Chapter 19
Taurine Improves Congestive Functions in a Mouse Model of Fragile X Syndrome

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Abstract Increased seizure susceptibility is a feature of the mouse model for fragile X that has parallels in the hyperarousal and prevalence of seizures in the fragile X syndrome. Our investigation of the basis for the increased seizure susceptibility of the fragile X mouse indicated a reduction in $GABA_A$ receptor expression and increased expression of glutamic acid decarboxylase (GAD), the enzyme responsible for GABA synthesis. Taurine-fed mice also show these GABAergic alterations. However, unlike fragile X mice, taurine-fed mice show a significant increase in memory acquisition and retention. This discordance implies that there may be divergent events downstream of the biochemical changes in the GABAergic system in these two mouse models. To investigate the divergence of these two models we fed taurine to fragile X mice. Our preliminary data shows that taurine supplementation to fragile X mice resulted in a significant improvement in acquisition of a passive avoidance task. Since taurine is an agonist for $GABA_A$ receptor, we suggest that chronic activation of $GABA_A$ receptors and the ensuing alterations in the GABAergic system may have beneficial effects in ameliorating the learning deficits characteristic of the fragile X syndrome.

Abbreviations *Tau*, taurine; *GAD*, glutamic acid decarboxylase; *WT*, wild type; *KO*, fragile X knockout; *IRB*, infrared beam

19.1 Introduction

The fragile X syndrome includes hyperarousal, hypersensitivity to sensory stimuli and an increased prevalence of seizures (Hagerman 2002; Wisniewski et al. 1991). The mouse model for this disorder (Bakker et al. 1994) has increased seizure susceptibility (Musumeci et al. 2000; Chen and Toth 2001; Yan et al. 2004) and this may be a direct parallel to elements of the syndrome that suggest reduced inhibition/increased excitability. Our investigations of the molecular basis of increased

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seizure susceptibility in the fragile X mouse indicated a reduction in $GABA_A$ receptor expression (El Idrissi et al. 2005). Since these receptors play a major role in inhibition, their reduction helps explain the increased seizure susceptibility of this mouse model for fragile X and suggest that the GABAergic system may be affected in the fragile X syndrome.

We also found increased expression of the enzyme responsible for the synthesis of GABA, the neurotransmitter agonist for $GABA_A$ receptors. This increase is likely to be a response of the brain to reduced inhibition – a response that has been observed in other models of elevated excitability (Riback et al. 1993). The excitability of neuronal circuits is kept within a normal range through feed-forward and -backward inhibition, mediated by inhibitory interneurons. These neurons continuously adjust their inhibitory output to match the level of excitatory input. Thus, when there is reduced inhibition of postsynaptic neurons, feedback from these neurons causes the presynaptic neurons to increase their inhibitory output. In the example of fragile X mouse brain, reduced $GABA_A$ receptor expression on postsynaptic membranes would induce an increase in GAD expression, thus increasing the bioavailability of GABA in presynaptic terminals. Therefore, increased GAD may represent a secondary response to the direct effects of Fmrp depletion.

In our previous studies (El Idrissi et al. 2003; El Idrissi and Trenkner 2004), we have shown that mice chronically supplemented with taurine in their drinking water showed biochemical changes in the GABAergic system similar to those observed in fragile X mouse, including reduced $GABA_A$ receptor and increased GAD expression as well as a lower threshold for seizure induction. However, unlike fragile X mice, taurine-fed mice showed a significant improvement in learning (acquisition and retention). The discrepancies in learning abilities signal dissimilarities between the two models. Therefore, we used a comparative approach, between the fragile X and taurine-fed mice, and examined divergent events downstream of the biochemical changes in the GABAergic system. Furthermore, we looked for neuronal markers that are differentially expressed in fragile X and taurine-fed mice that might explain the phenotypic discrepancies between these two mouse models (mainly learning deficit). This neuronal marker should show a correlation with at least some fragile X-specific features. Our preliminary data indicate that this neuronal marker could be somatostatin.

