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Kainic acid (KA)-induced seizures in Sprague-Dawley rats and the effect of dietary taurine (TAU) supplementation or deficiency

B. Eppler[1], T. A. Patterson[2], W. Zhou[1], W. J. Millard[1], and R. Dawson Jr.[1]

[1] Department of Pharmacodynamics, University of Florida, Gainesville, Florida, U.S.A.
[2] Division of Neurotoxicology, National Center for Toxicological Research/FDA, Jefferson, Arkansas, U.S.A.

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Summary. Male Sprague-Dawley rats received TAU supplementation (1.5% in drinking water) or TAU deficient diets for 4 weeks to test for a possible neuroprotective role of TAU in KA-induced (10mg/kg s.c.) seizures. TAU supplementation significantly increased serum and hippocampal TAU levels, but not TAU content in temporal cortex or striatum. TAU deficient diets did not attenuate serum or tissue TAU levels. Dietary TAU supplementation failed to decrease the number or latency of partial or clonic-tonic seizures or wet dog shakes, whereas a TAU deficient diet decreased the number of clonic-tonic and partial seizures. This study does not support previous observations of an anticonvulsant effect of TAU against KA-induced seizures. KA-treatment decreased α_2-adrenergic receptor binding sites and TAU content in the temporal cortex across all dietary treatment groups, supporting previous evidence of severe KA-induced damage and neuronal loss in this brain region.

Keywords: Amino acids – Taurine – Kainic acid – Epilepsy – Anticonvulsants – Neuroprotection – Excitatory amino acids

Introduction

Taurine (TAU) is a β-sulfur amino acid that is found in abundance in mammalian tissues. Many functions have been suggested for this ubiquitous molecule including improving cardiac function, modulating Ca^{2+} fluxes, neuroprotection and antioxidation, anticonvulsant properties, bile salt synthesis, and osmoregulation (Huxtable, 1992). The anticonvulsant and osmoregulatory properties of TAU (Huxtable, 1989, 1992; Yoshida et al., 1986) make this amino acid a good candidate for preventing seizures. TAU also has been suggested to be an inhibitory neurotransmitter (Huxtable, 1989; Barbeau et al., 1975; Davison and Kaczmarek, 1971; Sanberg et al., 1979), which may play a role in decreasing neuronal excitability during seizure

activity. TAU has been shown to be a potent agonist at both glycine and GABA receptors (Oja and Kontro, 1983; Huxtable, 1989).

TAU has been shown to have anticonvulsant properties in cobalt-induced seizures in cats and rats and in other experimental epilepsy models induced by ouabain, penicillin, or pentylenetetrazol (PTZ) (Barbeau et al., 1975; Izumi et al., 1975; Durelli et al., 1977; Durelli et al., 1979). It also has been reported that CNS TAU concentrations are decreased in vitamin B_6 deficient neonatal rats, an animal seizure model (Lombardini, 1992), and in human epilepsy (Van Gelder, 1978; Mutani et al., 1974). The reason for the decline in TAU, and perhaps the cause of seizures in the vitamin B_6-deficient seizure model, might be that less TAU is being synthesized since vitamin B_6 is a cofactor for cysteine sulphinic acid decarboxylase, the major biosynthetic enzyme for TAU (Lombardini, 1992). Other reports, however, have shown that dietary TAU supplementation in kittens did not change the threshold for PTZ seizures (Lehman, 1987). Intracerebroventricular (icv) injections of TAU showed a protective effect against nitrogen-induced convulsions, but not against PTZ- or hyperbaric oxygen-induced seizures (Lombardini, 1992). Malcangio et al. (1989) have reported a selective protective effect of TAU, since it was protective against convulsions induced by hypoxia, but not PTZ or hyperbaric oxygen. TAU has been shown to be an antiepileptic agent in certain forms of epilepsy in humans, and it is not toxic at high doses like other antiepileptic agents (Van Gelder et al., 1975). Currently it is unclear which type of seizures are most responsive to the anticonvulsant properties of TAU.

It has been reported that TAU concentrations are decreased in some brain areas in aging rats (Palkovits et al., 1990; Dawson and Wallace, 1992a) and previously we and others have found that aged rats were more sensitive to KA-seizure induction (Dawson and Wallace, 1992b; Wozniak et al., 1991). KA is a structural glutamate analog (Fariello et al., 1982; Olney et al., 1974) that has been isolated from sea weed *Digenea simplex* (Sperk, 1994). KA has been suggested as a model for temporal epilepsy (Nadler, 1981) which is resistant to many traditional therapeutic agents (Velísek et al., 1992). It has been observed that KA-induced seizures and neurotoxicity are reduced in the immature nervous system, a developmental stage when TAU levels are high (Stafstrom et al., 1992). KA has been shown to increase extracellular TAU in the piriform cortex 2–5 fold 60–90 minutes after systemic administration (Wade et al., 1987). Extracellular TAU also increases 5–6 fold in hippocampus 30–60 minutes after KA administration as determined by microdialysis (Menendez et al., 1989). Therefore, TAU might work as an endogenous protectant by suppressing neuronal excitability and cell swelling through its inhibitory and osmoregulatory actions, respectively.

The experiments in this study were designed to test if modifications in dietary TAU could predictably alter KA-induced seizures. We hypothesized that the behavioral and neurotoxic actions of KA in Sprague-Dawley (SD) rats might be attenuated due to TAU's ability to act as a regulator of brain osmolality and neuronal excitability. Therefore, we measured seizure sensitivity and amino acid and catecholamine concentrations and receptor binding as

biochemical markers for cell death to determine if TAU could reduce seizure activity or protect against the neurotoxic actions of KA.

