Chapter 21
Effects of Taurine on Anxiety-Like and Locomotor Behavior of Mice

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Abstract Taurine is one of the most abundant free amino acids especially in excitable tissues, with wide physiological actions. We have previously reported that chronic supplementation of taurine in drinking water to mice increases brain excitability, mainly through alterations in the inhibitory GABAergic system. In this study we investigated the effects of chronic versus acute taurine treatment on anxiety-like and locomotor behaviors using two behavioral tests: elevated plus-maze and open-field. These two test conditions generated different levels of anxiety, and both anxiolytic and anxiogenic effects of taurine could be assessed. We used two paradigms for taurine treatment: Acute injection versus chronic supplementation. In the open field test, taurine supplementation increased whereas taurine injection suppressed locomotor activity. We found that taurine supplementation induced an increase in the total distance traveled, the overall movement speed, the time the animals spent mobile, the number of line crossings, and the time the animals entered the center zone. In the elevated arm maze, taurine injection suppressed anxiety whereas taurine supplementation was anxiogenic. The major findings of this are two folds: First these results suggest that taurine might play a role in the modulation of anxiety and locomotor activity. Second, taurine when injected acutely had opposite effects than when administered chronically.

Abbreviation *Tau*, taurine; *KA*, kainic acid; *Inj,* injected

21.1 Introduction

Anxiety disorders are considered the most common psychiatric diagnoses, affecting between 10 and 30% of the general population. Excess anxiety can be debilitating and damage the quality of life. Benzodiazepines have been extensively used for the treatment of several forms of anxiety, although these compounds have well-known side-effects such as sedation, muscle relaxation, amnesia, and dependence (Rickels and Schweizer 1997). The development of new anxiolytic drugs has been an area of

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interest. In a search for new anxiolytic compounds, various types of non-traditional medicines have been used in the world today (Rex et al. 2002).

The GABAergic system plays a very important role in the regulation of anxiety. Since taurine interacts with GABAA receptors and mimics the actions of GABA, we determined the effects of taurine on this type of behavior. Furthermore, we investigated the relationship between taurine and anxiety in to two treatment paradigms: acute and chronic. We examined the effects of taurine on the behavior of mice in the elevated plus-maze, a most commonly used animal models of anxiety and in an open-field test that provides a simple method for measuring the response of an animal to an unfamiliar environment.

Taurine, 2-aminoethane-sulphonic acid, is a sulfur-containing amino acids found in relatively high concentrations in the central nervous system of mammals. Taurine has been shown to be essential for the development, survival, growth of vertebrate neurons (Hayes et al. 1975). Taurine deficiency has been confirmed in many neuropathological conditions, such as epilepsy (Barbeau et al. 1975; Joseph and Emson 1976), mental depression(Perry 1976), and the alcohol withdrawal syndrome (Ikeda 1977). Furthermore, we have shown that acute taurine injections increased the threshold of pharmacologically-induced convulsions (El Idrissi et al. 2003). Taurine (43 mg/kg, s.c) significantly increased the latency and decreased the duration of convulsions induced by kainic acid. While is well established that taurine acts as an agonist for $GABA_A$ receptors, chronic supplementation of taurine to mice, induces biochemical alterations in the inhibitory system (El Idrissi and Trenkner 2004). These alterations tend to increase neuronal excitability, eliciting therefore the opposite effects than acute injections of taurine. Thus, we examined in this study the effects of chronic versus acute treatment with taurine.

21.2 Methods

21.2.1 Open-Field Apparatus

The open-field used is a 60 × 60 cm square arena with 15 cm high walls. It is subdivided into 3 square sectors designated as outer, middle and inner zone. A male laboratory mouse is placed in the centre, covered by a small dome which was pulled up by an operator when the learners' recording activity begins. A video camera is positioned at about 1.5 m above the arena, immediately inside the vertical projection of a wall, covering the entire view of the arena. Animals were monitored for 10 min. After each run the mouse was returned to its home cage and the maze was cleaned with a damp sponge to remove any trace of odor.

21.2.2 Elevated-Plus Maze Apparatus

The elevated plus-maze used in this study comprised two opposing open arms (30 × 5cm) and two closed arms (30 × 5 × 15cm), which joined at a square central area

(5×5cm) to form a plus sign. The maze floor was constructed of black Plexiglas and the side/end walls (15cm height) of the enclosed arms of clear Plexiglas. To reduce the likelihood of falling-over, a slight raised edge (0.25 cm) around the perimeter of the open arms provided additional grip for the animals. The entire apparatus was elevated to a height of 45 cm above the floor by a single central support and four 25-W red fluorescent lights arranged as a cross at 100 cm above the maze were used as the source of illumination (Chen et al. 2003). Testing commenced by placing a mouse on the central platform of the maze facing an open arm. Its behavior on the plus-maze was recorded for 5 min by a vertically mounted video camera linked to a monitor and video recorder in an adjacent laboratory. After each run the mouse was returned to its home cage and the maze was cleaned with a damp sponge to remove any trace of odor.

