200mg/kg | 7 | 5,7143 | ,99235 | ,37507 | 4,10 | 6,60 | 1,000 |
| | taurine1000mg/kg | 7 | 7,4286 | ,54072 | ,20437 | 6,80 | 8,10 | 0,024* |

has no significant effect on mice reaction latency time.

- the dose of 1000mg/kbw taurine increase the latency time, statistically significant immediately after substance administration and after 30 minutes and 60 minutes of thermal noxious stimulation ($p<0,05$).

2. The hot-plate test results were presented in the graph number 2.

- in hot-plate test data analysis revealed no significant differences in effect mice reaction latency time, between the two doses of taurine, in the first 30 minutes after noxious stimulation.
- taurine 1000mg/kbw increase the latency time, statistically significant ($p>0,05$), only after 60 minutes of the thermal stimulation.

Hot plate taurine 1000mg/kg Latency (Seconds) (Graph no. 3)

According to the late effect observed for 1000mg/kbw taurine, we supplemental investigate the mice latency time reaction after 60 minutes of the noxious stimulation and we observe that this dose of taurine significant increase the latency time of the animals reaction ($p>0,05$).

3. The writhing test results were presented in the graph number 4.

Statistical analysis of the results obtained in writhing test shows that:

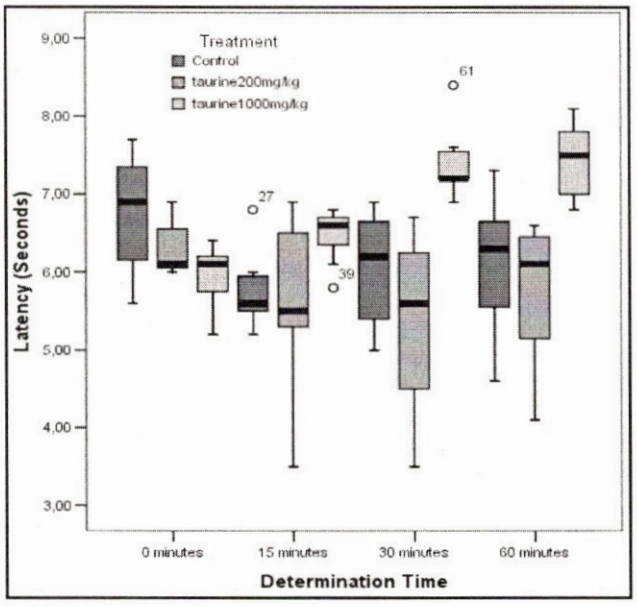
- intraperitoneal administration of taurine (200 and

**Table nr. II** **Hot plate Latency (Seconds)**

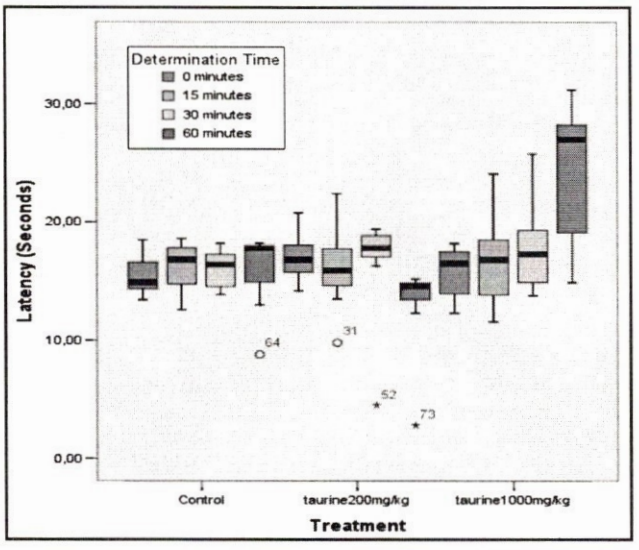
| Determination Time | | N | Mean | Std. Deviation | Std. Error | Minimum | Maximum | P Bonferroni |
|---|---|---|---|---|---|---|---|---|
| 0 minutes | Control | 7 | 15,5286 | 1,94997 | ,73702 | 13,40 | 18,50 | |
| | taurine200mg/kg | 7 | 17,0571 | 2,15086 | ,81295 | 14,20 | 20,80 | 0,598 |
| | taurine1000mg/kg | 7 | 15,7000 | 2,32236 | ,87777 | 12,30 | 18,20 | 1,000 |
| 15 minutes | Control | 7 | 16,1571 | 2,24415 | ,84821 | 12,60 | 18,60 | |
| | taurine200mg/kg | 7 | 16,1286 | 3,95631 | 1,49534 | 9,80 | 22,40 | 1,000 |
| | taurine1000mg/kg | 7 | 16,7429 | 4,39919 | 1,66274 | 11,60 | 24,10 | 1,000 |
| 30 minutes | Control | 7 | 16,0143 | 1,69059 | ,63898 | 13,90 | 18,20 | |
| | taurine200mg/kg | 7 | 16,2143 | 5,26480 | 1,98991 | 4,50 | 19,40 | 1,000 |
| | taurine1000mg/kg | 7 | 17,9000 | 4,13118 | 1,56144 | 13,80 | 25,80 | 1,000 |
| 60 minutes | Control | 7 | 15,7857 | 3,57977 | 1,35303 | 8,80 | 18,20 | |
| | taurine200mg/kg | 7 | 12,7429 | 4,48845 | 1,69647 | 2,80 | 15,20 | 0,815 |
| | taurine1000mg/kg | 7 | 23,9714 | 6,52909 | 2,46776 | 14,90 | 31,20 | 0,021* |

