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PREVENTIVE AND CURATIVE EFFECTS OF ETIFOXINE IN A RAT MODEL OF BRAIN OEDEMA

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SUMMARY

1. The aim of the present study was to test the hypothesis that increasing GABAergic neurotransmission is involved in the prevention or treatment of brain oedema. The study was conducted in the well-established rat triethyltin (TET) model of brain oedema and examined the effects of etifoxine, a compound that increases GABAergic neurotransmission through multiple mechanisms, including neurosteroid synthesis.

2. Daily oral administration of 3 mg/kg per day TET for 5 consecutive days strongly perturbed rat behaviour and induced reproducible cerebral oedema. Coadministration of etifoxine (2 × 25 or 2 × 50 mg/kg per day, p.o.) over the 5 days of TET treatment blocked the development of brain oedema and the increase in brain sodium content induced by TET, as well as reducing the increase in brain chloride content. Moreover, etifoxine inhibited the decrease in bodyweight, the neurological deficit and the altered locomotor activity induced by TET. At a lower dose (2 × 10 mg/kg per day, p.o.), etifoxine did not have any preventive effects.

3. To examine the curative effects of etifoxine, it was administered from the 4th day of TET treatment for 5 consecutive days, when brain oedema was already established. In these experiments, etifoxine (2 × 50 mg/kg per day, p.o.) significantly reduced cerebral oedema and the outcomes induced by TET treatment. Moreover, etifoxine reduced the mortality in response to TET treatment.

4. In conclusion, because etifoxine has a good safety profile as an anxiolytic, the results of the present study suggest that it is worth further clinical investigation as a neuroprotectant.

Key words: brain oedema, etifoxine, neuroprotection, neurotoxicant, triethyltin.

INTRODUCTION

The brain is known to be most vulnerable to noxious stimuli, such as hypoxia, ischaemia, oedema or neurointoxication, which occur as a consequence of cardiac arrest or head trauma, cerebral vasospasm, stroke or exposure to some neurotoxicants.[1–3] Brain oedema is a serious pathological condition characterized by an abnormal accumulation of water in brain tissue. This condition remains one of the unsolved problems in neurology and neurosurgery and its treatment is largely empirical.[3]

Among the various avenues that have been considered to achieve neuroprotection, the hypothesis of increasing cerebral GABA concentrations has received considerable attention.[4,5] Some GABA agents, such as clomethiazole, muscimol, tiagabine and vigabatrin, show clear effects in various animal models, whereas other potent $GABA_A$ receptor potentiators like barbiturates and benzodiazepines have effects that are either not reproducible or non-existent. The reasons for these differences are not understood and may be multifactorial. Some may be related to the complex interplay between GABAergic transmission and glutamatergic activity in the brain. Green *et al*. have suggested that enhancing GABA function decreases glutamatergic activity.[5] Conversely, studies like that of Kiewert *et al*. suggest that antagonism of GABA receptors may contribute, along with other mechanisms, to the neuroprotective effects of bilobalide, a constituent of *Ginkgo biloba*.[6] Among the GABA receptor potentiators, neurosteroids are of particular interest because compounds like progesterone and allopregnanolone are able to modulate $GABA_A$ and *N*-methyl-D-aspartate (NMDA) receptors and are associated with neuroprotection and the induction of neurite overgrowth.[7,8] For example, it has been shown in the rat that, after contusion injury of the frontal cortex, post-traumatic treatment with progesterone can improve the recovery of cognitive performance and behavioural deficits and decrease brain oedema.[9–13] In that context, the present study was undertaken to further test the hypothesis of the GABA pathway as an avenue for the prevention or treatment of brain oedema using etifoxine, a compound that increases GABAergic neurotransmission through multiple mechanisms.

Etifoxine is a clinically useful anxiolytic,[14,15] but it is chemically unrelated to benzodiazepines. Several studies have shown that etifoxine binds to $GABA_A$ receptors via an allosteric site that differs from that to which the benzodiazepines bind.[16,17] Functionally, etifoxine increases GABAergic neurotransmission by an allosteric effect on $GABA_A$ receptors and by an indirect mechanism involving the activation of the peripheral (mitochondrial)-type benzodiazepine receptor (PBR).[18,19] It was also shown recently that etifoxine increases the plasma and cerebral production of the neurosteroid allopregnanolone, a potent positive modulator of $GABA_A$ receptor function.[20]

Based on these multiple mechanisms of action, the preventive and curative effects of etifoxine were evaluated in the triethyltin (TET)-induced rat model of oedema. The TET rat model is well established because it has been characterized at multiple levels: physiologically, histologically and mechanistically.[21,22] To investigate

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The authors declare that they are employees of Biocodex, which markets etifoxine as an anxiolytic.

