

# Neuroprotective Properties of Topiramate in the Lithium-Pilocarpine Model of Epilepsy

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Received July 17, 2003; accepted October 14, 2003

## ABSTRACT

The lithium-pilocarpine model reproduces the main characteristics of human temporal lobe epilepsy. After status epilepticus (SE), rats exhibit a latent seizure-free phase characterized by development of extensive damage in limbic areas and occurrence of spontaneous recurrent seizures. Neuroprotective and antiepileptogenic effects of topiramate were investigated in this model. SE was induced in adult male rats by LiCl (3 mEq/kg) followed 20 h later by pilocarpine (25 mg/kg). Topiramate (10, 30, or 60 mg/kg) was injected at 1 and 10 h of SE. Injections were repeated twice a day for six additional days. Another group received two injections of diazepam on the day of SE and of vehicle for 6 days. Neuronal damage was assessed at 14 days after SE by cell counting on thionin-stained sections. Occurrence of spontaneous recurrent seizures (SRS) was

videorecorded for 10 h per day in other groups of rats. In diazepam-treated rats, the number of neurons was dramatically reduced after SE in all subregions of hippocampus and layers II–IV of ventral cortices. At all doses, topiramate induced a 24 to 30% neuroprotection in layer CA1 of hippocampus ( $p < 0.05$ ). In CA3b, the 30-mg/kg dose prevented neuronal death. All rats subjected to SE became epileptic. The latency (14–17 days) to and frequency of SRS were similar in topiramate- and diazepam-treated rats. The high mortality in the 30 mg/kg topiramate group (84%) was possibly the result of interaction between lithium and topiramate. In conclusion, topiramate displayed neuroprotective properties only in CA1 and CA3 that were not sufficient to prevent epileptogenesis.

Temporal lobe epilepsy (TLE) is one of the most common forms of intractable epilepsy. Patients affected often have similar clinical history, including an initial precipitating injury such as childhood febrile convulsions, status epilepticus (SE), or trauma. Between this injury and the emergence of recurrent complex partial seizures, there is usually a latent period of several years. Frequently associated with this epilepsy is the presence of hippocampal sclerosis (HS). HS is defined by specific neuronal loss throughout the hippocampus, with severe damage in the subiculum, CA1, CA4, and hilus in contrast with slighter damage in granule cells and relative sparing of CA3 and especially CA2 region. Human studies strongly support the view that HS probably initiates or contributes to the generation of most TLEs (Engel, 1996). However, there is a growing body of evidence that amygdala, limbic thalamus, and entorhinal cortex may be injured in

TLE (Juttila et al., 2001). The respective role of various hippocampal or extrahippocampal structures in the genesis of the disease remains unknown.

Animal models such as the pilocarpine or lithium-pilocarpine model of epilepsy may help to address this issue. Injection of pilocarpine, a muscarinic agonist, induces generalized convulsive SE in rodents, which represents the initial precipitating injury. After a latent period, adult rats exhibit spontaneous recurrent seizures (SRS) during the remainder of their life. The EEG and behavioral features of these seizures resemble those of complex partial seizures (Leite and Cavalheiro, 1995). When rats are pretreated with lithium chloride, SE can be produced by a substantially lower dose of pilocarpine, and rats display the same clinical and EEG features of SE as with pilocarpine alone (Honchar et al., 1983). Briefly, the injection of the convulsant leads first to cholinergic signs in about 5 to 10 min followed by the onset of partial seizures in the following 10 min. Usually, within 30 to 60 min after the injection of pilocarpine, partial seizures generalize; SE is characterized by continuous clinical and EEG seizures (Honchar et al., 1983; Dubé et al., 2001). The pilocarpine and lithium-pilocarpine models reproduce the temporal evolution of the disease (Turski et al., 1989; Dubé et

This work was supported by INSERM U 398 (Institut National de la Santé et de la Recherche Médicale), the Fondation pour la Recherche Médicale, and by a grant from Johnson and Johnson Pharmaceutical Research and Development, LLC (Raritan, NJ).

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Article, publication date, and citation information can be found at <http://jpet.aspetjournals.org>.

DOI: 10.1124/jpet.103.057091.

**ABBREVIATIONS:** TLE, temporal lobe epilepsy; SE, status epilepticus; HS, hippocampal sclerosis; Lipilo, lithium-pilocarpine; SRS, spontaneous recurrent seizures; TPM, topiramate; AMPA,  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid.

al., 2001). As in humans, the neuropathology includes HS (Turski et al., 1989; André et al., 2000, 2001, 2003; Roch et al., 2002a). However, neuronal damage is more marked and extensive than that found in most patients; in both models, neuronal death is very common in extrahippocampal areas such as the entorhinal, perirhinal, and piriform cortices; neocortex; numerous thalamic and amygdalar nuclei; the olfactory system; and the substantia nigra. The only difference between the two models is the damage in the parahippocampal cortices, which is more extended, sometimes total in the lithium-pilocarpine compared with the pilocarpine model (Persinger et al., 1988; Turski et al., 1989). Finally, the reduction of SRS frequency by antiepileptic drugs in the pilocarpine model parallels their activity against human complex partial seizures (Leite and Cavalheiro, 1995).

Because of the relevance of this model to human pathology, the lithium-pilocarpine model can be used to test the effects of drugs. Topiramate (TPM) is a new antiepileptic drug used as adjunctive therapy in refractory partial-onset seizures in adults (Yen et al., 2000), partial-onset seizures in children (Elterman et al., 1999), and generalized tonic-clonic seizures of nonfocal origin (Biton et al., 1999). TPM exhibits neuroprotective properties in several models of injury such as global ischemia (Edmonds et al., 2001), focal ischemia (Yang et al., 2000), or SE (Niebauer and Gruenthal, 1999). TPM has several mechanisms of action that may contribute to its anticonvulsant activity (Shank et al., 2000). It induces a voltage-dependent inhibition of sodium channels by stabilizing the inactivated state (Zona et al., 1997) and reduces L-type high-voltage-activated calcium current (Zhang et al., 2000). It increases chloride flux of GABA<sub>A</sub> channels by acting on a nonbenzodiazepine site (White et al., 2000) and antagonizes kainate-evoked currents (Gibbs et al., 2000). Last, it acts as an inhibitor of erythrocyte carbonic anhydrase, but the contribution of this property to its antiepileptic effect is controversial (Stringer, 2000).