Materials and methods

Animals and diets

Male SD rats obtained from Harlan (Indianapolis, IN) were 6 weeks old on arrival. The animals were randomly assigned to one of the following diets: control (CON, regular Purina rat chow #5012), TAU supplementation (TS, regular chow and 1.5% TAU in the drinking water), or TAU deficient (TD, TAU free Purina diet #5729C-M). There were 12 rats per dietary condition and the animals were on the diets for 4 weeks before KA treatment. The animals were kept on a 12-hour light-dark cycle (6 am–6 pm), housed individually in hanging metal cages, and provided food and water *ad libitum*. The estimated TAU concentration of 0.02% in control chow was confirmed by high performance liquid chromatography (HPLC) (0.029%). TAU concentrations in the special TAU free chow were below the limit of detection (0.004%). The stability of the 1.5% TAU solution was monitored by HPLC. The treatment timeline is outlined in Fig. 1.

KA-induced seizures

Seven rats per group received 10 mg/kg KA subcutaneously (sc) after 4 weeks on the diet. KA was suspended in sterile saline and the KA-treated animals were observed for two

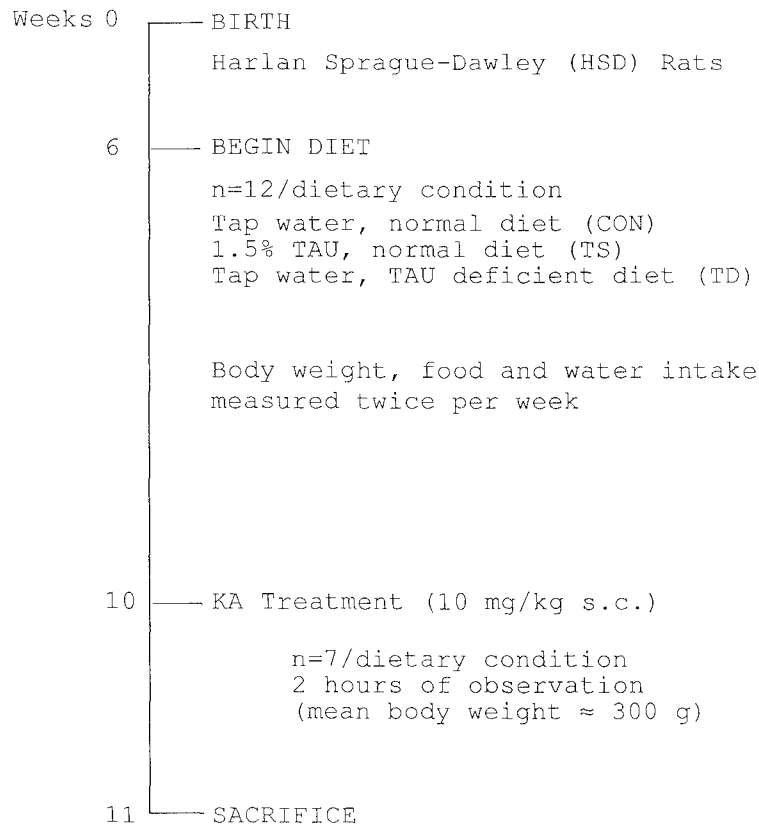

Fig. 1. Time line for the experimental procedures

hours. The latencies for wet dog shakes and clonic-tonic seizures and the total number of partial and tonic-clonic seizures were recorded for 2 hours as previously described (Dawson and Wallace, 1992b). The animals were allowed to recover for one week and mortality and weight changes were measured. Animals were sacrificed by decapitation and serum was collected. Tissue samples were collected and stored at −80°C until further analysis.

Food and water intake and body weights

Body weights and food and water intake were measured twice a week and food intake was corrected for spillage. KA treated animals were monitored daily for food and water intake and changes in body weight. Animals that demonstrated extreme weight loss were fed sweetened condensed milk to enhance survival.

Amino acid analyses

Amino acid levels were measured in several brain areas using HPLC with electrochemical detection (HPLC-ECD) (Wallace and Dawson, 1992b) to determine: (1) if KA would chronically deplete tissue TAU levels and (2) whether TAU would reduce the loss of amino acids. Tissue samples were homogenized in 0.2 M perchloric acid (PCA) with a Brinkman homogenizer PT10/305 and microcentrifuged at 12,400 rpm for three minutes. Serum samples were treated with methanol (1:9) to denature proteins and microcentrifuged for three minutes at 12,400 rpm. Serum TAU was measured to determine the effects of dietary TAU manipulations. The supernatants were analyzed for amino acid content after precolumn derivatization with o-phthalaldehyde. Tissue values were normalized with protein measurements using the method of Bradford (Bradford, 1976) or by wet weight.

Catecholamine analyses

Catecholamines were measured to index the effects of KA on presynaptic markers in the areas most severely affected by KA-induced seizure activity. Catecholamines were recovered from tissue homogenates via an alumina extraction. Briefly, alumina and 3 M Tris/1 mM EDTA were added to the PCA supernatants. The alumina was washed twice with HPLC-grade water after a 5-minute incubation with shaking (at room temperature) and transferred to a column made from large (1 ml) pipette tips and glass wool. The catecholamines were eluted from the column with 0.2 M perchloric acid. Dihydroxybenzylamine (DHBA) was used as an internal standard for determining recovery. The samples were analyzed using HPLC-ECD for norepinephrine (NE), dopamine (DA), and its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) as previously described (Kontur et al., 1984).

Receptor binding

PAC binding was used as a postsynaptic marker for neuronal loss in the brain areas known to be severely affected by KA. α_2-Adrenergic receptor binding was measured using p-aminoclonidine (PAC, α_2-adrenergic agonist) binding as previously described (Dawson and Wallace, 1989). Briefly, the tissue was homogenized in 50 mM Tris buffer and centrifuged at 40,000 × g for 10 min. and the pellet was resuspended in fresh buffer and centrifuged again. The pellet was resuspended and incubated with 2.5 nM ^{3}H-PAC at room temperature for 45 minutes (Total). Non-specific binding of ^{3}H-PAC (Blank) was determined by adding 10 μM yohimbine (α_2-adrenergic antagonist) and 2.5 nM ^{3}H-PAC. Samples were collected with a cell harvester (Brandel, Gaithersburg, MD) on GF/B

filters. Radioactivity of the filter was measured by liquid scintillation counting using a Beckman LS3801 counter (Beckman Instruments, Fullerton, CA). Specific binding was calculated by subtracting Total from Blank counts. Protein in the tissue homogenates was measured by the method of Bradford (1976).