**Table nr. III** **Writhing test (number of abdominal stretches)**

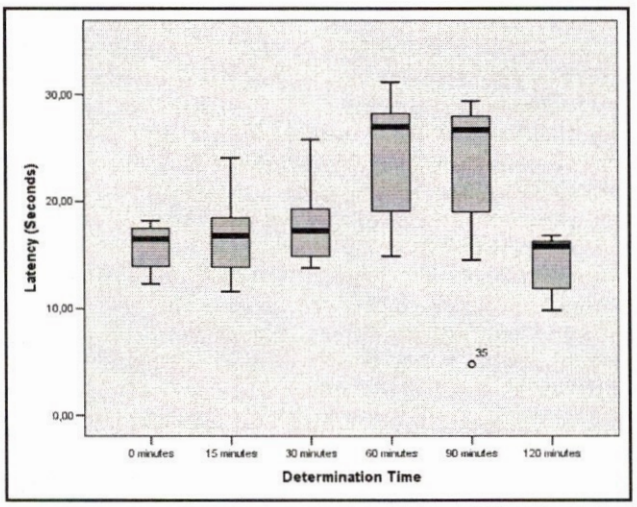
| Time interval | | N | Mean | Std. Deviation | Std. Error | Minimum | Maximum | P Bonferroni |
|---|---|---|---|---|---|---|---|---|
| 5-10 minutes | Control | 7 | 34,1429 | ,69007 | ,26082 | 33,00 | 35,00 | |
| | taurine200mg/kg | 7 | 27,5714 | 4,46681 | 1,68830 | 19,00 | 34,00 | 0,009* |
| | taurine1000mg/kg | 7 | 19,4286 | 4,19750 | 1,58651 | 13,00 | 24,00 | 0,000* |
| 10-15 minutes | Control | 7 | 33,8571 | 1,34519 | ,50843 | 32,00 | 35,00 | |
| | taurine200mg/kg | 7 | 29,0000 | 9,07377 | 3,42956 | 17,00 | 38,00 | 0,568 |
| | taurine1000mg/kg | 7 | 22,4286 | 6,99660 | 2,64447 | 14,00 | 31,00 | 0,015* |
| 15-20 minutes | Control | 7 | 33,1429 | 1,46385 | ,55328 | 31,00 | 35,00 | |
| | taurine200mg/kg | 7 | 21,0000 | 5,62731 | 2,12692 | 12,00 | 27,00 | 0,000* |
| | taurine1000mg/kg | 7 | 17,1429 | 5,01427 | 1,89521 | 11,00 | 25,00 | 0,000* |
| 20-25 minutes | Control | 7 | 32,8571 | ,89974 | ,34007 | 32,00 | 34,00 | |
| | taurine200mg/kg | 7 | 20,2857 | 3,40168 | 1,28571 | 15,00 | 24,00 | 0,000* |
| | taurine1000mg/kg | 7 | 13,5714 | 3,90969 | 1,47773 | 7,00 | 19,00 | 0,000* |
| 25-30 minutes | Control | 7 | 32,4286 | 1,13389 | ,42857 | 31,00 | 34,00 | |
| | taurine200mg/kg | 7 | 15,5714 | 4,64963 | 1,75739 | 8,00 | 20,00 | 0,000* |
| | taurine1000mg/kg | 7 | 11,2857 | 2,87021 | 1,08484 | 8,00 | 15,00 | 0,000* |
| 30-35 minutes | Control | 7 | 31,4286 | 1,51186 | ,57143 | 29,00 | 34,00 | |
| | taurine200mg/kg | 7 | 15,5714 | 3,77964 | 1,42857 | 11,00 | 22,00 | 0,000* |
| | taurine1000mg/kg | 7 | 9,4286 | 3,30944 | 1,25085 | 4,00 | 13,00 | 0,000* |