In this work, we studied the potential neuroprotective properties of TPM on neuronal loss induced by lithium-pilocarpine SE and on subsequent epileptogenesis. This study is in line with previous ones from our laboratory aimed at the identification of brain areas involved in the epileptogenic process (André et al., 2000, 2001, 2003).

## Materials and Methods

**Animals.** Adult male Sprague-Dawley rats provided by Janvier Breeding Center (Le Genest-St-Isle, France) were housed under controlled standard conditions (light/dark cycle, 7:00 AM–7:00 PM lights on), with food and water available ad libitum. All animal experimentation was performed in accordance with the rules of the European Community Council Directive of November 24, 1986 (86/609/EEC), and the French Department of Agriculture (License no. 67-97). The experiments were performed on a total number of 251 rats, weighing from 250 to 330 g.

**Experiment 1: Lithium-Pilocarpine-Induced Status Epilepticus and Topiramate Treatment.** At least 1 week after arrival or after surgery, all rats received lithium chloride (3 mEq/kg i.p.; Sigma-Aldrich, St. Louis, MO). On the following day, methylscopolamine bromide (1 mg/kg s.c.; Sigma-Aldrich) was administered to limit the peripheral effects of the convulsant. SE was induced by injecting pilocarpine hydrochloride (25 mg/kg s.c.; Sigma-Aldrich) 30 min after methylscopolamine.

The effects of increasing doses of TPM were studied in three groups of rats. The animals of the first group (21 rats) received 10

mg/kg TPM i.p., 1 h after the onset of SE (Lipilo-TPM10), whereas the animals of groups 2 (88 rats) and 3 (45 rats) received 30 and 60 mg/kg TPM (Lipilo-TPM30 and Lipilo-TPM60), respectively. Another group was injected with 2.5 mg/kg diazepam i.m. (DZP, Valium; Roche, France; Lipilo-DZP, 24 rats) at 1 h after the onset of SE, which is derived from our standard treatment to improve animals survival. The control group received lithium, and saline and vehicle instead of pilocarpine and TPM, respectively (saline-vehicle, six rats). The onset of SE, based on our previous experiments in this model, corresponds to the moment at which rats experience successive seizures without recovery. This corresponds to continuous spiking on the EEG and usually occurs between 30 and 60 min after pilocarpine administration. The Lipilo-TPM rats surviving SE received about 10 h after the first TPM injection a second i.p. injection of the same dose of TPM and were maintained under a twice daily TPM treatment for six additional days (s.c.). Lipilo-DZP rats received a second injection of 1.25 mg/kg DZP i.m. on the day of SE at about 10 h after the first one. Thereafter, Lipilo-DZP and saline-vehicle rats received twice daily an equivalent volume of vehicle (s.c.). The i.p. route was chosen on the day of SE to allow a rapid diffusion and action of TPM, whereas the s.c. route was preferred on the following days because a fast action of the drug was no longer as necessary and avoided the risk of lesions in the abdominal space related to repeated injections.

Within the total number of rats studied in experiment 1, a subgroup of 20 rats undergoing Lipilo-induced SE was used for hippocampal and cortical EEG recording. At least 1 week after arrival, rats were anesthetized by i.p. injections of 2.5 mg/kg DZP and 1 mg/kg ketamine chlorohydrate (Imalgene 1000; Rhone Merrieux, Lyon, France). Two single-contact recording electrodes were placed on the skull, one on each side of the parietal cortex and one bipolar deep recording electrode was placed in the right hippocampus (coordinates from lambda: AP, +4 mm; ML, –2 mm; DV, –4 mm; Paxinos and Watson, 1986). The animals were allowed to recover from surgery for 1 week. On the day of SE, the implanted animals were placed into Plexiglas boxes before methylscopolamine injection to perform a digital computer-based acquisition of cortical and hippocampal EEG baseline during 1 h (Coherence, Deltamed, France). Thereafter, they were subjected to SE induced by lithium and pilocarpine, as described above and were treated either with DZP, or with 10, 30, or 60 mg/kg TPM (5 in each group). The bilateral EEG cortical activity and the unilateral EEG hippocampal activity were recorded during the whole duration of SE and concurrent behavioral changes were noted. Thereafter, the rats were used for the study of epileptogenesis and Timm staining as described below.

**Experiment 2: Lithium-Pilocarpine and Pilocarpine-Induced Status Epilepticus.** For this study, we performed both lithium-pilocarpine and pilocarpine-induced SE on an additional group of animals. In this study, 25 rats received methylscopolamine bromide (1 mg/kg s.c.). Injection of pilocarpine hydrochloride (400 mg/kg s.c.), 30 min after methylscopolamine, provoked SE. Then, three animals were treated with DZP, three with 10 mg/kg TPM, 14 with 30 mg/kg TPM, and five with 60 mg/kg TPM. In addition, 14 rats underwent lithium-pilocarpine SE, as described above, and were all treated with 30 mg/kg TPM as described above.

**Experiment 3: Blood Gases and pH Measurement.** To test the hypothesis that the very high mortality of Lipilo-TPM30 rats could reflect an interaction of TPM with lithium and result in the acidification of the rats, we measured blood gases in a subgroup of animals subjected either to Lipilo- (seven rats) or pilocarpine-induced SE (10 rats). All rats received an injection of 30 mg/kg TPM 1 h after the onset of SE. For this part of the study, a femoral artery was catheterized with polyethylene tubing under light isoflurane anesthesia in 16 rats. The catheter was threaded under the skin, up to the back of the hindpaw, to allow free access to the catheter without disturbing rat movements. The animals were allowed to recover from surgery in their home cage for 18 to 24 h before the onset of the experiment. On the day of SE, blood sampling was performed to measure arterial

blood pH, pO<sub>2</sub>, and pCO<sub>2</sub> by means of a blood gas analyzer (model 158; Corning Medical and Scientific, Halstead, UK).