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the preventive effects of etifoxine, it was administered from the onset of TET administration and for all 5 days of TET treatment. To investigate its curative effects, etifoxine was administered from the 4th day of TET treatment, when the oedema and its outcomes were already established. The importance of investigated these two types of effect is underlined by numerous animal and clinical studies of neuroprotectants that show the existence of a 'time-window for treatment'.[23]

METHODS

Animals

Male Wistar rats (Janvier Breeding, Le Genest Saint Isle, France) were housed in air-conditioned rooms that were temperature (22 ± 2°C) and hygrometry (50 ± 20%) controlled under a 12 h light–dark cycle (lights on 0700 hours). Food (SAFE, Augy, France) and filtered tap water were available *ad libitum*. Experiments were performed at least 4 days after the rats had arrived in the laboratory. All experiments were performed in accordance with the recommendations of the International Association for the Study of Pain (IASP) Committee for Research and Ethical Issues guidelines,[24] the European Community regulations and ethical policies (no. 86/609/CEE; http://ec.europa.eu/food/fs/aw/aw_legislation/scientific/86-609-eec_fr.pdf) and French government guidelines (authorization A60-02, July 2005; http://www.ddsv60@agriculture.gouv.fr).

Triethyltin-induced oedema

Rats (200–250 g) were used in groups of 10. Triethyltin oedema was induced according to the method of Linee *et al*.,[22] with some modification. Triethyltin (3 mg/kg per day; 1 mL/100 g) was administered orally for 5 consecutive days from Day 0 to Day 4 at 0800 hours. Bodyweight, neurological score and behavioural outcomes were recorded every day. On the Day 5, or at the end of the protocol, rats were killed and their brains removed quickly and weighed (wet weight).

Measurement of cerebral water and electrolyte content

Wet brains were dried for 72 h at 90°C in an oven. Cerebral water content was determined by measuring the percentage difference in brains weight before and after drying. Before determining cerebral electrolyte content, each dried brain was digested in 10 mL of 1 mol/L nitric acid (HNO_3) for 1 week and homogenized at room temperature. Then, the brain suspension was centrifuged at 9000 *g* for 10 min at 4°C (Allegra 21R Centrifuge; Beckman, Villepinte, France). The supernatant was taken and filtered through polytetrafluorethylene (PTFE) syringe 0.45 μm filters (Merck, VWR, Fontenay-sous-Bois, France). Cerebral sodium, potassium, calcium and chloride content was determined by ion chromatography (761 Compact IC chromatograph; Metrohm, Courtaboeuf, France) and expressed as μEq/g dry weight. Standard curves were constructed using standard sodium, potassium, calcium and chloride solutions (1 g/L). The detection levels for sodium, potassium, calcium and chloride ions were 20, 30, 30 and 10 μg/L, respectively. Samples were diluted 1 : 10 in distilled water before measurement of ion concentrations.

Neurological score

The neurological score was evaluated before treatment and then on every day of the study as described by Linee *et al*.[22] on the following four-point scale: 0, no observable trouble (normal); 1, loss of spontaneous and exploratory activities, but the rat retains its motor ability (mild); 2, loss of the clutching reflex when the rat is pushed (moderate); 3, loss of the righting reflex, coma with eventual death (severe).[22] These evaluations were not conducted in a blinded fashion.

Locomotor activity

Locomotor activity was recorded before treatment and at the end of the study. Each rat was placed into an Opto-Varimex (Columbus, Columbus, OH, USA) activity monitor that allows recording of locomotion through 15 photoelectric cells positioned on the walls of a rectangular chamber (302 × 290 × 100 mm) for 15 min. Locomotor activity was expressed in arbitrary units as the number of cells crossed (with each crossing in front of a cell corresponding to 1 unit).

Drugs and treatment

Etifoxine (Biocodex, Gentilly, France) was dissolved in a 1% solution of Tween 80. Pilot studies indicated that this percentage of Tween 80 did not produce any toxic symptoms. Triethyltin bromide (97%; Sigma, Saint Quentin Fallavier, France) was dissolved in distilled water and administered orally (by gavage) at a dose of 3 mg/kg per day once a day (at 0800 hours); etifoxine was administered orally twice a day (at 0900 and 1600 hours) for 5 consecutive days from Day 0 to Day 4. In experiments examining the curative effects of etifoxine, it was administered from Day 3 for 5 consecutive days. Control rats in experiments examining the preventive and curative effects of etifoxine were treated with the vehicle only.

Statistical analysis

All data are expressed as the mean±SEM. One-way analysis of variance (ANOVA) followed by post hoc comparisons based on Bonferoni or Holm-Sidak methods were used to determine the significance of differences between groups. Differences were considered to be significant when $P < 0.05$.