19.2 Methods

19.2.1 Passive Avoidance Test

We conducted the repetitive training passive avoidance test when the animals were 8 weeks of age. The test was carried out during the light phase (13:00–17:00 hrs), and each animal was housed individually during the test. The apparatus has a bright and a dark compartment with a computer-controlled door between them. The delivery of electric shocks (0.5 mA for 2 sec) and the raising and lowering of the door

and the latencies at which the animals stepped into the dark from the bright compartment were controlled by the computer. Each animal was gently placed in the light compartment for 10 sec, after which the guillotine door was raised and the time the animal waited before crossing to the dark (shock) compartment was recorded as the latency. The trial ended when an animal waited more than 180 sec to cross to the dark side, or if it received an electrical shock in the dark side after crossing. Once the animal crossed with all four paws to the dark compartment, the door was closed and a 0.5 mA foot shock was delivered for 2 sec. This shuttle box apparatus has 8 infrared sensors on each side that allow to measure locomotor activity by measuring the number of infrared beams breaks.

19.2.2 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.

19.3 Results

19.3.1 Behavioral Consequences of Taurine on Fragile X Mice

To test if there were changes induced by taurine on the cognitive function of fragile X mice, we measured learning ability using a repetitive training passive avoidance test. In this paradigm, we tested the ability of mice to learn to avoid a mild electrical foot shock (0.5 mA). We gave the mice six repetitive trials and measured their learning ability. Retention of the learned task was determined 24 hrs later. The apparatus has a bright and a dark compartment with a computer-controlled door between them. The delivery of electric shocks and the raising and lowering of the door and the latencies at which the animals stepped into the dark from the bright compartment were controlled by the computer. Each animal was gently placed in the light compartment for 10 sec, after which the guillotine door was raised and the time the animal waited before crossing to the dark (shock) compartment was recorded as the latency. The trial ended when an animal waited more than 180 sec to cross to the dark side, or if it received an electrical shock in the dark side after crossing. Once the animal crossed with all four paws to the dark compartment, the door was closed and a 0.5 mA foot shock was delivered for 2 sec. This shuttle box apparatus has 8 infrared sensors on each side that allow measure of locomotor activity. On the first trial, all mice had the same level of activity as measured by the number of infrared beams breaks. By the third trial, KO-Tau mice were relatively more active than WT mice (Fig. 19.1). The Baseline activity of KO-Tau mice was similar to all other groups, if not these mice were slightly hypoactive. The hyperactivity emerges

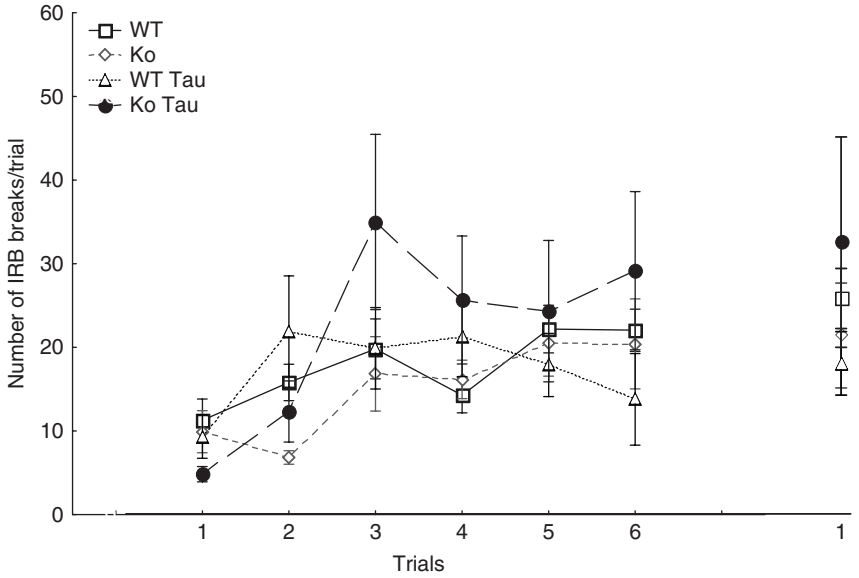

Fig. 19.1 Stress-Induced activity in KO-Tau mice. Activity is measured as the number of infrared beam (IRB) breaks. The graph shows activity over six trials and 24hrs later. KO-Tau mice have very low baseline activity and become very active following the foot shock. All mice were 2 months old. Taurine (0.05%) was supplemented in the drinking water for four weeks prior to the test. WT, $n = 20$; KO, $n = 12$; WT-Tau, $n = 12$; KO-Tau, $n = 12$