Statistics

When appropriate, a test for outliers (Dixon and Massey, 1969) was applied prior to further analysis. Mann-Whitney-U or ANOVA were performed followed by the Bonferroni multiple comparisons test. Significance was set at the 95% confidence interval ($p < 0.05$).

Results

Fluid and water intake were not significantly altered by the dietary conditions (data not shown). Weight gain was significantly greater in the TS animals ($p < 0.05$) when compared to the deficient group (Fig. 2).

The latencies to the first wet dog shake or clonic-tonic seizure after KA treatment were not different between the groups (Fig. 3A). Dietary condition had no effect on the number of wet dog shakes (data not shown). The number of tonic-clonic and partial seizures, however, was significantly decreased ($p < 0.05$) in the TD group as compared to the TS group (Fig. 3B). The one week survival rates of the animals were: CON = 5/7, TS = 4/7, TD = 5/7. There was

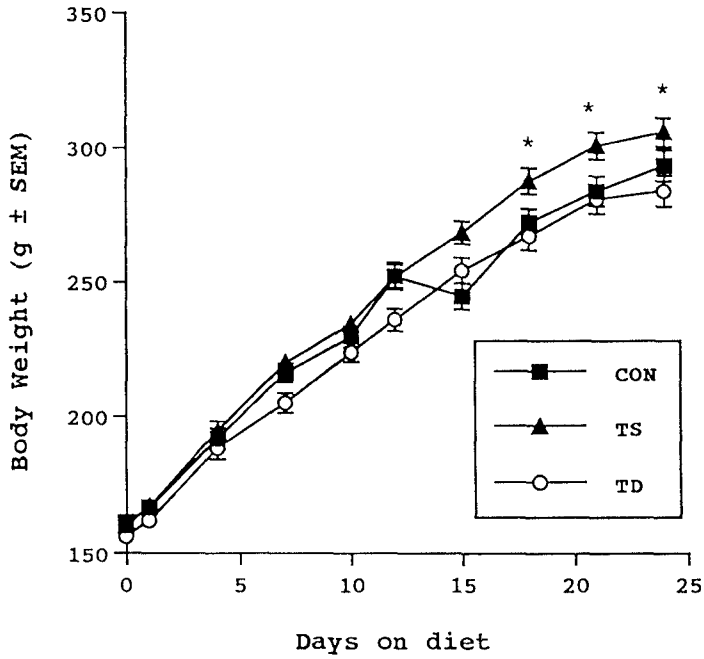

Fig. 2. Body weights of male SD rats on normal (*CON*), TAU supplemented (*TS*), and TAU deficient (*TD*) diets. Weights were measured twice weekly. The data are represented as means ± SEM (n = 12/group) in grams. *$p < 0.05$ vs. TD

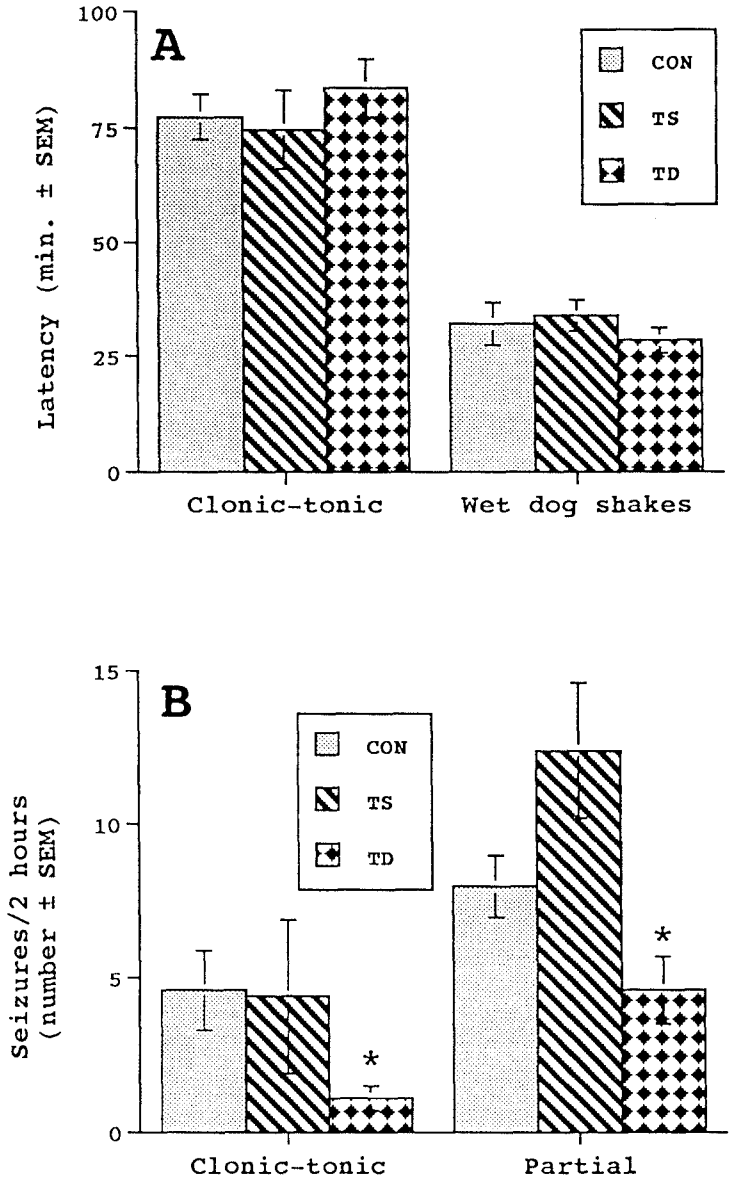

Fig. 3. Seven animals per group received KA sc. (10 mg/kg) after 4 weeks on the diet. The latencies and number of seizures were observed and recorded for two hours. Data represent mean ± SEM for 7 observations/group. The latency of clonic-tonic seizures and wet dog shakes (**A**) and the number of tonic-clonic and partial seizures in a 2-hour period (**B**) were recorded. Dietary manipulations had no effect on the latency of clonic-tonic seizures or wet dog shakes (**A**). TD animals had significantly (*$p < 0.05$) less tonic-clonic and partial seizures compared to the supplemented (*TS*) animals (**B**)

no difference in survival or weight loss (data not shown) due to KA treatment between the groups.