RESULTS

Bodyweight

In experiments examining the preventive effects of etifoxine, bodyweight increased in the saline-treated group by an average of 25.0 ± 0.9 g over the 5 day period (Fig. 1a). Administration of 3 mg/kg per day TET significantly inhibited the increase in weight gain and resulting in a decrease in bodyweight of 1.4 ± 2.4 g over the 5 days. Etifoxine administered alone at doses ranging from 2 × 10 to 2 × 50 mg/kg per day had no significant effect on bodyweight compared with the saline-treated group.

Concomitant oral administration of 2 × 10 mg/kg per day etifoxine with TET did not inhibit the decrease in bodyweight induced by TET. However, coadministration of higher doses of etifoxine (2 × 25 and 2 × 50 mg/kg per day) with TET blocked the TET-induced decrease in bodyweight and, in these groups, an increase in bodyweight was observed of 17.6 ± 3.0 and 19.9 ± 2.6 g, respectively.

In experiments examining the curative effects of etifoxine, TET was administered from Day 0 to Day 4 and etifoxine was administered from Day 3 to Day 7. In these experiments, bodyweight had decreased in the TET-treated group by 26.4 ± 2.2 g on Day 7, whereas in the saline-treated group bodyweight had increased by 56.1 ± 1.0 g (Fig. 1b). In these studies, etifoxine was administered at the highest dose used in the first series of experiments (2 × 50 mg/kg per day), which was shown to significantly inhibit the effects of TET on bodyweight. In this second series of experiments, 2 × 50 mg/kg per day etifoxine did not completely inhibit the effects of TET on bodyweight, although it did significantly attenuate them, with the TET + etifoxine-treated group exhibiting an increase in bodyweight of 12.5 ± 5.6 g on Day 7. In this series of experiments,

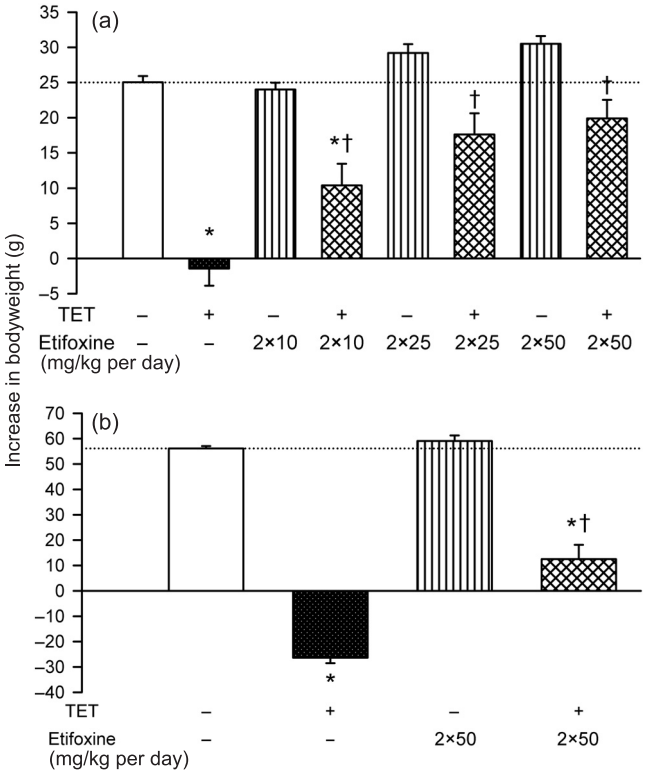

Fig. 1 (a) Preventive and (b) curative effects of etifoxine on the bodyweight of rats treated or not with 3 mg/kg per day triethyltin (TET). (a) In experiments investigating the preventive effects of etifoxine, TET and etifoxine were coadministered orally for 5 consecutive days and bodyweight was measured on the 5th day. (b) In experiments investigating the curative effects of etifoxine, TET was administered for 5 days and etifoxine was given from the 4th day, when the oedema was established. Changes in bodyweight were determined as the difference in bodyweight from the first day until the end of the treatment. Data are the mean±SEM ($n = 10$). $^{*}P < 0.05$ compared with untreated control; $^{\dagger}P < 0.05$ compared with TET alone.