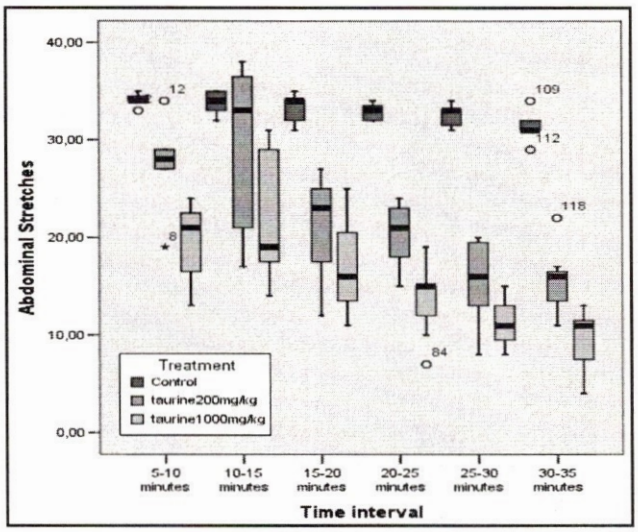

***Graph no. 1***



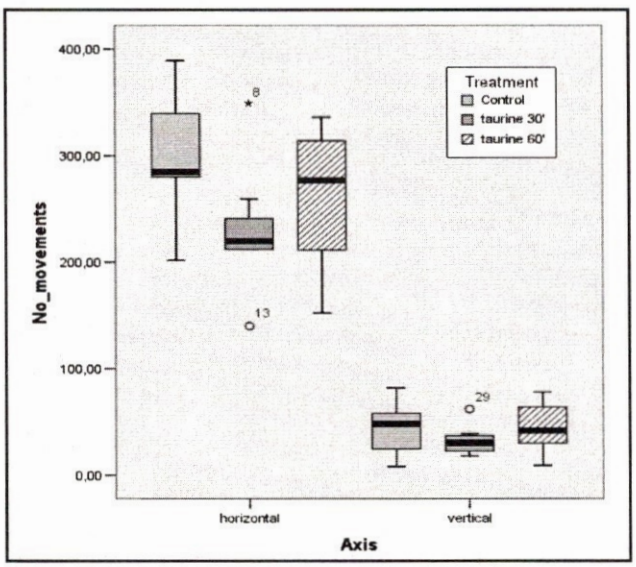

***Graph no. 2***




***Graph no. 3***




***Graph no. 4***




***Graph no. 5***

**Table nr. IV** **Activity cage (Number of movements)**

| Axis | | N | Mean | Std. Deviation | Std. Error | Minimum | Maximum | P Bonferroni |
|---|---|---|---|---|---|---|---|---|
| horizontal | Control | 7 | 302,1429 | 62,58442 | 23,65469 | 202,00 | 389,00 | |
| | taurine 30' | 7 | 230,8571 | 63,18341 | 23,88108 | 140,00 | 349,00 | 0,166 |
| | taurine 60' | 7 | 259,4286 | 69,25281 | 26,17510 | 152,00 | 336,00 | 0,706 |
| vertical | Control | 7 | 43,2857 | 26,89928 | 10,16697 | 8,00 | 82,00 | |
| | taurine 30' | 7 | 32,7143 | 15,05229 | 5,68923 | 18,00 | 62,00 | 1,000 |
| | taurine 60' | 7 | 45,2857 | 24,49295 | 9,25747 | 9,00 | 78,00 | 1,000 |

1000mg/kbw), resulted in a significant and dose-dependent reduction of the writhes number ($p<0,05$).

4. The Activity results were presented in the graph number 5.

Statistical analysis of the results obtained in Activity Cage test shows that:

- in our experimental conditions both doses of taurine do not influence both global motor behavior and the number of escape attempts which could correspond somehow to agitation in humans.

## Discussions:

Checking the scientific literature data, one can conclude that the most of relevant communicated studies about the influence of the taurine influence in nociceptive processes have been few and inconclusive.

Literature data shows that taurine is found in the developing brain in concentrations up to four times that found in adult brain. (Huxtable R. et al., 1992) Since taurine acts as a suppressor of neuronal activity in the developing brain, during the phase when other regulatory systems are not fully developed, it is thought that deficiency of taurine, at this stage, might contribute towards, or predispose the individual to epilepsy. Taurine has been shown in human trials to have an anticonvulsive effect.