21.2.3 Data Analysis

Data were recorded as digital video clips using an analog-digital converter. The movies were analyzed using AnyMaze software. Tracking of the animal was based on contrast relative to background. Different zones were labeled and indicated on the monitor. Two tracking points were specified one the head and the other the center of gravity of the animal. An excel spreadsheet was generated containing all the parameters specified.

21.2.4 Statistic Analysis

Multifactorial analysis of variance was used to identify overall condition effects. Significant changes were determined by post hoc comparisons of means using the Tukey HSD test. Significance was set at a confidence level of 95%. Data are presented as mean $\pm$ SEM.

21.3 Results

21.3.1 Effects of Taurine on Locomotor Activity in an Open-Field

Open-field test provides a simple method for measuring the response of an animal to an unfamiliar environment and can be used to detect emotionality, anxiety and/or responses to stress in animals. Using this behavioral test, we found that taurine injection (43 mg/kg, s.c) decreased all parameters measured in the open-field test when compared to controls. On the other hand, taurine supplementation in the drinking water (0.05%) for four weeks induced the opposite effects than acute injection. Table 21.1 shows the different parameters measured. Injection of taurine (43 mg/kg, s.c) 15 min before the test significantly decreased the total distance

Table 21.1 Effects of taurine on locomotor activity

	Con	Tau-Fed	Tau-inj
Total distance traveled 9 m	69.83	72.81	30.36
Overall average speed (m/s)	0.12	0.12	0.05
Total time mobile (s)	473.24	481.53	285.44
Total time immobile (s)	126.76	118.47	314.56
Total mobile episodes	34.79	33.29	31.00
Total immobile episodes	34.21	33.00	30.36
Number of line crossing	208.71	250.00	101.00
Total distance traveled by the head	89.31	96.91	42.25

Con controls, *Tau-Fed* taurine-fed (0.05% for 4 weeks), *Tau-inj* taurine-injected (43 mg/kg, s.c). All mice were 2 months old. Taurine (0.05%) was supplemented in the drinking water for 4 weeks. control, n=6; Tau-fed, n=7, Tau-inj, n=7.

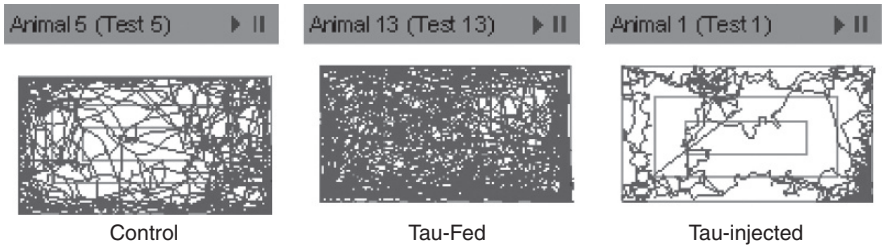

Fig. 21.1 Representative track plots depicting locomotor activity in an open-field. Mice were tracked for 10 min. Control mice spent most of the time around the periphery of the apparatus. On the other hand, taurine-fed mice moved significantly more. Injection of taurine (43 m/kg s.c) significantly suppressed locomotor activity and mice spent most of the time immobile in the corner of the platform

traveled, the average speed, the time the animal spent moving, and the number of line crossings. On the other, supplementation of taurine in the drinking water did not affect all parameters. However, the animals spent more time moving and therefore traveled longer distances. In doing so the taurine-fed mice crossed more lines than controls, although the speed of travel was the same as controls. Figure 21.1 shows a representative tracking plot of a control, a taurine-fed and a taurine-injected mice during the open-field test. Control mice (saline injected) spent more time around the periphery of the apparatus whereas taurine supplemented mice showed a significant increase in locomotor activity. On the other hand taurine injected mice showed a drastic reduction in the overall locomotor activity spending the bulk of the test time immobile around the corner of the apparatus.