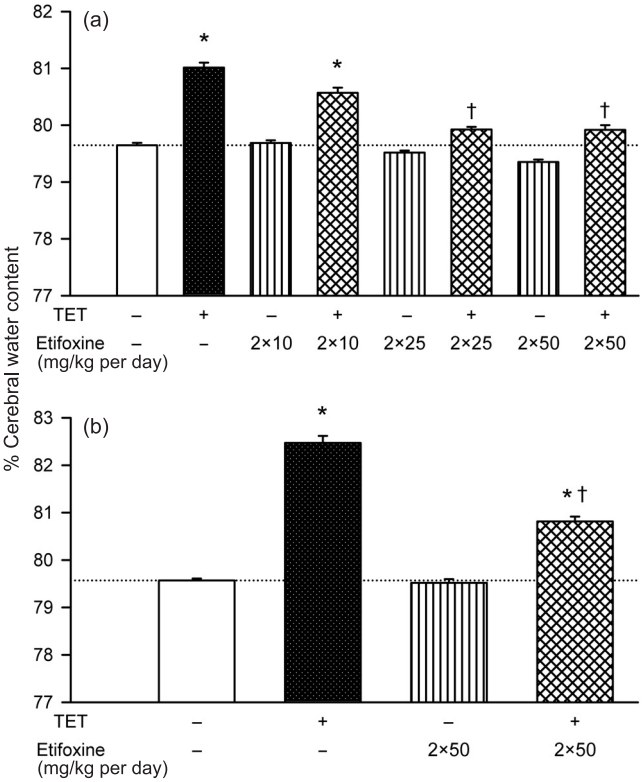

Fig. 2 (a) Preventive and (b) curative effects of etifoxine on cerebral water content in rats treated or not with 3 mg/kg per day triethyltin (TET). Rats were treated as described for Fig. 1. Data are the mean±SEM ($n = 10$). $^{*}P < 0.05$ compared with untreated control; $^{\dagger}P < 0.05$ compared with TET alone.

the extent of the oedema in the TET + etifoxine-treated group was still greater than that in the control group.

Cerebral water content

As shown in Fig. 2a, oral administration of 3 mg/kg per day TET for 5 consecutive days significantly increased cerebral water content from 79.65 ± 0.04% (saline-treated group) to 81.02 ± 0.09%. Etifoxine administration (2 × 10 and 2 × 50 mg/kg per day) alone resulted in no significant change in cerebral water content compared with that in the saline-treated group.

Concomitant oral administration of 2 × 10 mg/kg per day etifoxine and TET had no siginificant effect on the increased cerebral water content observed with TET treatment alone. The higher doses of etifoxine (2 × 25 and 2 × 50 mg/kg per day) significantly blocked the increase in cerebral water content induced by TET (79.93 ± 0.05 and 79.92 ± 0.08% cerebral water content, respectively).

In experiments examining the curative effects of etifoxine, TET treatment markedly increased cerebral water content on Day 7 from 79.57 ± 0.04% in the saline-treated group to 82.47 ± 0.15% (Fig. 2b). Etifoxine (2 × 50 mg/kg per day) did not totally suppress the TET-induced increase in cerebral water content but did reduce it significantly by 60% to 80.81 ± 0.10%.

Neither TET nor etifoxine alone, nor their combination, significantly altered brain dry weight compared with that of the saline-treated group. For example, in experiments examining the preventive effects of etifoxine, brain dry weight in the saline-, TET-, etifoxine (2 × 50 mg/kg per day)- and TET + etifoxine-treated groups was 281 ± 3, 284 ± 4, 290 ± 3 and 284 ± 3 mg, respectively.

Cerebral electrolyte content

In experiments examining the preventive effects of etifoxine, brain sodium content in the saline-treated group was 250 ± 5 μEq/g (Fig. 3a). Five days treatment with TET was associated with a significant increase in brain sodium content (334 ± 8 μEq/g). Etifoxine alone (2 × 10 and 2 × 50 mg/kg per day) had no significant effect on brain sodium content compared with that of the saline-treated group. Concomitant oral administration of etifoxine (2 × 10 mg/kg per day) and TET did not suppress the TET-induced increase in brain sodium content. However, coadministration of 2 × 25 and 2 × 50 mg/kg per day etifoxine blocked the TET-induced increase in brain sodium content (281 ± 4 and 272 ± 8 μEq/g, respectively).

Treatment with TET also significantly enhanced brain chloride content from 173 ± 3 μEq/g in the saline-treated group to 230 ± 5 μEq/g (Fig. 4a). Etifoxine administration (2 × 10 and 2 × 50 mg/kg per day) alone had no significant effect on brain chloride content compared with that in the saline-treated group. Concomitant oral