**Quantification of Neuronal Damage.** Quantification of cell densities was performed at 14 days after SE on eight Lipilo-DZP, six Lipilo-TPM10, six Lipilo-TPM30, seven Lipilo-TPM60, and six saline-vehicle rats taken from experiment 1. Animals were deeply anesthetized with 1.8 g/kg pentobarbital i.p. (Doletal; Vétquinol, Lure, France). Brains were then removed and frozen. Serial 20- $\mu$ m slices were cut in a cryostat and air-dried during several days before thionine staining. In all rats, all coronal sections containing the hippocampus from the anterior to the posterior level were taken. Readings were performed at two different levels, i.e., 1) the anterior hippocampus and piriform cortex, and 2) ventral hippocampus and entorhinal cortex. The antero-posterior level of the sections was selected according to stereotaxic coordinates of the rat brain atlas of Paxinos and Watson (1986) and were identical in each animal (-3.30 from bregma for the anterior hippocampus and piriform cortex, -4.16 from bregma for the median thalamus and hippocampus, and -5.60 from bregma for ventral hippocampus and entorhinal cortex).

Quantification of cell density was performed with a 1-cm<sup>2</sup> 10  $\times$  10-box microscopic grid. The grid of counting was placed on a well defined area of the cerebral structure of interest, and counting was carried out with a microscopic enlargement of 200- or 400-fold defined for each single cerebral structure. Cell counts were performed twice on each side of three adjacent sections for each region by a single observer unaware of the animal's treatment. The number of cells obtained in the 12 counted fields in each cerebral structure was averaged. Neurons touching the inferior and right edges of the grid were not counted. Counts involved only neurons with cell bodies larger than 10  $\mu$ m.

**Study of Epileptogenesis.** Starting 1 week after SE induced by lithium-pilocarpine, a subset of rats that survived SE in experiment 1 (eight Lipilo-DZP, seven Lipilo-TPM10, seven Lipilo-TPM30, and 10 Lipilo-TPM60 rats) were observed by daily videorecording (Sony) 10 h a day. The onset of SRS was determined and the frequency of SRS was recorded over 1 month after the first SRS.

The effects of TPM on the EEG were investigated on the surviving implanted animals (two in each treatment group, DZP, 10, 30, or 60 mg/kg TPM) during 6-h recording sessions on the first 3 days after SE. Thereafter, implanted animals were videomonitoring 10 h per day and the recording of the electrographic activity was performed twice a week for 2 h.

**Timm Staining.** At 2 months after the onset of SRS determined by daily videorecording, mossy fiber sprouting was studied on the chronically epileptic rats, including the implanted animals and on five additional saline-vehicle rats. Animals were deeply anesthetized and perfused transcardially with saline followed by 100 ml of 1.15% (w/v) Na<sub>2</sub>S in 0.1 M phosphate buffer, and 100 ml of 4% (v/v) formaldehyde in 0.1 M phosphate buffer. Brains were removed from the skull, postfixed in 4% formaldehyde during 4 h, and 40- $\mu$ m coronal sections were cut on a sliding vibratome and mounted on gelatin-coated slides. The following day, sections were developed in the dark in a 26°C solution of 50% (w/v) arabic gum (160 ml), 2 M sodium citrate buffer (30 ml), 5.7% (w/v) hydroquinone (80 ml), and 10% (w/v) silver nitrate (2.5 ml) during 42 min. The sections were then rinsed with tap water at 40°C during at least 45 min, rinsed rapidly with distilled water, and allowed to dry. They were dehydrated in ethanol and coverslipped. Mossy fiber sprouting was evaluated according to criteria previously described in dorsal hippocampus (Cavazos et al., 1991), which are as follows: 0, no granules between the tips and crest of the DG; 1, sparse granules in the supragranular region in a patchy distribution between the tips and crest of DG; 2, more numerous granules in a continuous distribution between the tips and crest of DG; 3, prominent granules in a continuous pattern between tips and crest, with occasional patches of confluent granules between tips and crest; 4, prominent granules that form a confluent dense laminar band between tips and crest; and 5, confluent dense

laminar band of granules that extends into the inner molecular layer.

**Data Analysis.** Significant differences in mortality rates of the different groups were evaluated by  $\chi^2$  test and odds ratio, using the CIA software (London, UK). Statistical analysis of neuronal damage and epilepsy between the different groups was performed by means of an analysis of variance followed by a posthoc Dunnett's test for multiple comparisons using the Statview software (SAS Institute, Cary, NC).

## Results

### Behavioral Characteristics of Status Epilepticus

Within the total number of 216 rats subjected to lithium-pilocarpine SE, 17 did not develop SE and were removed from the study. Within 5 min after pilocarpine injection, rats developed diarrhea, piloerection, and other signs of cholinergic stimulation. During the following 15 to 20 min, rats exhibited head bobbing, scratching, chewing, and exploratory behavior. Recurrent seizures started around 15 to 20 min after pilocarpine administration. These seizures that associated episodes of head and bilateral forelimb myoclonus with rearing and falling, progressed to SE at about 35 to 40 min after pilocarpine, as described previously (Dubé et al., 2000, 2001). No overt behavioral difference was noted between Lipilo-DZP and Lipilo-TPM groups.

SE induced by pilocarpine alone was performed on 25 rats. These rats showed more pronounced signs of cholinergic stimulation (chewing, salivation), but the other behavioral signs of SE were identical to those of rats subjected to lithium-pilocarpine.

### EEG Characteristics of the SE, Latent, and Chronic Phases

**EEG Patterns during Lithium-Pilocarpine-Induced SE.** Within 5 to 10 min after the injection of pilocarpine, wave oscillations of very low amplitude superimposed on the normal background EEG activity in the cortex, whereas theta rhythm (6–7 Hz) occurred in the hippocampus. By 15 to 30 min, high voltage fast activity superimposed over the hippocampal theta rhythm and isolated high voltage spikes were recorded only in the hippocampus, whereas the activity of the cortex did not substantially change. By 20 to 45 min after pilocarpine injection, animals developed typical electrographic seizures with high voltage fast activity present in both the hippocampus and cortex, which first occurred as bursts of activity preceding seizures, and were followed by continuous trains of high-voltage spikes and polyspikes. From the onset of SE, the amplitude of the EEG progressively increased, whereas the frequency decreased until the administration of DZP or TPM at 1 h after onset of SE. At about 3 h of SE, the hippocampal and cortical EEG was characterized by bursts of spikes or periodic epileptiform discharges in the TPM groups. There was no difference between TPM10, TPM30, and TPM60 groups. In DZP-treated rats, the amplitude of the hippocampal and cortical EEG was reduced and the EEG was characterized by periodic epileptiform discharges in cortex and hippocampus. By 6 h of SE, the EEG amplitude decreased and came back to baseline levels in the cortex and hippocampus in DZP-treated rats, whereas periodic epileptiform discharges were still present in both structures of TPM-treated rats. At 9 h of SE, all

groups presented the same pattern of EEG with periodic epileptiform discharges (Fig. 1).