The serum TAU content was significantly ($p < 0.05$) higher in the supplemented group when compared to controls and the deficient animals. KA-

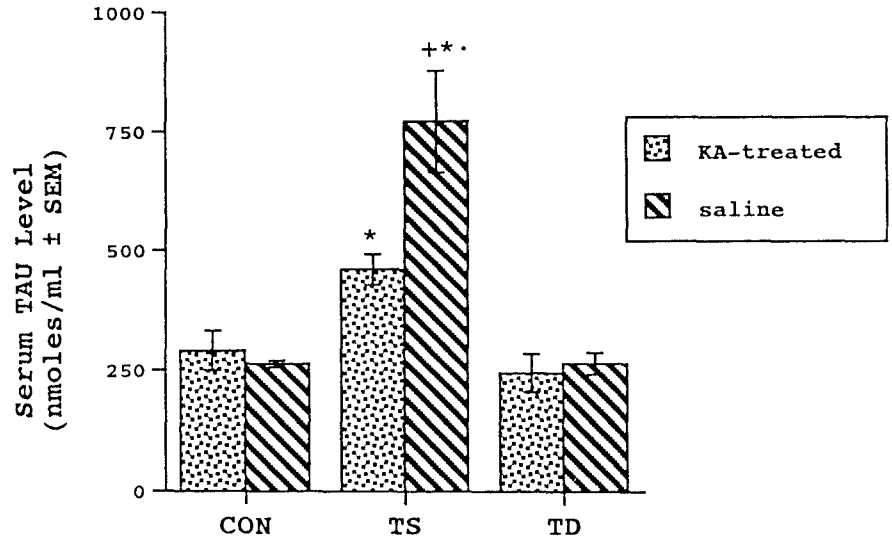

Fig. 4. Serum TAU levels were measured by HPLC-ECD as discussed in Material and methods. Values are represented as nmoles/ml serum ± SEM). $^{+}p < 0.05$ vs. CON, $^{*}p < 005$ vs. TD, and $^{\cdot}p < 0.05$ vs. KA-treated. N = 4–6/group

Table 1. Amino acid levels in brain (μmoles/g tissue ± SEM)

	Brain region treatment		TAU	ASP	GLU
temporal cortex	CON	saline	4.97 ± 0.12	1.42 ± 0.11	8.34 ± 0.23
		KA-treated	3.13 ± 0.23[1]	1.23 ± 0.08	4.39 ± 0.15[1]
	TS	saline	5.47 ± 0.24	1.31 ± 0.09	8.42 ± 0.46
		KA-treated	4.18 ± 0.19[1]	1.24 ± 0.08	4.48 ± 0.57[1]
	TD	saline	5.07 ± 0.21	1.51 ± 0.08	8.45 ± 0.24
		KA-treated	4.05 ± 0.34[1]	1.22 ± 0.08	5.23 ± 0.93[1]
hippocampus	CON	saline	4.87 ± 0.19	1.11 ± 0.10	7.55 ± 0.18
		KA-treated	4.02 ± 0.20	0.28 ± 0.19[1]	4.74 ± 0.54[1]
	TS	saline	5.66 ± 0.26[2]	0.99 ± 0.06	7.41 ± 0.35
		KA-treated	5.36 ± 0.12[2,3]	0.98 ± 0.07[3]	5.54 ± 0.59
	TD	saline	4.67 ± 0.21	0.94 ± 0.06	6.84 ± 0.21
		KA-treated	4.71 ± 0.16	0.93 ± 0.08[3]	6.06 ± 0.54

n = 4–6/group.
[1] $p < 0.05$ vs. saline; [2] $p < 0.05$ vs. TD; [3] $p < 0.05$ vs. CON.

treated animals in the TS group had lower TAU levels when compared to the saline-treated animals in the TS group (Fig. 4).

Amino acid levels were analyzed in the temporal cortex, hippocampus (Table 1) and striatum which have previously been shown to be severely affected by KA injections (McGeer and McGeer, 1982; Schwob et al., 1980; Sperk et al., 1985, Ben-Ari, 1985). TAU and GLU were significantly ($p < 0.05$) depleted in the temporal cortex by KA treatment in all dietary groups,

whereas in the hippocampus KA-treatment only depleted GLU in the CON diet group. Dietary TAU supplementation only caused a significant ($p < 0.05$) increase in TAU levels in the hippocampus compared to TAU deficient animals, but not in temporal cortex. There was no change in ASP levels in temporal cortex, but KA treatment significantly decreased ASP levels in hippocampus of CON animals, and ASP levels in KA-treated CON rats were also significantly lower compared to KA-treated TS and TD animals (Table 1). Dietary conditions did not have an effect on TAU or GLU in the striatum (data not shown).

NE content in the hippocampus and temporal cortex was not significantly changed by KA injections or dietary manipulations (data not shown). Agonists that activate α_2-adrenergic receptors have been shown to attenuate KA-acid induced seizures (Baran et al., 1985). Therefore, we assessed the neuroprotection of postsynaptic neurons that express α_2-adrenergic binding sites by using radioligand binding methods. KA-induced seizures significantly ($p < 0.01$) reduced ^{3}H-PAC binding (Fig. 5) in the temporal cortex of all dietary groups: 82% in CON, 71% in TS, and 76% in TD. In a separate study, a group ($n = 12$) of adult SD rats were treated with KA (10 mg/kg) and seizure severity significantly ($p < 0.005$) correlated with the loss of α_2-adrenergic binding sites ($r = -0.85$). These results indicate a decrease in postsynaptic α_2-adrenergic receptor population in the temporal cortex after KA treatment.