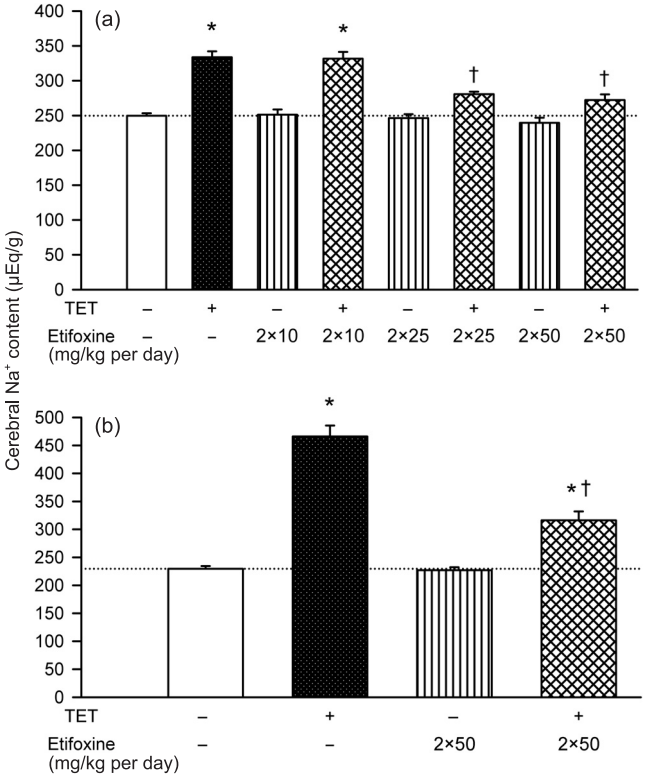

Fig. 3 (a) Preventive and (b) curative effects of etifoxine on cerebral sodium ion content in rats treated or not with 3 mg/kg per day triethyltin (TET). Rats were treated as described for Fig. 1. Data are the mean±SEM ($n = 10$). *$P < 0.05$ compared with untreated control; †$P < 0.05$ compared with TET alone.

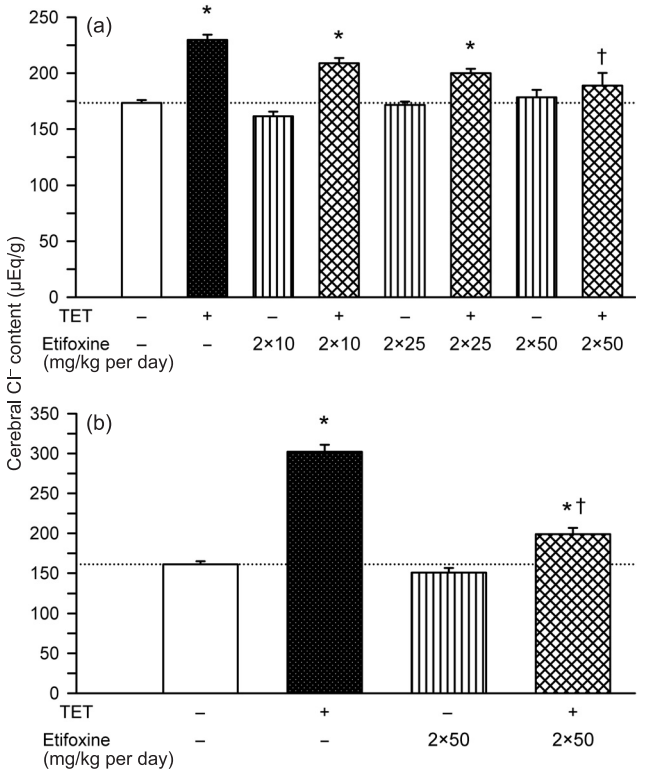

Fig. 4 (a) Preventive and (b) curative effects of etifoxine on cerebral chloride ion content in rats treated or not with 3 mg/kg per day triethyltin (TET). Rats were treated as described for Fig. 1. Data are the mean±SEM ($n = 10$). *$P < 0.05$ compared with untreated control; †$P < 0.05$ compared with TET alone.

administration of etifoxine (2 × 10 or 2 × 25 mg/kg per day) and TET did not suppress the TET-induced increase in brain chloride content. However, coadministration of 2 × 50 mg/kg per day etifoxine and TET inhibited the TET-induced increase in brain chloride content (189 ± 11 μEq/g).

In experiments examining the curative effects of etifoxine, brain sodium content in the saline-treated group was 230 ± 5 μEq/g (Fig. 3b). Treatment with TET significantly increased brain sodium content to 466 ± 19 μEq/g on Day 7. Oral administration of 2 × 50 mg/kg per day etifoxine from Day 4 did not completely inhibit the TET-induced increase in brain sodium content, although it did significantly reduced it by approximately 60% (to 316 ± 16 μEq/g).

In addition, TET significantly enhanced the brain chloride content from 161 ± 4 μEq/g in the saline-treated group to 302 ± 9 μEq/g (Fig. 4b). Administration of 2 × 50 mg/kg per day etifoxine with TET did not totally suppress the TET-induced increase in brain chloride content, although it did reduce it significantly by approximately 65% to 199 ± 8 μEq/g.

Brain potassium and calcium content was also determined, but none of the treatments modified the levels of these electrolytes.

Neurological score

In experiments examining the preventive effects of etifoxine (Fig. 5a), administration of 3 mg/kg per day TET for 5 consecutive days induced a neurological deficit starting from the Day 4 and yielding a score of 2.6 ± 0.1 on Day 5, which is close to the maximum score of 3. Etifoxine alone did not affect the general behaviour of the rats.