**EEG Patterns during the Latent and Chronic Phases.** The EEG patterns during the latent period were similar in Lipilo-DZP and Lipilo-TPM10, TPM30, or TPM60 rats. At 24- and 48 h after SE, the EEG was still disturbed in all groups on background activity of decreased amplitude and frequency. Frequent spikes and spike-and-waves occurred on a baseline of low amplitude. Periodic epileptiform discharges were still present. In all rats, at 3 to 7 days after SE, the EEG returned to baseline rhythmicity, with occasional occurrence of low-amplitude, fast activities and atypical 4- to 5-Hz spikes-and-waves. During this period, in all groups, isolated spikes and abnormalities tended to cluster progressively until the occurrence of SRS. The recovery of the EEG was slower in animals treated with 10 mg/kg TPM than in DZP-treated animals with a tendency to the presence of rhythmic activities and slow spikes on the hippocampal and cortical EEG. No difference in recovery times was noted between Lipilo-TPM30, Lipilo-TPM60, or Lipilo-DZP groups. In some Lipilo-TPM30 or Lipilo-TPM60 rats, EEG abnormalities were less frequent than in Lipilo-DZP and Lipilo-TPM10 rats, whereas in other Lipilo-TPM30 or Lipilo-TPM60 rats, EEG recordings were similar to Lipilo-DZP and Lipilo-TPM10 rats.

During the chronic period, no further evolution of interictal EEG activity was noted, outside of the occurrence of electrographic seizures.

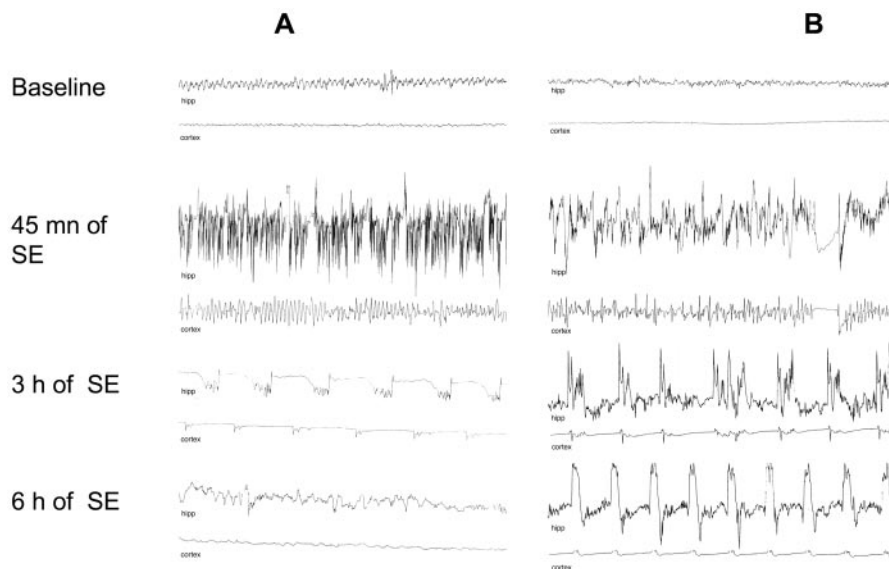
### Mortality Induced by SE

**Experiment 1: Mortality Induced by Lithium-Pilocarpine SE.** We ran a first experiment in which we intended to test the effects of increasing doses of TPM compared with a standard treatment with DZP on neuronal damage and epileptogenesis. In this experiment, during the first 48 h after SE, the degree of mortality varied with the treatment: 17% (5/24) of Lipilo-DZP rats, 38% (8/21) of Lipilo-TPM10 rats (estimate of odds ratio 2.34 compared with Lipilo-DZP), 84% (74/88) of Lipilo-TPM30 rats (estimate of odds ratio 20.09 compared with Lipilo-DZP) and 62% (28/45) of Lipilo-TPM60 rats died (estimate of odds ratio 6.26 compared with Lipilo-DZP). Most animals died during the night after the

onset of SE. The rate of mortality was abnormally high in Lipilo-TPM30 rats but not in Lipilo-TPM10 or TPM60 ones, which suggested that this high mortality was not only reflecting a lack of control of SE by TPM. The rate of mortality in Lipilo-TPM30 rats was also much higher than in rats subjected to pilocarpine-induced SE in which there was neither a high nor TPM dose-dependent mortality (DeLorenzo et al., 2002); thus, we wondered whether TPM may interact with lithium and compared animals subjected to Lipilo- or pilocarpine-SE and treated with 30 mg/kg TPM, which seemed to be a critical dose.

**Experiment 2: Comparison between the Mortality Induced by Lipilo- or Pilocarpine-Induced SE.** Therefore, we performed a first experiment in which we compared the effects of the three doses of TPM on the mortality linked to pilocarpine-induced SE with Lipilo-induced SE in a group treated with 30 mg/kg TPM. In this experiment, we induced SE with pilocarpine alone in 25 rats that received DZP ( $n = 3$ ), 10 mg/kg TPM ( $n = 3$ ), 30 mg/kg TPM ( $n = 14$ ), or 60 mg/kg TPM ( $n = 5$ ). These groups were compared with rats subjected to Lipilo SE and treated with 30 mg/kg TPM ( $n = 14$ ). In the pilocarpine groups, the mortality was null in groups treated with DZP and 10 or 60 mg/kg TPM and reached 14% (2/14 rats) in the 30 mg/kg TPM group. In the rats subjected to Lipilo SE and receiving 30 mg/kg TPM, the mortality was much higher 46% (6/13 rats), although not significantly different (estimate of odds ratio 0.17 compared with pilo-TPM30). Thus, there was a tendency to increased mortality when SE was induced by lithium plus pilocarpine. This may suggest a deleterious effect of lithium associated with TPM on survival.