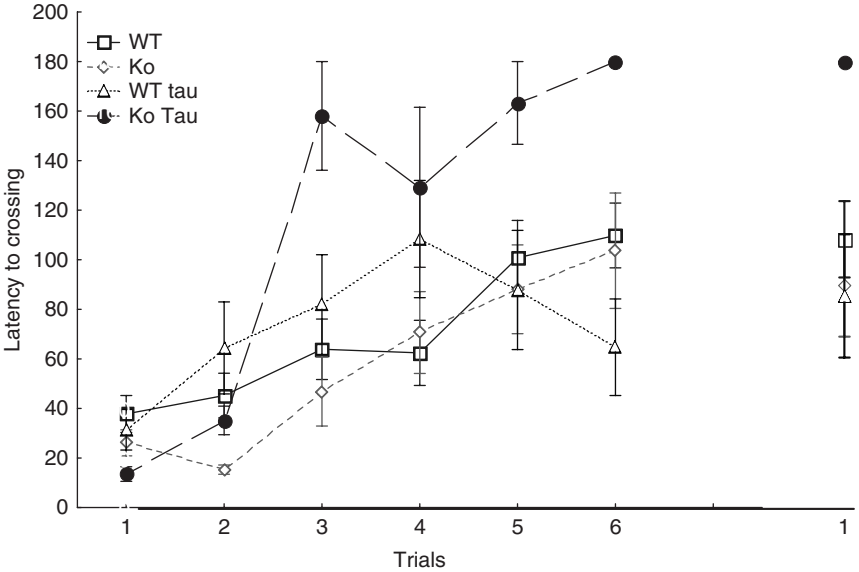

Fig. 19.2 Taurine enhances learning in KO-Tau mice. Learning is expressed by increases in the latency to cross to the dark side of the box. The graph shows latencies over six trials and 24 hrs later. KO-Tau mice were the only group to completely learn the task and they retained the same learning level 24 hrs later

after these mice were exposed to stress (foot shock). This increase in activity is probably induced by stress and should be further investigated in the context of stress and psychostimulant-induce locomotor and behavioral sensitization. If these mice are hypersensitive to stress then this sensitivity might be mediated through the dopaminergic and adrinergic systems and could help to make these mice more vigilant and hence improve their cognitive function as demonstrated in Fig. 19.2 (i.e. improvement in passive avoidance test through the increase in latency to choice).

Despite stress-induced hyperactivity, it took only two trials for the KO-Tau group to learn the task and by the end of the sixth trial, all mice in this group had a perfect score. There were no significant differences between the other groups throughout the 6 trials (WT, KO and WT-Tau). Interestingly, 24 hrs later all mice retained the same level of learning, with an impressive perfect score for the KO-Tau group. Thus, the appearance of cognitive improvement correlates well with the appearance of hyperactivity in the KO-Tau mice. The learning of the passive avoidance task was associated with fewer errors. Figure 19.3 shows, as expected, that all groups made 100% errors on the first trial and overall on the first day. By the end of the sixth trial, there were no significant differences between groups except the KO-Tau group that did exceptionally well making no errors at all by the sixth trial. Similarly this graph shows the rapid stress-induced learning in the KO-Tau group. After 24hrs