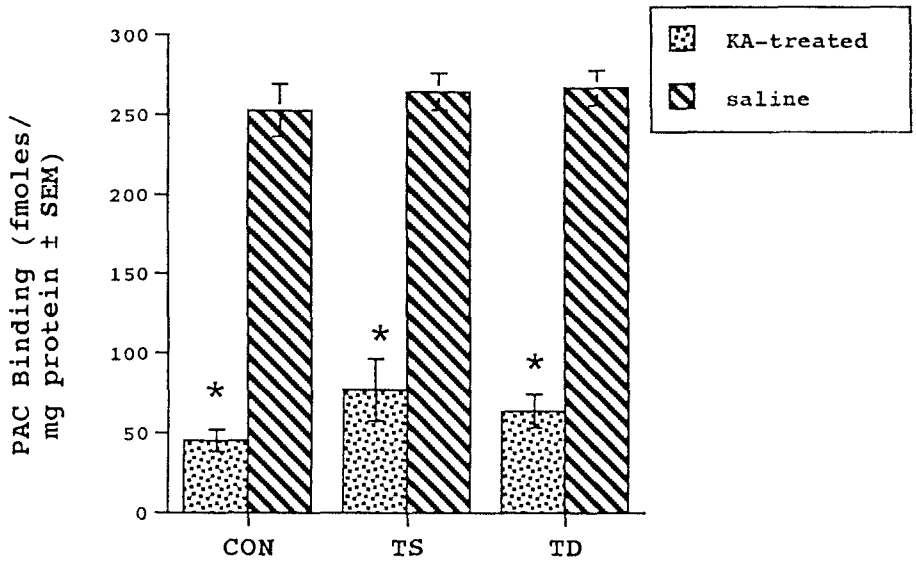

Fig. 5. α_2-adrenergic (*PAC*) binding in temporal cortex of KA-lesioned rats on CON, TS, and TD diets was measured (fmoles/mg protein) as explained in Material and methods. Each bar represents the mean value ± SEM. *$p < 0.05$ vs. KA-treated animals ($n = 4$–5/group)

Discussion

This study did not confirm previous reports by other investigators of TAU's anticonvulsant activity (Wada et al., 1975; Uemura, 1991; Huxtable, 1992; Huxtable, 1989, Yoshida et al., 1986; Izumi, 1974). Oral TAU administration did increase serum levels by 3-fold in SD rats after 4 weeks of 1.5% TAU supplementation. This observed increase in serum concentration of TAU, however, did not prevent or reduce KA-induced seizure activity in male SD rats. Dietary TAU restriction did not decrease serum TAU levels or exacerbate seizure activity which was the expected result. Instead, rats in the TAU deficient group showed a decrease in the number of partial and tonic-clonic seizures in a 2-hour period. We did not monitor seizures that may have occurred after the initial 2 hour period, so we do not know if the TD group exhibited a delay in the full expression of seizure activity.

The reason for the negative results observed in the TAU supplemented group could be that the oral delivery of TAU in rats could not increase the brain tissue content to a level that is protective, whereas other routes of administration (iv, icv) or oral TAU administration in humans, might accomplish the increase necessary for protection. Dietary TAU supplementation increased hippocampal, but not cortical TAU levels; however the increase might not have been sufficient to have a protective effect. TAU enters the brain via two saturable transport mechanisms and a third non-saturable mechanism, diffusion, across the blood-brain barrier (BBB) (Huxtable, 1989). TAU supplementation increased serum TAU levels from 263μM to 773μM, and the Km for the high affinity transport system has been reported to range from 50 to 9,400μM in rat brain (Huxtable, 1989). This suggests the TAU serum concentration reached in our study might be below the optimal concentration necessary to saturate the transporter and significantly elevate brain TAU concentrations to a level that might be protective. Furthermore, we have shown that dietary TAU supplementation decreases the activity of the biosynthetic enzymes (cysteine sulphinic acid and cysteine dioxygenase) in the liver which could decrease endogenous TAU production (Eppler and Dawson, 1998). A higher percentage of TAU supplementation and/or an increase in the duration of supplementation might be required to show efficacy for TAU against KA seizures when it is delivered orally.

Taurine has been shown to partially protect against KA-induced neurotoxicity (Sanberg et al., 1979). In the Sanberg study (1979), TAU was dissolved in the drinking water to a final concentration of 0.9% and given to rats for 22–34 days, and KA (3nmol) was administered via unilateral stereotaxic injections. The markers for protection measured were enzyme activities (glutamic acid decarboxylase, choline transferase, glutaminase, tyrosine hydroxylase, and cysteine sulphinic acid decarboxylase) and not latencies or number of seizures. The protective effect was very slight and only significant in choline acetyltransferase activity. Since the measures of the protective effect of TAU and the KA concentration and mode of administration were different between Sanberg's and our study, it is difficult to explain the discrepancy in the results. Another study showed partial protection by

TAU against KA-induced seizures (Fariello et al., 1982). The KA acid concentration and administration was the same as in the present study (10 mg/kg, systemic injection), but TAU was administered subcutaneously for three consecutive days, twice daily at a concentration of 200 mg/kg (Fariello et al., 1982). TAU decreased the number of seizures and wet dog shakes and prevented KA-induced neuronal loss in hippocampus and piriform cortex. Serum TAU levels were not reported in this study and it is unclear whether this route of administration increased serum TAU levels to a higher concentration than we observed in our study. Again, the difference in the methods and route of administration of TAU makes it difficult to compare results, and we did not examine brain sections for neuronal loss. In summary, we were unable to duplicate previous observations of a protective effect of TAU against KA-induced seizures or neurotoxicity, but this fact is likely due to differences in the methodology and study design.