21.3.2 Effects of Taurine on Anxiety-Like Behavior

To assess the effects of taurine on anxiety-like behavior, we used an elevated-plus maze. The elevated plus-maze is a well-established animal model for testing anxiolytic drugs (Dawson and Tricklebank 1995; Kulkarni and Reddy 1996). In the test,

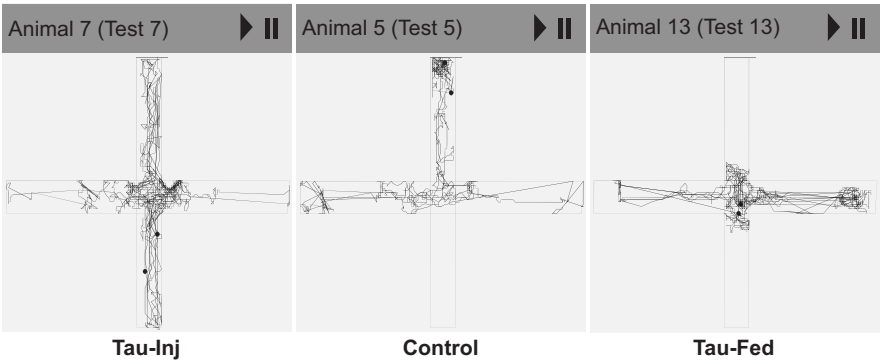

Fig. 21.2 Representative track plots showing motoric activity in an elevated plus maze. Mice were tracked for 5 min. Control mice spent approximately 20% of the test time exploring the open arm of the apparatus, shown here in the vertical position. On the other hand, taurine-injected (43 m/kg s.c) mice spent significantly more time exploring the open arm of the maze. Supplementation of taurine in drinking water (0.05% for 4 weeks) heightened the anxiety level and in some case the mice did not explore the open arm and spent all the time in the closed arm (shown here in the horizontal position)

the percentage of entries into the open arms and of the time spent in open arms have generally been used as indices of the anxiety.

Consistent with the open-field data, injection of taurine reduced the overall speed of locomotion (Table 21.1). However, the effects of taurine were more pronounced

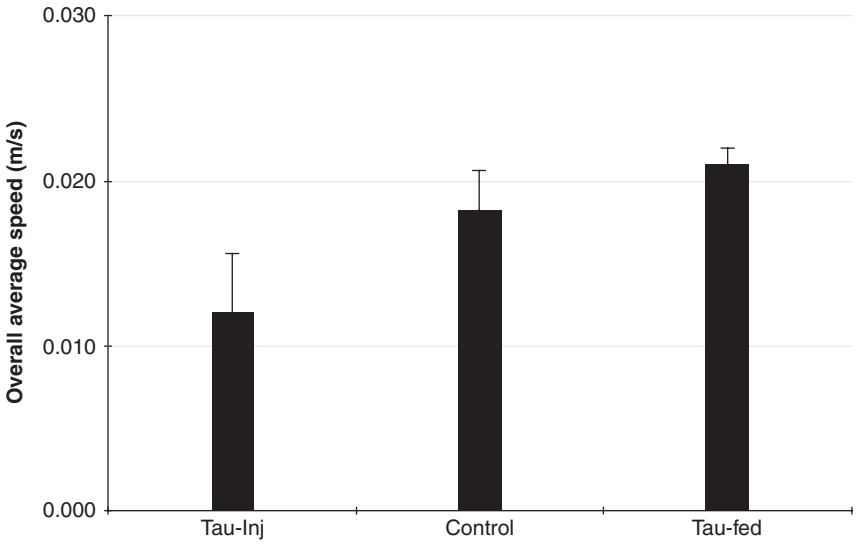

Fig. 21.3 Effects of taurine on speed of movement in the elevated plus maze. All mice were 2 months old. Taurine (0.05%) was supplemented in the drinking water for 4 weeks. Data represent mean ± SD. control, *n*=6; Tau-fed, *n*=7, Tau-inj, *n*=7

on anxiety–like behavior. In contrast to taurine-fed mice, taurine-injected mice spend more time in the open arm and less time in the closed arm (Fig. 21.2). In some cases, the anxiety level was so high in the taurine-fed mice that they did not explore the open arm at all. Thus, it seems that taurine, depending on the time and duration of treatment may have completely opposite effects on anxiety-like behavior. Acute injection of taurine has an axiolytic effect whereas chronic supplementation of taurine has an anxiogenic effect.

Consistent with the open-field data, injection of taurine reduced and supplementation of taurine to the drinking water increased the overall speed of locomotion in the elevated plus maze (Fig. 21.3). Therefore, the two behavioral test used in this study are consistent on the effects of taurine on locomotor activity. Acute taurine injection suppresses locomotor activity whereas chronic supplementation of taurine increases locomotor activity.