**Experiment 3: Effect of Lithium-Pilocarpine- and Pilocarpine-Induced SE on Arterial Blood Gases and pH.** The possibly combined effect and/or interaction of lithium with TPM was hypothesized to be due to a decrease in blood pH of the animals. Therefore, we ran another experiment in which we measured arterial blood gases and pH during SE, induced either by pilocarpine (10 rats) or by lithium-pilocarpine (seven rats). All rats received an injection of 30 mg/kg TPM at 1 h after the onset of SE as in all other experiments described in this report. SE occurred in 9 of 10 pilocarpine



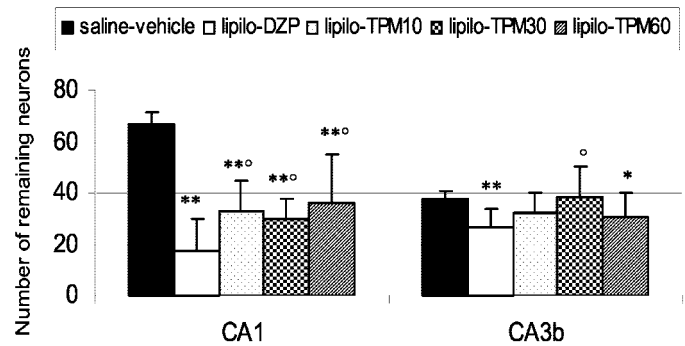
**Fig. 1.** EEG patterns of a Lipilo-DZP rat (A) and a Lipilo-TPM60 rat (B) recorded during SE. All DZP-treated rats exhibited an EEG pattern similar to that of the Lipilo-DZP rat presented here (A). At all doses, the TPM-treated rats had an EEG pattern close to that of the Lipilo-TPM60 rat shown here (B), where periodic epileptiform discharges never receded.

rats. Four pilocarpine rats died early after the onset of SE (between 90 and 150 min of SE) probably because of the severity of the convulsions, with no obvious changes in blood gases. The remaining pilocarpine animals survived without experiencing any significant changes in blood gases. Among the seven Lipilo animals, one Lipilo rat died during the night after the onset of SE, as usually seen in the other Lipilo-TPM30 rats dying in the course of our experiments. This rat showed a transient drastic decrease in pH (6.93 at 210 min) accompanied by a decrease in pCO<sub>2</sub> and an increase in pO<sub>2</sub>. Remaining Lipilo animals survived without experiencing any significant changes in blood gases. In this experiment, the mortality of Lipilo rats (14%) was lower than rates recorded in the other experiments reported here (46–84%). Lower mortality could be due to the anesthetics animals received for catheterization the day before SE, which are enzymatic inductors possibly modifying pilocarpine, lithium, and topiramate metabolism and pharmacokinetics. However, because the only rat that died during the course of SE was also the only one experiencing a decrease in blood pH, acidification of the animals may be one of the factors contributing to the high mortality occurring during the night after the onset of Lipilo SE.

### Effect of Topiramate on Neuronal Damage in Hippocampus and Cortex

In Lipilo-DZP rats compared with saline-vehicle rats, the number of surviving neurons was massively decreased in the CA1 region of the dorsal hippocampus (75% cell loss in the pyramidal cell layer), whereas the CA3 region was less extensively damaged (54% cell loss in CA3a and 30% in CA3b). In the dentate gyrus, Lipilo-DZP rats experienced extensive cell loss in the hilus (73%), whereas the granule cell layer did not show overt damage. In the piriform cortex, cell loss was total in layer III, which was no longer visible and reached 52% in layer II in Lipilo-DZP rats. In the entorhinal cortex, layers II and III-IV underwent slight, nonsignificant cell loss (8 and 15%, respectively) (Table 1).

In the hippocampus of Lipilo-TPM animals, cell loss was reduced compared with Lipilo-DZP rats in the CA1 pyramidal layer in which the cell loss reached 55 to 44% in the pilo-TPM10, 30 or 60 animals. This difference was statistically significant at all TPM doses (Table 1; Figs. 2 and 3). In the CA3 pyramidal layer, there was a tendency to a slight neuroprotection induced by TPM, mainly in CA3b, but the



**Fig. 2.** Effects of topiramate treatment on the number of neuronal cell bodies in CA1 and CA3b areas of the hippocampus of rats after Lipilo-induced SE. Saline-vehicle animals were treated with lithium and saline instead of pilocarpine. They did not experience SE and showed no damage. Lipilo-DZP and Lipilo-TPM animals were subjected to lithium-pilocarpine SE and received for 7 days two daily injections of DZP or 10, 30, or 60 mg/kg TPM, respectively. All animals were sacrificed at 14 days after SE or saline injection in controls. Values, expressed as the number of neuronal cell bodies in each area of interest, represent means  $\pm$  S.D. \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ , statistically significant differences from saline-vehicle. °,  $p < 0.05$ , statistically significant differences from Lipilo-DZP rats, Mann-Whitney  $U$  test.

difference with the Lipilo-DZP group was only significant at the dose of 30 mg/kg TPM (Table 1; Fig. 2). In the dentate gyrus, cell loss in the hilus was similar in Lipilo-TPM (67–70%) and Lipilo-DZP animals (73%). In the piriform cortex, the treatment with TPM did not afford any neuroprotection compared with DZP. In the entorhinal cortex, neuroprotection was not induced by TPM administration. Instead, there was a slight worsening of cell loss in Lipilo-TPM10 rats compared with Lipilo-DZP rats in both layers II (24% more damage) and III-IV (20% more damage). At the other doses of TPM, cell loss in the different layers of entorhinal cortex was similar to the one recorded in Lipilo-DZP rats (Table 1).

### Occurrence of Spontaneous Recurrent Seizures

All the rats studied until the chronic phase developed SRS with a similar latency. The latency was  $16.4 \pm 7.7$  days in Lipilo-DZP rats ( $n = 8$ ),  $16.9 \pm 10.2$  days in Lipilo-TPM10 rats ( $n = 7$ ),  $14.0 \pm 4.9$  days in Lipilo-TPM30 rats ( $n = 7$ ), and  $14.3 \pm 11.4$  days in Lipilo-TPM60 rats ( $n = 10$ ). The difference between the four groups was not statistically significant. None of the saline-vehicle rats ( $n = 5$ ) developed SRS.