Other common antiepileptic drugs such as carbamazepine, phenobarbital, clonazepam, and valproate do not decrease seizure activity in rats when administered i.p. 10–13 min. before KA exposure (Velísek et al., 1992). These findings might be an indication that KA seizures may be due to mechanisms not attenuated by TAU and other antiepileptic drugs. It has been reported that TAU can increase KA damage when used in low concentrations (0.1–1.0 mM) in a fetal rat brain culture model (Tang et al., 1996). Thus, it still remains to be established if KA-induced seizure activity can be attenuated by oral TAU administration in rats and if so, by what mechanisms.

Mannitol is an osmotic agent that has been shown to reduce brain edema and reduces markers of brain damage due to KA (Baran et al., 1987). TAU efflux from neurons is important to reduce cell swelling (Huxtable, 1989), and it was anticipated that TAU supplementation might have increased the ability of neurons to osmoregulate under these excitotoxic conditions. If KA-induced seizure activity had caused a TAU efflux to prevent cell swelling, the increase in extracellular TAU levels might acutely cause a concomitant intracellular decline. We did show a seizure-induced decline in tissue TAU concentration in cortical samples, but not in hippocampus. Our study looked at the chronic effects of KA-treatment whereas another study by Menendez et al. (1989) looked at acute effects of GLU agonists. Menendez et al. found an acute increase of extracellular TAU concentration, measured by microdialysis, in the hippocampus after KA exposure. We analyzed the tissue samples 7 days after the KA exposure and did not see that same effect chronically. Dawson et al. (1992b) analyzed hippocampal TAU content 2 hours after KA exposure and also failed to find a decline. The extracellular TAU is probably taken back up into cells after recovery from the insult. A chronic decline in the TAU level, as we observed in the cortex, is probably an indication of cell loss and not of an osmoregulatory response to KA.

The high serum TAU levels in the TS group could have contributed to an unfavorable concentration gradient for TAU efflux from swollen neurons or caused a downregulation in the TAU transporter. This could have compromised TAU-mediated osmoregulation and, therefore, contributed to the observed results. TAU activation of chloride channels is also thought to

contribute to cell swelling and increase excitatory amino acid (EAA) toxicity in some models (Tang et al., 1996). Thus, even though TAU is an osmotically active molecule, increasing dietary TAU did not prevent neuronal loss because TAU may not have reached a protective level in the damaged brain areas or chronic exposure to high dietary levels of TAU may downregulate TAU synthesis and transporter function.

A decrease in dietary TAU intake did not significantly alter the TAU concentration in serum or brain tissue, indicating that an adaptive mechanism might compensate for the dietary TAU deficiency. Studies using pyridoxal-depleted animals, depleting the coenzyme of cysteine sulphinic acid decarboxylase, failed to decrease the tissue TAU content (Hope, 1957; Nyffenegger et al., 1960; Jacobsen and Smith, 1968) and one study even showed an increase in TAU content in muscle and spleen (Nyffenegger et al., 1960). The rat is a prolific synthesizer of TAU, whereas humans do not exhibit the same high biosynthetic capacity (Huxtable, 1989; Gaull, 1989). Previous studies in our laboratory have attempted to elucidate the compensatory mechanisms activated by dietary TAU deficiency in aged, male Fisher 344 rats (Eppler and Dawson, 1998). The biosynthetic enzymes cysteine sulphinic acid decarboxylase and cysteine dioxygenase are not upregulated in the liver by dietary TAU depletion, as might be expected (Eppler and Dawson, 1998). There are large enzymatic activity differences between species (Huxtable, 1989), and even among strains of rats (Eppler and Dawson, 1998; Bagley et al., 1995). Therefore, the compensatory mechanism activated in the animals that are deprived of TAU in the diet remains to be elucidated and the mechanism might not be the same in different tissues or species. Another area that should be examined in the future is the possible upregulation of brain TAU transporters to maintain TAU levels when TAU is deficient in the diet. The kidney is known to dynamically regulate TAU transporter expression (Han et al., 1997), but this response has not been studied extensively in other tissues. The TD group may have increased TAU transporters in response to the low dietary TAU or increased TAU synthesis from methionine or cysteine via a biosynthetic pathway other that the cysteine sulphinic acid pathway. Other studies in our laboratory have shown that TD diets can improve renal function in aging F344 rats (Dawson et al., 1996) suggesting a yet unidentified adaptive mechanism.

The adaptive mechanism responsible for functional improvement under TAU-free dietary conditions might be responsible for the decreases in seizure number observed in this study. Even though a TAU deficient diet is apparently beneficial at the behavioral level, it did not have the same effect at a cellular level, such as protection of the α_2-adrenergic binding sites in the cortex. As mentioned previously, we cannot rule out that TD diets resulted in a delay in the expression of seizure activity beyond the 2 hour observation period. The non-significant increase in the latency for tonic-clonic seizures in animals on TD diets seen in figure 3A might be an indication of this possible delay in seizure activity.

Our study confirmed previous reports (McGeer and McGeer, 1982; Schwob et al., 1980; Sperk et al., 1985; Ben-Ari, 1985) of extensive damage in

temporal cortex by KA treatment. We assessed this damage by measuring α_2-adrenergic binding sites in cortical samples. Cell bodies that are destroyed due to KA-induced seizure activity have postsynaptic α_2-adrenergic receptors. Noradrenergic neurons projecting into the area with seizure activity remain intact, as indicated by the failure to detect a decline in NE concentrations. Norepinephrine (NE) has been shown to be acutely depleted by KA (Baran et al., 1985; Nelson et al., 1980; Dawson and Wallace, 1992) suggesting its release in response to KA receptor activation; however, chronically the NE levels come back to control levels (Nelson et al., 1980). The importance of the postsynaptic α_2-adrenergic binding sites in seizure activity is reflected in the observations that α_2-adrenergic agonists can attenuate KA-induced seizures (Baran et al., 1985) and that interference with central noradrenergic neurotransmission lowers seizure thresholds (Killian and Frey, 1973). We found that KA-induced lesions significantly decreased binding sites, and this depletion of α_2-adrenergic binding sites seemed to be more severe in animals on the CON diet; however, this was not statistically significant. Studies in our laboratory have shown that incubation of cortical membranes with TAU significantly increases α_2-adrenergic binding sites in vitro (Green et al., 1998). Dietary TAU supplementation, however, did not attenuate the loss of α_2-adrenergic binding sites because it did not significantly increase TAU levels in the temporal cortex.