21.4 Discussion

Taurine is one of the major constituents of the free amino acid pool in the CNS (Shaw and Heine 1965; Guidotti et al. 1972). Taurine has a heterogeneous distribution in the brain with high levels found in cerebral cortical areas, hippocampus, caudate-putamen, and in cerebellum. Taurine enters the brain via a high affinity, saturable, sodium and chloride dependent carrier from blood to the endothelial cell (Benrabh et al. 1995). Once in the brain taurine may exert several biological effects. With respect to it neuromodulatory effects, taurine has been shown to interact with $GABA_A$ and glycine receptors. In recent years, the agonistic action of taurine on the inhibitory neurotransmission has been the focus of several studies. Taurine has been shown to be a low affinity agonist for $GABA_A$ receptors. Moreover, it has been found that taurine interacted with $GABA_A$ receptor-linked benzodiazepine receptor binding sites (Medina and DeRobertis 1984). Since the GABAergic system plays an important role in modulating anxiety-like behaviors and Diazepan/benzodiazepan, a typical anxiolytic drugs interact with $GABA_A$ receptor to the same binding site as taurine, we sought to determine the anxiety-modulatory role of taurine. Taurine binds to, and mimics the effects of GABA on the $GABA_A$ receptors. However, the functional consequence of taurine interaction with the inhibitory system is highly dependent on the duration of treatment. This is consistent with our finding of the effects of taurine on seizure threshold. Acute injection of taurine prior to seizure induction with kainic acid elevated seizure threshold exerting an anti-epileptic effect, whereas chronic supplementation of taurine has a pro-epileptic effect and lowered seizure threshold. We used the same treatment with taurine (acute and chronic) and evaluated the effects of taurine on anxiety and locomotor activity.

There are two major findings associated with the current work. Most importantly, taurine produced behavioral effects in both elevated plus maze and the open-field. The effect of taurine however was dependent on the treatment paradigm. Acute injection of taurine (43 mg/kg, s.c) has an anxiolytic effect whereas chronic supplementation in drinking water (0.05% for 4 weeks) had an anxiogenic effect.

Since acute injection of taurine activates $GABA_A$ receptors and chronic supplementation of taurine induces alteration in the inhibitory system, these data may suggest that taurine acting through the GABAergic system modulate anxiety-like behaviors. It has been shown that the strychnine-sensitive glycine receptors function to modulate anxiety-like behaviors *in vivo* (Danober and Pape 1998; McCool and Botting 2000). Since taurine interacts with these receptors as well, it is conceivable that the interaction of taurine with both inhibitory systems and the cooperative actions of these two systems is responsible for the observed effects of taurine on anxiety-like behaviors. Previous work has shown that $GABA_A$ receptor activation in the amygdala, either directly with agonist (Bueno et al. 2005) or indirectly with benzodiazepine agonist (Zangrossi and Graeff 1994), causes anxiolysis while inhibition with receptor antagonists cause anxiogenesis (Sanders and Shekhar 1995). Given that both glycine and $GABA_A$ receptors are ligand-gated chloride channels, we hypothesized that $GABA_A$ and/or glycine receptors activation by taurine would also produce anxiolytic effects under the conditions of our behavioral assays. On the other hand, chronic supplementation of taurine in drinking water, which increases neuronal excitability, produces anxiogenic effects.

Taurine elicited slightly distinct effects in the open-field and the elevated-plus maze where acute injection of taurine suppressed locomotor activity and chronic taurine supplementation increased locomotor activity in the open-field test. This is consistent with previous findings. In open-field test, injection of taurine significantly decreased ambulation levels, increased latency scores, and increased thigmotaxis(Sanberg and Ossenkopp 1977). While it may seem that acute taurine injection may be anxiogenic, since the number of visits to the center zone of taurine-injected mice was drastically reduced in the open-field test (Fig. 21.1), we think that this anxiogenic-like effect is a consequence of reduced locomotor activity. The assay-dependent taurine effects may also reflect distinct neurobiological contributions to anxiety-like behaviors expressed in each apparatus. The plus maze and open-field test share many characteristics. They are both perceived as unconditioned responses to naturally aversive environments; and, anxiety-like behaviors expressed in both assays are sensitive to many of the same pharmacological manipulations (Belzung and Griebel 2001; Bourin and Hascoet 2003; Rodgers and Dalvi 1997; Wall and Messier 2001). Despite these similarities, anxiety-like behavior is clearly multidimensional (Ramos and Mormede 1998); and, the unique environments in each assay are likely to recruit unique neurobiological processes. Thus, the test-specific effects of taurine may merely provide an additional demonstration of the complex and multidimensional character of anxiety.

21.5 Conclusion

In summary, this study shows that taurine regulates both locomotor and anxiety-like behavior in mice. Acute taurine injection reduced locomotor activity and anxiolytic. Chronic taurine supplementation induced a state of neuronal hyper-excitability characterized by increased ambulatory levels and heightened anxiety.

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