TABLE 1

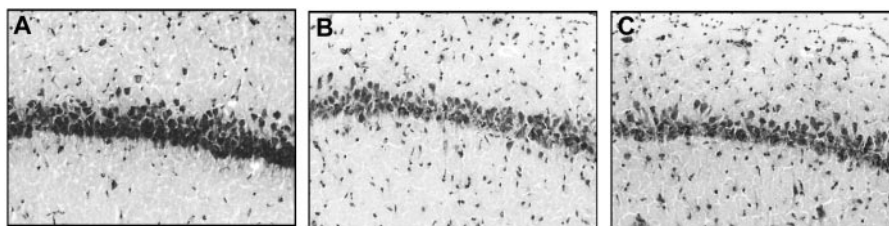
Effects of topiramate treatment on the number of neuronal cell bodies in the hippocampus and cerebral cortex of rats subjected to lipilo-SE

	Saline-Vehicle ( $n = 6$ )	Lipilo-DZP ( $n = 8$ )	Lipilo-TPM10 ( $n = 6$ )	Lipilo-TPM30 ( $n = 6$ )	Lipilo-TPM60 ( $n = 7$ )
<b>Hippocampus</b>					
Area CA1	66.9 $\pm$ 4.4	17.0 $\pm$ 13.1**	32.8 $\pm$ 11.7**†	33.1 $\pm$ 7.5**†	36.6 $\pm$ 18.7**†
Area CA3a	51.1 $\pm$ 4.9	23.4 $\pm$ 7.3**	27.4 $\pm$ 7.6**	32.3 $\pm$ 13.5*	30.1 $\pm$ 14.1*
Area CA3b	37.7 $\pm$ 2.7	26.3 $\pm$ 7.5**	31.9 $\pm$ 8.0	40.0 $\pm$ 11.6†	30.6 $\pm$ 9.2*
Dentate gyrus hilus	67.0 $\pm$ 7.3	17.9 $\pm$ 4.6**	19.8 $\pm$ 5.5**	22.2 $\pm$ 6.8**	20.6 $\pm$ 7.3**
<b>Cerebral cortex</b>					
Piriform, layer II	28.0 $\pm$ 2.0	13.3 $\pm$ 8.6**	7.3 $\pm$ 7.2**	13.0 $\pm$ 11.0*	11.3 $\pm$ 9.3**
Piriform, layer III	28.5 $\pm$ 2.4	0 $\pm$ 0**	0 $\pm$ 0**	0.7 $\pm$ 1.6**	0 $\pm$ 0**
Dorsal entorhinal, layer II	22.2 $\pm$ 2.4	20.3 $\pm$ 8.8	15.0 $\pm$ 11.0	21 $\pm$ 10.7	19.0 $\pm$ 10.2
Dorsal entorhinal, layers III/IV	23.0 $\pm$ 3.0	19.6 $\pm$ 11.1	15.0 $\pm$ 18.5	16.8 $\pm$ 9.7	13.7 $\pm$ 13.1
Ventral entorhinal, layer II	22.2 $\pm$ 3.4	22.0 $\pm$ 10.8	13.2 $\pm$ 11.5	16 $\pm$ 15.1	17.0 $\pm$ 11.3
Ventral entorhinal, layers III/IV	22.2 $\pm$ 4.9	12.4 $\pm$ 12.2	4.0 $\pm$ 6.7**	5.3 $\pm$ 9.2*	6.7 $\pm$ 10.4*

Values, expressed as the number of neuronal cell bodies in each area of interest, represent means  $\pm$  S.D. of the number of animals written in parentheses.

\*  $P < 0.05$ , \*\*  $P < 0.01$ , statistically significant differences from saline-vehicle rats.

†  $P < 0.05$ , statistically significant differences from lipilo-DZP rats.



**Fig. 3.** Cell density in hippocampal pyramidal layer CA1 in a saline-vehicle rat (A), a Lipilo-DZP rat (B), and a Lipilo-TPM60 rat (C). In the saline-vehicle rat, the density of the CA1 pyramidal cell layer stained with thionine seems very dense (A), whereas the thickness of the layer and the density of neuronal cell bodies seem largely reduced in the rat subjected to Lipilo SE and treated with DZP (B). In the rat subjected to Lipilo SE and treated with 60 mg/kg TPM (C), the thickness of the pyramidal cell layer and the density of cells are increased compared with DZP-treated rats but remain reduced compared with those of the saline-vehicle control animals (A).

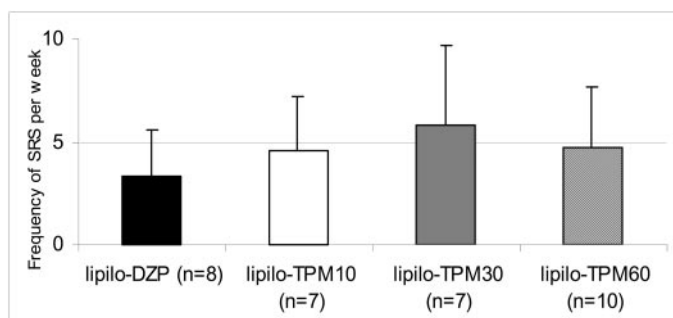
Although the difference between groups was not statistically significant because of the variability between the animals in each group, there was a tendency to an increase in the number of seizures per week in the animals exposed to TPM. This was mainly true in the Lipilo-TPM30 group in which the number of seizures per week was increased by 74% over the values recorded in Lipilo-DZP rats during the 4 weeks of observation (Fig. 4).

### Mossy Fiber Sprouting in Hippocampus

No animal of the saline-vehicle group showed any sprouting (score 0). All rats exhibiting SRS in Lipilo-DZP and Lipilo-TPM groups showed Timm staining in the outer molecular layer of the dentate gyrus (scores 2–5). Timm staining was present both on the upper and lower blades of the dentate gyrus (Fig. 5). The mean value of the Timm score in the upper blade reached  $2.9 \pm 0.7$  in Lipilo-DZP rats ( $n = 8$ ),  $3.3 \pm 0.4$  in Lipilo-TPM10 rats ( $n = 7$ ),  $3.3 \pm 0.4$  in Lipilo-TPM30 rats ( $n = 6$ ), and  $3.3 \pm 0.4$  in Lipilo-TPM60 rats ( $n = 8$ ). The values recorded in the Lipilo-TPM rats were not statistically significantly different from the values in the Lipilo-DZP group.

## Discussion

The present experiments show that TPM treatment during 1 week after Lipilo-induced SE 1) reduced neuronal death in some hippocampal areas but 2) did not prevent the appearance of SRS or decrease their frequency.