Concomitant oral administration of 2 × 10 mg/kg per day etifoxine and TET slightly reduced the neurological deficit induced by TET on Day 5, although the effect was not signficant and levels were not restored to those comparable to the control group. Coadministration of 2 × 25 and 2 × 50 mg/kg per day etifoxine strongly and significantly inhibited the neurological deficit induced by TET, resulting in levels corresponding to a slow reduction of spontaneous activity.

In experiments examining the curative effects of etifoxine, TET markedly altered the general behaviour of rats, with a neurological score reaching the maximum level of 3 (Fig. 5b) and with severe morbidity approaching mortality for half the rats from Day 6 onwards. Etifoxine (2 × 50 mg/kg per day) from the Day 3 of TET intoxication significantly inhibited the deleterious effects of TET: the neurological score was progressively reversed and decreased towards levels in the control group. Moreover, no mortality was observed.

Locomotor activity

Administration of 3 mg/kg per day TET for 5 consecutive days significantly reduced locomotor activity recorded on Day 5 to a level of 54% (Fig. 6a). Etifoxine given alone did not modify locomotor activity.

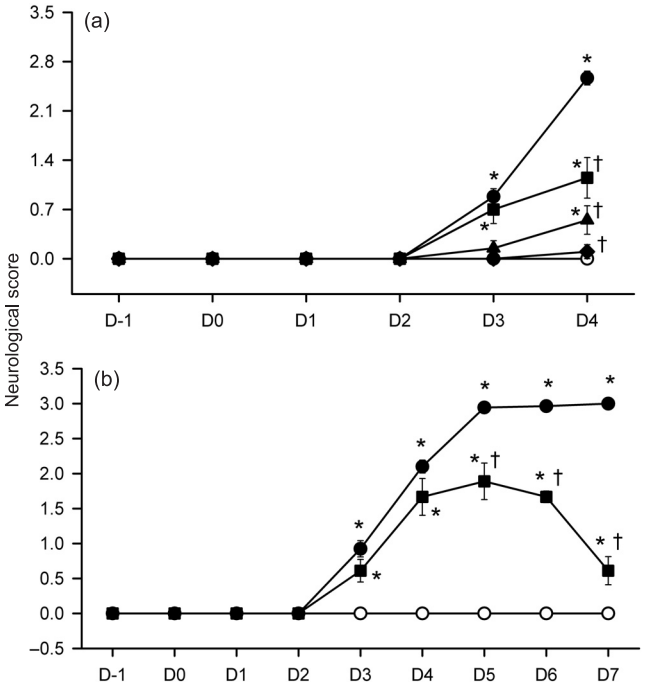

Fig. 5 (a) Preventive and (b) curative effects of etifoxine on the neurological deficits induced by triethyltin (TET) in rats. (a) In experiments investigating the preventive effects of etifoxine, 3 mg/kg per day, p.o., TET was administered alone (●) or with 2 × 10 (■), 2 × 25 (◆) or 2 × 50 mg/kg per day (▲), p.o., etifoxine for 5 consecutive days. (○), control. (b) In experiments investigating the curative effects of etifoxine, 3 mg/kg per day TET was administered alone (●) or with 2 × 50 mg/kg per day, p.o., etifoxine (■), which was given from the 4th day until the 8th day. (○), control. Data are the mean±SEM (n = 10). *P < 0.05 compared with untreated control; †P < 0.05 compared with TET alone.

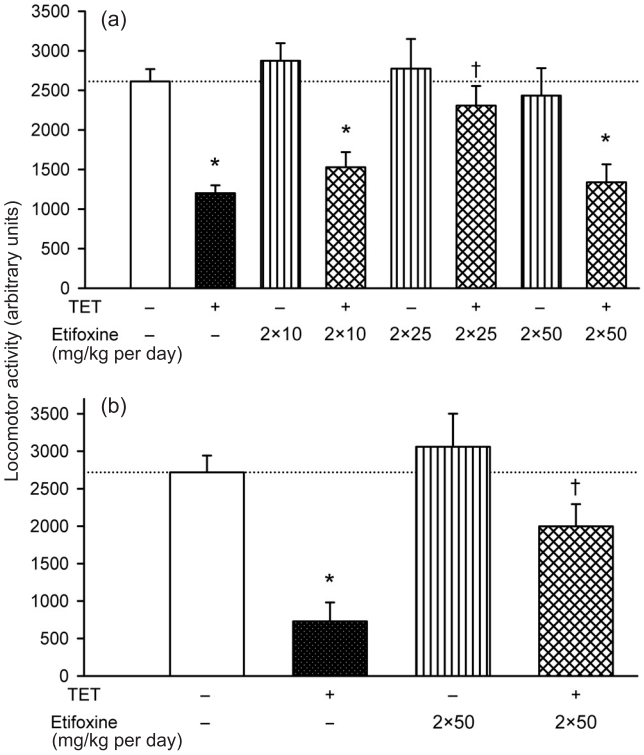

Fig. 6 (a) Preventive and (b) curative effects of etifoxine on locomotor activity in rats treated or not with 3 mg/kg per day triethyltin (TET). Rats were treated as described for Fig. 1. Data are the mean±SEM (n = 10). *P < 0.05 compared with untreated control; †P < 0.05 compared with TET alone.