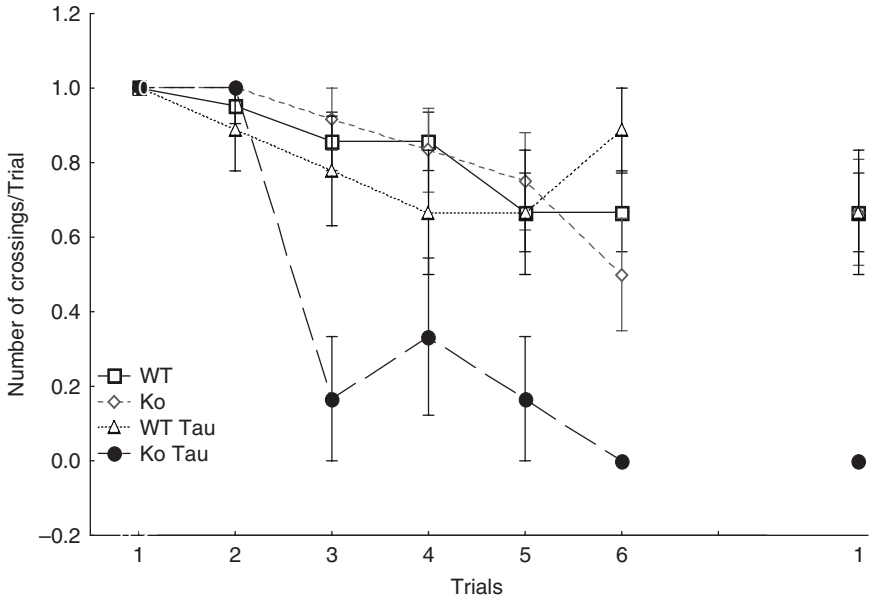

Fig. 19.3 Taurine enhances learning in KO mice. Learning was associated with decreased errors. Errors are defined by the number of attempts to cross to the dark side before the end of the trial. The graph shows errors in the six trials and 24 hrs later. All mice made the same number of errors in the first trial of the first day. KO-Tau mice were the only group to completely learn the task and they retained the same learning level 24 hrs later

later, there was clear separation between the KO-Tau mice and the other groups (WT, KO, WT-Tau). The KO-Tau were the only mice that made no errors by the end of the sixth trial and retained this memory when tested 24 hrs later. Thus, supplementation of taurine in drinking water to KO mice which increases motoric activity also seem to have a positive effect on learning/memory mechanisms studied by a passive avoidance behavior model. Interestingly, in this test the effects of taurine are more conspicuous only in the KO mice that have deficits in the inhibitory system.

19.4 Discussion

The excitability of neuronal circuits is controlled by inhibitory GABAergic interneurons. In this study, we used two models of hyper-excitability the fragile X mouse, where hyper excitability is genetically induced, and the taurine-fed mice where hyper-excitability can be induced by supplementing taurine in drinking water (0.05%) for 4 continuous weeks then we tested the effects of increased neuronal excitability on GABAergic plasticity. We found that like fragile X KO mice, taurine-fed mice showed increased susceptibility to KA-induced seizures (El Idrissi et al. 2003). Associated with this increased state of brain excitability, we found some biochemical changes in the GABAergic system, mainly, increase in the expression and activity of the enzyme responsible for GABA synthesis, glutamic acid decarboxylase (GAD). We also found a reduced expression of the β subunit of $GABA_A$ receptors. These biochemical and functional changes were similar in both mouse models. In addition, supplementing taurine in the drinking water resulted in an increase in somatostatin expression in both the WT and KO mice.

The neonatal brain contains high levels of taurine (Huxtable 1989; Sturman 1993). As the brain matures its taurine content declines and reaches stable adult concentrations that are second to those of glutamate, the principal excitatory neurotransmitter in the brain. Taurine levels in the brain significantly increase under stressful conditions (Wu et al. 1998), suggesting that taurine may play a vital role in neuroprotection. A possible mechanism of taurine's neuroprotection lies in its calcium modulatory effects (El Idrissi and Trenkner 1999; El Idrissi and Trenkner 2003; El Idrissi and Trenkner 2004) and agonistic role on GABAA receptors (El Idrissi et al. 2003; El Idrissi and Trenkner 2004).

Taurine has been shown to play a role in neurotransmission, but taurine does not satisfy the criteria of a classical neurotransmitter. However, there is increasing evidence supporting a functional interaction between GABA, glycine and taurine (Kuriyama and Hashimoto 1998; El Idrissi and Trenkner 2004). Taurine has been shown to increase plasma membrane chloride conductances by affecting bicuculine-sensitive chloride channels (del Olmo et al. 2000; Mellor et al. 2000). Taurine has also been shown to act as a partial agonist of $GABA_A$ receptors in synaptic membranes (Quinn and Harris 1995). In addition to modulating neuronal transmission, the observed effects of taurine on the up-regulation of somatostatin expression are not well understood and could be mediated at the transcription level. The

levels of somatostatin and somatostatin-positive neurons were increased in the brain of taurine-fed mice (Levinskaya et al. 2006). Numerous experimental and clinical studies have demonstrated that somatostatin neurotransmission plays an important role in the modulation of several brain functions, including learning and memory processes. It is possible that the increase in somatostatin levels observed after taurine supplementation may be responsible for the increased learning observed in fragile X mice. Taurine supplementation therefore could be beneficial as a naturally occurring pharmaco-therapeutic agent for the improvement of learning in fragile X mice and perhaps patients.

19.5 Conclusion

In summary, this study shows that taurine supplementation to fragile X mice induced a significant increase in acquisition and retention of a hippocampal-dependent memory task, interpreted here as improvement in cognitive functions. The taurine enhancing effects are mediated though interaction with, and modification of the GABAergic system.

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