**Fig. 4.** Effects of topiramate treatment on the frequency of spontaneous seizures after Lipilo-induced SE. Saline-vehicle animals (not represented) received lithium and saline instead of pilocarpine; they did not develop SE. Lipilo-DZP and Lipilo-TPM animals were subjected to lithium-pilocarpine SE and received for 7 days two daily injections of DZP or 10, 30, or 60 mg/kg TPM, respectively, and were observed by videomonitoring for 4 weeks after the occurrence of the first spontaneous seizure. All animals showed spontaneous recurrent seizures. Although Lipilo-TPM animals exhibited a tendency to exhibit more frequent seizures than Lipilo-DZP rats, the difference was not statistically significant. Values represent means  $\pm$  S.D.

### Interaction between Lithium and Topiramate

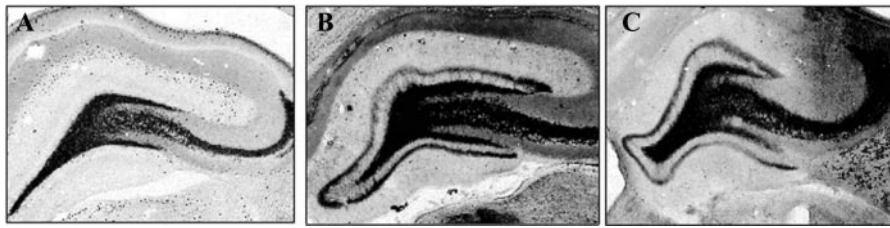
In the present study, mortality in rats treated with TPM30 during Lipilo-induced SE was abnormally high. Compared with Lipilo-DZP rats, mortality increased in all Lipilo-TPM rats, possibly because TPM did not control SE as well as DZP. Surprisingly, however, the mortality rate was peaking at the 30-mg/kg and not at the 10-mg/kg dose, which should have occurred in the latter case. Moreover, data on the effects of TPM on pilocarpine-induced SE did not report such high mortality but in the latter experiment, TPM was given concurrently with DZP (DeLorenzo et al., 2002), whereas we used monotherapy and compared DZP and TPM effects. Thus, we hypothesized a possible interaction of TPM with lithium. We recorded a greater mortality in Lipilo-TPM30 than in pilocarpine-TPM30 rats; however, the difference was not statistically significant. Our data suggest possible additive deleterious effects of TPM and lithium that would need to be explored more in depth. Lithium has a broad spectrum of biological effects and many interactions, especially with antiepileptic drugs, have occurred (Jope, 1999). In humans, the interaction between lithium and TPM was reported (Pininti et Zelinski, 2002). Finally, TPM is effective on the manic phase of bipolar disorders as lithium, which is the standard treatment of this disease, so both may act on a same biological pathway (Maidment, 2002).

A hypothesis favored in our study was acidosis of the animals. Indeed, as with TPM that inhibits carbonic anhydrase, lithium can lead to acidification. In rats, Levine et al. (2001) showed a moderate nephrotoxicity in fasted rats induced by lithium at a dose 3-fold lower than the one used in the present study. These data are consistent with the hypothesis that lithium could alter renal function and aggravate the acidification due to TPM. Indeed, measurement of blood gases showed blood acidification in a rat subjected to Lipilo-TPM30 SE that died afterwards. The other TPM-treated rats experienced only minor, transient acidification and survived.

### Neuroprotective Effects of Topiramate

The extension and location of neuronal loss in Lipilo-DZP rats observed here are in accordance with previous studies (Honchar et al., 1983; Dubé et al., 2001).

Compared with DZP, all doses of TPM induced a significant neuroprotection of CA1 pyramidal cells but the extent of protection was not dose-dependent. In CA3, neuroprotection was significant only at the 30-mg/kg dose. However, the neuroprotection afforded by TPM is incomplete, leaving over 30% cell loss, whereas, in the pilocarpine model, TPM totally suppressed the 10% cell loss recorded in that area (DeLorenzo et al., 2002). Our data are in agreement with other



**Fig. 5.** Effects of diazepam (B) or topiramate (60 mg/kg; C) treatment on mossy fiber sprouting in the dentate gyrus after Lipilo-induced SE. In the saline-vehicle rat, Timm staining does not show any mossy fiber sprouting (A). The intensity of sprouting in the rat subjected to Lipilo SE and treated with DZP (B) or with any dose of TPM is of the same intensity whether it is concurrent or not with marked hippocampal sclerosis, as seen in C. The degree of hippocampal sclerosis varies with the rats but is present in all groups, whether they were treated with DZP or any TPM dose.

studies on TPM neuroprotective properties. In global ischemia in rats and gerbils, TPM protects also CA1 (Edmonds et al., 2001). After SE induced by electrical stimulation, 20 to 80 mg/kg TPM decrease silver staining density in CA1, CA3, and the hilus (Niebauer and Gruenthal, 1999). In the present experiment, no protection in the hilus was observed. This discrepancy may be explained by the fact that silver staining was performed 3 days after SE, whereas most neuronal death in the hilus occurs within 24 h after Lipilo-induced SE (André et al., 2001; Roch et al., 2002a).

The mechanisms underlying the neuroprotective effect of TPM are not fully understood. Modulation of GABA<sub>A</sub> receptors (White et al., 2000), AMPA/kainate receptors (Gibbs et al., 2000), L-type high-voltage-activated calcium channels (Zhang et al., 2000), and voltage-dependent sodium channels (Zona et al., 1997) mediate antiepileptic effects and could contribute to neuroprotection. However, the degree of cell death is not directly related to the severity of SE. In this study, DZP seems to be more effective than TPM in resolving EEG activity but DZP does not protect CA1 as well as TPM. This suggests that neuroprotection provided by TPM may result from the reduction of excessive neuronal activity but also from action on related neurotoxic processes. The action of TPM on AMPA/kainate and L-type high-voltage-activated calcium channels is highly susceptible to interfere with the excitotoxic cascade because both types of channels are permeable to Ca<sup>2+</sup> (Weiss and Sensi, 2000). Moreover, the benefits of TPM may arise from the additive or synergistic effect of its multiple mechanisms of action (Niebauer et Gruenthal, 1999).