Concomitant oral administration of 2 × 10 or 2 × 50 mg/kg per day etifoxine and TET did not inhibit the TET-induced decrease in locomotor activity. Only coadministration of 2 × 25 mg/kg per day etifoxine and TET significantly blocked the effects of TET on locomotor activity.

In experiments examining the curative effects of etifoxine, TET strongly reduced locomotor activity to a level of 73% compared with that in the saline-treated group (Fig. 6b) on Day 7. In this series, 2 × 50 mg/kg per day etifoxine significantly inhibited and reversed the effects of TET on locomotor activity.

DISCUSSION

The search for potential therapies for brain oedema has been ongoing for several decades and has relied on a variety of animal and cellular models. Among these, the TET rat model is well established because it has been characterized at multiple levels, including physiologically, histologically and mechanistically.[21,22] In this model, intraperitoneal or oral administration of the neurotoxicant is associated with reproducible weight loss, neurological deficit and oedema.[22,25–27] Chronic oedema appears progressively and disappears after the intoxication is stopped. Oedema formation can be followed experimentally because it is characterized by an increase in total brain water content and the accumulation of fluid and sodium ions between the myelin layers, with a more severe injury of the white matter.[28] Triethyltin intoxication has been described as a useful model for the study of vacuolar degeneration of central myelin or demyelination associated with oedema.[29] Central myelin is the site for various pathological conditions, such as ischaemia, inflammation or intoxication. At the biochemical level, TET inhibits a number of metabolic activities in the brain, such as mitochondrial respiration and oxidative phosphorylation,[30] and alters intracellular concentrations of organic osmolytes, which are involved in ion and volume regulation and osmotic balance in astrocytes,[31] leading to astrocytic swelling. In this model, etifoxine prevents the full development of the cytotoxic oedema, significantly reducing the extent of the oedema and its outcomes after it has been established (curative model).

In experiments examining the preventive effects of etifoxine, 5 consecutive days of oral administration of TET (3 mg/kg) increased cerebral water content (oedema), increased sodium and brain chloride content and induced behavioural and neurological deficits. Oral coadministration of etifoxine (2 × 25 mg/kg per day) for 5 days during TET intoxication blocked the development of cerebral oedema, inhibited the diminution of the bodyweight increase and markedly diminished the neurological deficit and the reduction in locomotor activity induced by TET. A lower dose of etifoxine (2 × 10 mg/kg per day) had no effect on the TET-induced changes, whereas a higher dose (2 × 50 mg/kg per day) had no further effect on the TET-induced changes. These results suggest that a threshold level was reached with the middle dose of etifoxine because that dose reversed the alterations induced by TET. In the literature, some studies have shown that TET increases brain sodium content[22,25,27] without affecting potassium brain content,[22] or even enhancing[27] or

decreasing it.[25] In the present study, coadministration of etifoxine with TET blocked the TET-induced increases in both brain sodium and chloride content.

In the second series of experiments examining the curative effects of etifoxine, etifoxine was administered from Day 3 after the beginning of TET intoxication, when the oedema was established, in order to determine whether it could reverse the cerebral oedema induced by TET. In this series of experiments, administration of 3 mg/kg per day TET for 5 consecutive days resulted in a more severe oedema measured on Day 7 compared with the measures obtained on Day 4 in the preventive study. For example, the percentage of cerebral water content increased by 1.37% on Day 4 and by 2.90% on Day 7. Moreover, there were no deaths on Day 4, but mortality appeared thereafter, with a 50% of rats dead by Day 7. In this second series of experiments, etifoxine was administered at a dose of 2×50 mg/kg per day, the highest dose used in the preventive study, because exposure to TET was longer in the curative study (8 days) than in the preventive study (5 days). Etifoxine, given from the Day 3 until Day 7, did not completely suppress the effects induced by TET, but did significantly reduce the TET-induced decrease in bodyweight by 50%, the TET-induced increase in cerebral water content by 60%, the TET-induced increase in cerebral sodium content by 60% and the TET-induced increase in cerebral chloride content by 65%. Moreover, etifoxine markedly inhibited the neurological symptoms and deficits in locomotor activity, as well as completely preventing TET-induced mortality.