KA-treatment decreased TAU and GLU levels in the cortex, but not hippocampus. EAA such as GLU and ASP play a role in seizure activity and are released in response to KA treatment (Dawson and Wallace, 1992b). The decline in GLU levels, therefore, is a reflection of a massive release due to KA-treatment and chronically may be an indication of neuronal and glial loss in the observed brain areas. The TAU decline might also be an indication of cellular loss since a decrease in the number of cells containing TAU would cause an overall decrease in TAU concentration in that tissue. TAU and GLU are the most abundant amino acids in the brain (Huxtable, 1989) and one would except neuronal loss to be reflected as a chronic loss of their concentration in brain tissue. The KA-induced neuronal loss as indicated by these markers could not be significantly diminished or enhanced by dietary TAU manipulations.

In summary, our study does not support previous observations of a protective role for TAU in KA-induced seizures. This might de due to the fact that serum and tissue levels of TAU reached in our experiments were not sufficient to protect from the KA-mediated brain damage. Further studies are needed to assess chronic adaptive changes in brain to either TAU supplementation or deprivation. Our study confirms previous reports demonstrating significant cell loss due to KA-induced seizures (Schwob et al., 1980; McGeer and McGeer, 1982; Ben-Ari, 1985) by specifically showing a significant decline in postsynaptic α_2-adrenergic binding sites in the temporal cortex. The mechanism of KA seizures is resistant to pharmacotherapy and cannot be prevented by many classical antiepileptic drugs or TAU. Future studies will be required to optimize TAU delivery to the brain in order to fully exploit the potential of TAU as an antiepileptic agent.

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References

Bagley P, Hirschberger LL, Stipanuk MH (1995) Evaluation and modification of an assay procedure for cysteine dioxygenase activity: high-performance liquid chromatography method for measurement of cysteine sulfinate and demonstration of physiological relevance of cysteine dioxygenase activity in cysteine metabolism. Analyt Biochem 227: 40–48

Baran H, Sperk G, Hörtnagel H, Sapetsching G, Hornykiewicz O (1985) α_2-Adrenoceptors modulate kainic acid-induced limbic seizures. Eur J Pharmacol 113: 263–269

Baran H, Lassmann H, Sperk G, Seitelbeger F, Hornykiewicz O (1987) Effect of mannitol treatment on brain neurotransmitter markers in kainic acid-induced epilepsy. Neuroscience 21/3: 679–684

Barbeau A, Inoue N, Tsukada Y, Butterworth RF (1975) The neuropharmacology of taurine. Life Sci 17: 669–678

Ben-Ari Y (1985) Limbic seizure and brain damage produced by kainic-acid: mechanisms and relevance to human temporal lobe epilepsy. Neuroscience 14/2: 375–403

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248–254

Davison AN, Kaczmarek LK (1971) Taurine- a possible neurotransmitter. Nature 234: 107–108

Dawson Jr R, Wallace DR (1989) Central and peripheral actions of α_2-adrenergic agonists on renal function in Long-Evans and Brattleboro rats. Pharmacology 39: 240–252

Dawson Jr R, Wallace DR (1992a) Taurine content in tissues from aged Fischer 344 rats. Age 15: 73–81

Dawson Jr R, Wallace DR (1992b) Kainic acid-induced seizures in aged rats: neurochemical correlates. Brain Res Bull 29: 459–468

Dawson Jr R, Eppler B, Patterson TA, Shih D, Liu S (1996) The effect of taurine in a rodent model of aging. In: Huxtable RJ et al (eds) Taurine 2. Plenum Press, New York, pp 37–50

Dixon WJ, Massey FJ (1969) Introduction to statistical analysis. McGraw-Hill, New York

Durelli L, Mutani R, Quattrocolo G, Delsedime M, Buffa C, Fassio F, Valentini C, Fumero S (1977) Relationships between electroencephalographic pattern and biochemical picture of the cobalt epileptogenic lesion after cortical superfusion with taurine. Exp Neurol 54: 489–503

Durelli L, Mutani R, Delsedime M, Quattrocolo G, Buffa C, Mazzarino M, Fumero S (1979) Electroencephalographic and biochemical study of the antiepileptic actions of taurine administered by cortical superfusion. Exp Neurol 52: 30–39

Eppler B, Dawson Jr R (1998) The effects of aging on taurine content and biosynthesis in different strains of rats. In: Huxtable RJ et al (eds) Taurine 3. Plenum Press, New York, pp 55–61

Fariello RG, Golden GT, Pisa M (1982) Homotaurine (3 aminopropanesulfonic acid; 3APS) protects from the convulsant and cytotoxic effect of systematically administered kainic acid. Neurology 32: 241–245

Gaull GE (1989) Taurine in pediatric nutrition: review and update. Pediatrics 83/3: 433–442

Green P, Dawson Jr R, Wallace D, Owens J (1998) Treatment of rat brain membranes with taurine increases radioligand binding. In: Huxtable RJ et al (eds) Taurine 3. Plenum Press, New York, pp 377–383