The physiological role of taurine in the central nervous system remains obscure. Recent attempts to localize the sites of taurine biosynthesis in the brain demonstrated immunostains of cysteine sulfonate decarboxylase in rows like oligodendrocytes and cells around the Purkinje cells in the cerebellum. (Almarghini K. et al., 1991) Activity of the enzyme was also detected in glial cells fractions enriched with oligodendrocytes and astrocytes. The high taurine concentrations in brain suggest an important role of transport along-side the biosynthesis of taurine. (Liu Q.R. et al., 1992) (Bitoun M. et al., 2000)

Taurine regulates the most basic of cell functions - genetic transcription (Maar T.E. et al., 1998). It was demonstrated in mice that taurine acts as both an osmoregulator (to balance cell volume) and neuromodulator (protecting against over-excitation that may lead to cell death). Taurine prominently concentrated in glial cells in the supraoptic nucleus is probably involved in the inhibition of supraoptic nucleus vasopressin neurons by peripheral hypotonic stimulus, via activation of neuronal glycine receptors. These data suggest the implication of taurine and glial cells in the regulation of the whole-body fluid balance, through the modulation of vasopressin release. (Deleuze C. et. al., 1998) (Oja S.S. et al., 1996)

Taurine is found abundantly in tissues that are excitable, rich in membranes, and that generate oxidants. Thus, it is the most prevalent of all the amino acids in the tissues comprising the skeletal and cardiac muscles and the brain.

Taurine is essential for vision, directly to execute muscular motion and control, and indirectly to prevent disorders such as diabetes and cancer.

Taurine is also the precursor to valuable analogs and derivative substances. Recently, the value of the taurine precursor hypotaurine as a potent antioxidant has been discovered. If present in sufficient concentration where oxidation commonly occurs (like the brain), hypotaurine may protect against oxidative cellular damage. (Aruoma O.I. et al., 1988) The sulfinyl group in the hypotaurine molecule is responsible for its efficiency as a radical scavenger. The process through which hypotaurine proceeds to taurine has been shown to effectively scavenge free radicals.

Taurine is now being explored for its capacity to protect tissue against oxidative stress. In cerebellar neurons, stimulation by excitatory agents was effectively countered by taurine. While taurine did not directly decrease the levels of free radicals, it did increase cell viability. This may become an important alternate protective mechanism to offering protection against free radical damage. (Shioda R. et al., 2002) (Aruoma O.I. et al.1988)

Taurine has been shown to act as a preventive treatment against the disturbances associated with hypoxia. Taurine modulates the enzymes involved in energy metabolism in the brain, restoring adenine and ATP while reducing ADP and AMP levels. Through its ability to preserve the glutathione peroxidase system, recognized for its potent antioxidant capabilities, taurine protects cells from lipid peroxidation and deleterious membrane structure changes.

In the cerebral cortex, exposure to glutamate and aspartate causes intracellular swelling directly proportional to the increase in taurine. The release of taurine, prompted by the excitatory amino acids, is a function of the movement of ions and water across cell membrane. Calcium causes a release of taurine, GABA, aspartate, glutamate, glycine, and alanine in hypoxia, the proportions of which are guided by potassium-induced modifications to the cell membrane. Levels of taurine release are also modulated by the presence of both potassium and sodium ions. Ions regulate the release of taurine in the hypoxic or ischemic state, and an increased release of taurine may act to preserve neurons (12). (Sergeeva O.A. et al., 2003)

Taurine release is enhanced by N-methyl-D-aspartate (NMDA), an agonist (promoter) of glutamate receptors. Nitric oxide is the messenger that prompts NMDA to evoke taurine release. (Albrecht J., 1998).

Certain electrophysiologic studies show that acute pain stress reduced taurine levels in the hypothalamus and the

lower brainstem nuclei but not in the cortical areas. (Palkovits M. et al., 1986) Furthermore, in the presence of taurine a concentration-dependent enhancement of [3H]GABA release was observed. (Whitton P.S. et al., 1988)

## Conclusions:

1. Using tests for somatic we determined taurine at the dose at 1000mg/kbw could produce antinociceptive actions 60 minutes after the thermal noxious stimulation.

2. In visceral pain model used, both doses of taurine (200mg/kbw and 1000mg/kbw) exhibited significant antinociceptive effects.

3. Both doses of taurine administered in our experiment (200mg/kbw and 1000mg/kbw) do not influence the global motor behavior in mice.

4. These results may be due to the small number of the animal used in the experiment.

5. However, further researches are needed in order to establish the effective doses of taurine and its interaction between opioid, glutamatergic and serotoninergic pathways.

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