The apparently selective effect of etifoxine on sodium and chloride content in both the preventive and curative modes of administration is of interest because etifoxine did not significantly affect ion content in the dried brain of rats not treated by TET. This cannot be explained at present on the basis of the known effects of etifoxine or other neurosteroids on ion transport.

Numerous clinical trials of neuroprotection for ischaemic stroke have been unsuccessful, but fewer than one-half of the neuroprotective treatments were administered within the 4–6 h window within which efficacious clinical neuroprotection is considered possible.[23] The protective effect of etifoxine when administered to rats after the beginning of the neurointoxication, when problems were apparent, are of interest from a clinical perspective because they suggest that the window of opportunity extends some time after the appearance of symptoms.

These effects of etifoxine can be understood on the basis of its known mechanisms of action on GABA function, including an interaction with the tert-butylbicyclophosphorothionate (TBPS) binding site of the $GABA_A$ receptor complex, leading to a facilitation of GABAergic inhibition,[16,18] and binding to the PBR, initiating neurosteroid biosynthesis.[20] Thus, etifoxine enhances $GABA_A$ receptor function by two known mechanisms: (i) an allosteric effect; and (ii) by an indirect mechanism involving activation of the PBR.

With respect to a potential contribution of the first mechanism of action, it is interesting to note that TET inhibits synaptosomal [^{3}H]-GABA uptake in mouse forebrain,[32] although an *in vivo* study revealed that TET administered during postnatal development did not change brain GABA concentrations in the rat (although the dose of TET used was lower than that needed to cause intramyelin oedema).[33] It is known that increasing GABAergic transmission decreases glutamatergic activity in the brain and this may be associated with neuroprotective effects against brain ischaemia;[4,5] thus, etifoxine may exert its effects by acting at the GABA receptor to reduce neuronal excitotoxicity triggered by amino acid release.

With respect to activation of the PBR, it is known that the PBR plays an important role in steroidogenesis by regulating the entry of cholesterol into the steroidogenic pathway.[34,35] Etifoxine increases rat brain concentrations of several neurosteroids, including pregnenolone, progesterone and allopregnanolone,[20] which have demonstrated neuroprotective properties. Progesterone decreased cerebral oedema, cell death mediators, inflammatory cytokines and reactive gliosis in a model of rat traumatic brain injury.[36] Progesterone also improved clinical and histological outcomes in a rat model of spinal cord injury[37] and ameliorated neurological function in a rat model of focal cerebral ischaemia.[38] Progesterone has promyelinating effects, as documented in the mouse sciatic nerve and in cocultures of sensory neurons and Schwann cells, promotes myelination in the brain[39] and stimulates myelin formation in organotypic slice cultures of 7-day-old rat and mouse cerebellum.[40] In a recent review of the neuroprotective effects of neurosteroids, Wojtal *et al*. concluded that these effects are multifactorial, including both genomic and non-genomic mechanisms, and that these steroids have a promising role in the management of neurodegenerative conditions.[8] Because etifoxine has been shown to increase brain and plasma levels of key neurosteroids in Wistar rats[20] and to exhibit adequate oral absorption,[41] it can be assumed that its protective effects against oedema and behavioural toxicity induced by TET are mediated by stimulation of neurosteroids biosynthesis.

In assessing the relevance of these findings, one must take into consideration the well-known gap between the results of animal and clinical studies observed in neuroprotection research.[23] Because a number of compounds with different mechanisms of action (e.g. *Ginkgo biloba* extract,[27] vincamine derivatives[25] or the monoamine oxidase inhibitor moclobemide[26]) exhibit protective efficacies in the experimental model used in the present study, it is possible that other pathways may contribute to the mechanism(s) of action of etifoxine.

The TET-induced oedema model has relevance in investigations of potential protective properties of candidate drugs against oedema provoked by a neurotoxicant. However, this model has its limitations: it does not explore the spectrum of potential neuroprotective effects of the candidate drug and investigations should be conducted in other models, such as the ischaemic or traumatic brain injury models. Moreover, the mechanism of action of etifoxine should be explored in further detail because several mechanistic options are possible.

Thus, a number of additional studies are indicated, including: (i) the effects of etifoxine on histological and biochemical parameters altered during oedema; (ii) the potential neuroprotective effect of etifoxine in other models of cerebral ischaemia, stroke and traumatic spinal or brain injury;[42–44] and (iii) *in vitro* studies assessing the effects of etifoxine on the neurotoxicity of TET on human fetal neuron and astrocyte cultures,[45] as well as on TET-induced cell death in cultured oligodendrocytes.[32] Finally, it should be noted that the established clinical use of etifoxine as an anxiolytic and its favourable safety profile are positive characteristics that will facilitate its clinical evaluation.

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