Han X, Budreau AM, Chesney RW (1997) Functional expression of rat renal cortex taurine transporter in *Xenopus leavis* oocytes: adaptive regulation by dietary manipulation. Pediatr Res 41/5: 624–631
Hope DB (1957) The persistence of taurine in the brain of pyridoxine-deficient rats. J Neurochem 1: 364–369
Huxtable RJ (1989) Taurine in the central nervous system and the mammalian actions of taurine. Prog Neurobiol 32: 471–533
Huxtable RJ (1992) Physiological actions of taurine. Physiol Rev 72/2: 101–163
Izumi K, Igisu H, Fukuda T (1974) Suppression of seizures by taurine-specific or non-specific? Brain Res 76: 171–173
Izumi K, Igisu H, Fukuda T (1975) Effects of edetate on seizure suppressing actions of taurine and GABA. Brain Res 88: 576–579
Jacobsen JG, Smith LH Jr (1968) Biochemistry and physiology of taurine and taurine derivatives. Physiol Rev 48/2: 424–511
Killian M, Frey HH (1973) Central monoamines and convulsive thresholds in mice and rats. Neuropharmacol 12: 681–692
Kontur P, Dawson JR R, Monjan AA (1984) Manipulation of mobile phase parameters for HPLC separation of endogenous monoamines in rat brain tissue. J Neurosci Methods 11: 5–18
Lehman A (1987) Pentylenetetrazol seizure threshold and extracellular levels of cortical amino acids in taurine-deficient kittens. Act Physiol Scand 131: 453–458
Lombardini JB (1992) Review: Recent studies on taurine in the central nervous system. In: Lombardini JB (ed) Taurine. Plenum Press, New York, pp 245–251
Malcangio M, Bartolini A, Ghelardini C, Bennardini F, Malmberg-Aiello P, Franconi F, Giotto A (1989) Effects of ICV taurine on the impairment of learning, convulsions and death caused by hypoxia. Psychopharmacology 98: 316–320
McGeer PL, McGeer EG (1982) Kainic acid: the neurotoxic breakthrough. Crit Rev Toxicol 10/1: 1–26
Menendez N, Herreras O, Solis JM, Herranz AS, Del Rio RM (1989) Extracellular taurine increase in rat hippocampus evoked by specific glutamate receptor activation is related to the excitatory potency of glutamate agonists. Neurosci Lett 102: 64–69
Mutani R, Bergamini L, Fariello R, Delsedime M (1974) Effects of taurine on cortical acute epileptic foci. Brain Res 70: 170–173
Nadler JV (1981) Kainic acid as a tool for the study of temporal lobe epilepsy. Life Sci 29: 2031–2042
Nelson MF, Zazcek R, Coyle JT (1980) Effects of sustained seizures produced by intrahippocampal injection of kainic acid on noradrenergic neurons: evidence for local control of norepinephrine release. J Pharmacol Exp Ther 214: 694–702
Nyffenegger E, Lauber K, Aebi H (1960) Die Taurineausscheidung normaler und B_6-avitaminotischer Ratten nach Ganzkörperbestrahlung. Biochem Zeitschr 333: 226–235
Oja SS, Kontro P (1983) Taurine. In: Lajtha A (ed) Handbook of neurochemistry, vol 3. Plenum, New York, pp 501–533
Olney JW, Rhee V, Lan Ho O (1974) Kainic acid: a powerful neurotoxic analogue of glutamate. Brain Res 77: 507–512
Palkovits M, Banay-Schwartz M, Lajtha A (1990) Taurine levels in brain nuclei of young adult and aging rats. In: Taurine: functional neurochemistry, physiology, and cardiology. Wiley-Liss, Inc. New York, pp 45–51
Sanberg PR, Staines W, McGeer EG (1979) Chronic taurine effects on various neurochemical indices in control and kainic acid-lesioned neostriatum. Brain Res 161: 376–370
Schwob JE, Fuller T, Price JL, Olney JW (1980) Widespread patterns of neuronal damage following systemic or intracerebral injections of kainic acid: a histological study. Neuroscience 5: 991–1014

Sperk G, Lassmann H, Baran H, Seitelberger F, Hornykiewicz O (1985) Kainic acid-induced seizures: dose-relationship of behavioural, neurochemical and histopathological changes. Brain Res 338: 289–295

Sperk G (1994) Kainic acid seizures in the rat. Prog Neurobiol 42: 1–32

Stafstrom CE, Thompson JL, Holmes GL (1992) Kainic acid seizures in the developing brain: status epilepticus and spontaneous recurrent seizures. Dev Brain Res 65: 227–236

Tang XW, Deupree DL, Liu L, Wu JY (1996) Effect of GABA, taurine and chloride-channel blockers on excitatory amino acid-induced neurotoxicity. J Neurochem 66 [Suppl]1: S80

Uemura S, Ienaga K, Higashiura K, Kimura H (1991) Effects of intraamygdaloid injection of taurine and valyltaurine on amygdaloid kindled seizure in rats. Jpn J Psychiatry Neurol 45/2: 383–385

Van Gelder NM, Sherwin AL, Sacks C, Andermann F (1975) Biochemical observations following administration of taurine to patients with epilepsy. Brain Res 94: 297–306

Van Gelder NM (1978) Taurine, the compartmentalized metabolism of glutamic acid, and the epilepsies. Can J Physiol Pharmacol 56: 362–374

Velísek L, Kubová H, Velísková J, Mares P, Ortová M (1992) Action of antiepileptic drugs against kainic acid-induced seizures and automatisms during ontogenesis in rats. Epilepsia 33/6: 987–993

Wada JA, Osawa T, Wake A, Corcoran ME (1975) Effects of taurine on kindled amygdaloid seizures in rats, cats, and photosensitive baboons. Epilepsia 16: 229–234

Wade JV, Samson FE, Nelson SR, Pazdernik TL (1987) Changes in extracellular amino acids during soman- and kainic acid-induced seizures. J Neurochem 49/2: 645–649

Wozniak DF, Steward GR, Miller JP, Olney JW (1991) Age-related sensitivity to kainate neurotoxicity. Exp Neurol 114: 250–253

Yoshida M, Izumi K, Koja T, Fukuda T, Munekata E, Nakanishi T (1986) Inhibitory effect of taurine on wet-dog shakes produced by [D-ALA^2, MET^5]enkephalinamide with reference to effects on hippocampal epileptic discharges. Neuropharmacology 25/12: 1373–1378

Authors' address: Dr. B. Eppler, Department of Pharmacodynamics, College of Pharmacy, University of Florida, JHMHC Box 100487, Gainesville, FL 32610, U.S.A.

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