In the piriform and ventral entorhinal cortices, neuronal damage was extensive in all groups and similar or greater in TPM- than in DZP-treated rats. Thus, TPM seems more neuroprotective in hippocampus than in cortices. The selective pattern of neuroprotection recorded in this study has similarities with the pattern of distribution of AMPA/kainate receptors, suggesting that the neuroprotective effect of TPM may relate to its property to inhibit AMPA/kainate activation. Indeed, the density of AMPA/kainate receptors is higher in hippocampus than in cortex, with highest densities in CA1 and CA3 (Ozawa et al., 1998) where TPM exhibits neuroprotective effects. Besides, the timing of neuronal death induced by Lipilo SE may be critical. Previous studies from our group reported that neuronal death is rapid and fully developed by 24 h in the ventral cortices and hilus, whereas the process is delayed in CA1 and CA3 (Roch et al., 2002a). Thus, TPM may not be effective in the protection of cortices in which damage occurs very rapidly during SE. Conversely, in hippocampal CA1 and CA3 areas where neuronal loss is more progressive,

the 7-day TPM treatment is effective, possibly by interfering with delayed neuronal injury processes leading to death.

### Effect of Topiramate on Epilepsy: Relation with Neuronal Damage

**No Effect of Topiramate on Epilepsy.** All animals became epileptic whatever the treatment, TPM or DZP. The frequency of SRS after TPM treatment was not significantly different from that observed after DZP. Thus, the treatment with TPM does not seem to modify the epileptogenesis induced by Lipilo SE. Conversely, DeLorenzo et al. (2002) reported that the total prevention of 10% neuronal loss by TPM in CA1 was sufficient to prevent epileptogenesis. The difference between the two models may reflect the more moderate neuronal loss in parahippocampal cortices in the pilocarpine compared with the Lipilo model (Persinger et al., 1988; Turiski et al., 1989) but DeLorenzo et al. (2002) did not explore neuronal damage in areas outside the hippocampus.

After experimental SE in animals and in hippocampus of patients with TLE, mossy fiber sprouting was reported. Sprouting of mossy fibers, i.e., of granule cell axons, parallels formation of new synapses, mainly excitatory, on granule cells. These new circuits could mediate recurrent excitation of granule cells and underlie hippocampal hyperactivity and SRS (Buckmaster et al., 2002). In the present study, Timm score was identical in Lipilo-DZP and -TPM groups. The similar latency to SRS and epileptic behavior are concordant in all groups, confirming that mossy fiber sprouting may indeed represent a critical reorganization underlying the occurrence of SRS.

**Involvement of Hippocampus in Epileptogenesis.** The lack of significant differences in the epileptic outcome between groups may have two causes.

First, protection of CA1 pyramidal cell layer (and CA3, to some extent) by TPM is insufficient to prevent the occurrence of SRS. Indeed, the protection of CA1 by TPM is partial and not total. Thus, it remains possible that the extent of protection by TPM in CA1 (30% neuronal loss remaining) was not sufficient to prevent epileptogenesis. However, in the lithium-pilocarpine model, even the complete protection of CA1 after chronic caffeine treatment did not prevent epileptogenesis (Rigoulot et al., 2003). On the other hand, in the pilocarpine model, DeLorenzo et al. (2002) reported that the total prevention of 10% neuronal loss by TPM in CA1 was sufficient to prevent epileptogenesis.

Second, damage in CA1 and CA3 is not critical in the epileptogenic process. Hippocampal pyramidal cells are glutamatergic and excitatory. CA1 pyramidal cells receive inputs from CA3 and project on the adjacent subiculum and on

deep layers of the entorhinal cortex (Amaral and Witter, 1995). Although network reorganizations also take place in the CA1 region (Lehmann et al., 2000), the present study suggests that CA1 pyramidal layer seems to act more as a relay in the entorhinal-hippocampal loop than as an area promoting epilepsy. In fact, the hippocampal area most suspected to be involved in TLE is the dentate gyrus. In the lithium-pilocarpine model of epilepsy, metabolic studies performed in our group have highlighted a relative hypermetabolism of the surviving neurons in the hilus of the dentate gyrus of epileptic rats during both the latent and interictal chronic phases. These data support the role of this area in the genesis and maintenance of SRS (Dubé et al., 2000, 2001).

**Involvement of Ventral Cortices in Epileptogenesis.** All groups of animals became epileptic after damage in all layers of piriform cortex and layers III/IV of ventral entorhinal cortex. Entorhinal cortex is the gate-keeper region controlling the bidirectional information flow to and from hippocampus (Schwarcz and Witter, 2002) and may be a potentially important site for generation and propagation of seizures. Indeed, patients that are still experiencing seizures after surgery have an epileptogenic focus involving lateral and posterior temporal cortex. Cases of TLE where damage to the entorhinal cortex is obvious without clear hippocampal pathology have been reported by Gastaut (Scharfman et al., 1998). Finally, ventral cortices, both piriform and entorhinal, are injured early during Lipilo SE (Roch et al., 2002a), and this injury is predictive of further epilepsy (Roch et al., 2002b). However, a lesion limited to layer III of the entorhinal cortex induces long-lasting abnormalities of the hippocampal synaptic response but no SRS (Scharfman et al., 1998). This suggests that damage in other limbic areas such as the one recorded here in the hippocampus is necessary to trigger epileptogenesis, which highlights the role of the high excitability of the hippocampal-entorhinal loop in epileptogenesis (Stoop and Pralong, 2000). It may be that a minimal extent of damage in the basal cortices is necessary to trigger the epileptogenesis. Neuronal loss in the latter structures is more moderate in the pilocarpine than the Lipilo model (Persinger et al., 1988; Turski et al., 1989). This may explain why DeLorenzo et al. (2002) suppressed epileptogenesis with 30 mg/kg TPM in the pilocarpine model by only preventing the 10% neuronal loss in CA1. But, it must be noted that these authors did not explore neuronal damage in areas outside of the hippocampus. Moreover, they associated TPM with DZP, whereas TPM was given alone and compared with DZP in the present study.

## Conclusions

This study demonstrated that 1) the administration of TPM and lithium may have additive deleterious effects; 2) 1-week administration of 10 to 60 mg/kg TPM after Lipilo SE protects partly neurons of Ammon's horn, mainly CA1; and 3) SRS occur in TPM-treated rats, suggesting that protection of CA1 is not sufficient to prevent epileptogenesis.

## Acknowledgments

TPM was a gift from Johnson and Johnson Pharmaceutical Research and Development, LLC. (Raritan, NJ).

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