Experimental Investigations

A STUDY OF THE EFFECTS OF LAMOTRIGINE ON MICE USING TWO CONVULSIVE TESTS

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ABSTRACT
The aim was to study the effects of lamotrigine on bicuculline and pentylenetetrazol models of epilepsy.

Material and methods: Mice divided in 8 groups (n = 6) were pretreated intraperitoneally 30 min before pentylenetetrazol (50 mg/kg) or bicuculline (1 mg/kg) with saline 0.1ml/10 g body weight or lamotrigine 10 mg/kg, 15 mg/kg or 20 mg/kg, respectively. The seizure intensity and latency to the seizures 60 min after bicuculline or pentylenetetrazol injection were observed. The following scale for seizure intensity was used: 1 - excitation; 2 - body tremor; 3 - clonic seizures of forelimbs; 4 - heavy clonic seizures with rotations; 5 - tonic seizures of forelimbs; 6 - tonic seizures of limbs.

Results: The controls showed bicuculline-induced seizure intensity up to 5. Lamotrigine in the higher doses used decreased the seizure intensity (p < 0.05). Lamotrigine in all doses studied did not change the latency period of the first bicuculline seizure compared with the control. Controls treated with pentylenetetrazol showed seizure intensity up to 4. Lamotrigine in the highest dose decreased the pentylenetetrazol-induced seizure intensity (p < 0.05). Lamotrigine in all studied doses increased the latency to the first pentylenetetrazol-induced seizure compared with the controls (p < 0.05). Both convulsing drugs influence the brain GABA-ergic transmitter system by competitively blocking GABA_A receptors. Lamotrigine inhibits glutamate transmission and sodium channels. Both neurotransmissions – glutamate and GABA are closely related in seizure control. The conclusion is that lamotrigine has an anticonvulsive effect on both bicuculline and pentylenetetrazol seizure models, suppressing seizure intensity and influencing the latency to the first seizure.

Key words: lamotrigine, pentylenetetrazol, bicuculline

INTRODUCTION
Lamotrigine (LTG) is a third generation anticonvulsant drug frequently used in the therapy of epilepsy. Its main mechanism of action is blockade of sodium channels.1 LTG is an antiepileptic drug, a glutamate release inhibitor, with action at the neuronal voltage-gated sodium channels.2 Recent data showed that LTG inhibits postsynaptic AMPA receptors and glutamate release in the dentate gyrus.3

LTG is efficient against various types of experimental seizure models in adult rodents, including generalized tonic-clonic seizures induced by pentylenetetrazol (PTZ).4 PTZ is believed to interact with the picrotoxin site of the GABA_A-receptor.5 Bicuculline (BIC) acts competitively by blocking the action of the GABA_A-receptors.6

The aim of the present study was to evaluate the effects of LTG on two convulsive tests, PTZ and BIC, in mice.

MATERIAL AND METHODS
We used 48 white male mice weighing 25-30 g divided in 2 series, 4 groups in each series (n = 6). Two experimental series were used:
1. Experimental series I:

PTZ test was performed with the PTZ at a dose of 50 mg/kg subcutaneously. The groups were treated as follows: a) saline (0.1ml/10 g b.w.) i. p. + 30 min later PTZ s.c.; b) LTG at a dose of 10 mg/kg i.p. and 30 min later PTZ s.c.; c) LTG at a dose of 15 mg/kg i.p. and, 30 min later, PTZ s.c.; d) LTG at a dose of 20 mg/kg + PTZ s.c.

2. Experimental series II:

BIC test was performed using BIC at a dose of 1 mg/kg s.c. The mice were treated as follows: a) saline 0.1ml/10g b.w. intraperitoneally + 30 min later BIC s.c.; b) LTG at a dose of 10 mg/kg i.p. and 30 min later BIC s.c.; c) LTG at a dose of 15 mg/kg i.p. and 30 min later BIC s.c.; d) LTG at a dose of 20mg/kg + BIC s.c.

In both tests we evaluated the seizure intensity and the latency period to the first seizure (in minutes) for 1 hour after seizure agents injection. The seizure intensity was observed using the following scale:

0 – normal behavior
1 – light excitation
2 – excessive grooming
3 – clonic seizures of forelimbs
4 – heavy clonic seizures of the limbs with turning of the body
5 – clonic seizures mixed with tonic seizures of the forelimbs
6 – full tonic seizures of the limbs with apnea.

Chemicals: pentylenetetrazol (Sigma); lamotrigine (GlaxoWellcome); bicuculline (Fluka AG).

Statistics

All values were given as mean ± SEM. The comparison between groups was made by Student’s t-test and analysis of variance one-way (ANOVA) in the Excel and Instat GRAPHPAD computer programs. A value of p < 0.05 was considered as statistically significant. The proportions were compared using Chi-square test.

Results

Effects of Lamotrigine on PTZ Test

Control group of mice showed seizure intensity of up to 4 by the evaluation scale, i.e. predominantly well expressed clonic seizures. LTG administered at doses of 10 or 15 mg/kg did not have any significant effect on the seizure intensity. LTG at a dose of 20 mg/kg decreased the seizure intensity (p < 0.05) (Fig. 1).

Most of the animals in the control group had clonic seizures, only 16.7% had tonic seizures and died. There were no changes in the incidence of tonic seizures and rate of mortality between controls and LTG-treated mice (Table 1).

The mean latency to the first seizure in controls was 8 min. Mice treated with LTG in all of the doses used increased the latency to the first seizures (p < 0.05) (Fig. 2).

Figure 1. Anticonvulsiv effect of LTG on PTZ-induced seizures in mice.
Most of the mice injected with saline and BIC showed seizure intensity of up to 5 on the scale. Mice with LTG in the lower dose (10 mg/kg) showed no change in the seizure intensity, but in the higher doses (15 and 20 mg/kg) decreased the seizure intensity (p < 0.05) (Fig. 3).

The controls (BIC only) 66.66% had tonic seizures, but LTG-treated groups showed significant decrease of tonic seizures (at a dose of 10 mg/kg – 16.66%, at doses 15 and 20 mg/kg, respectively – 0%) (Table 2).

The mortality rate in the control group injected with BIC was 66.66%. Mice treated with 20 mg/kg of LTG significantly decreased the mortality rate to 0% (p < 0.05).

**Table 1. Effects of LTG on PTZ-induced seizures in mice – number and percentage of animals with seizures and mortality**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Clonic seizure n, (%)</th>
<th>Tonic seizure n, (%)</th>
<th>Mortality n, (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control – saline</td>
<td>6</td>
<td>4 (66.6)</td>
<td>1 (16.6)</td>
<td>1 (16.6)</td>
</tr>
<tr>
<td>Lamotrigine 10 mg/kg</td>
<td>6</td>
<td>4 (66.6)</td>
<td>1 (16.6)</td>
<td>1 (16.6)</td>
</tr>
<tr>
<td>Lamotrigine 15 mg/kg</td>
<td>6</td>
<td>4 (66.6)</td>
<td>2 (33.3)</td>
<td>2 (33.3)</td>
</tr>
<tr>
<td>Lamotrigine 20 mg/kg</td>
<td>6</td>
<td>4 (66.6)</td>
<td>0 (0)</td>
<td>2 (33.3)</td>
</tr>
</tbody>
</table>

* p < 0.05 compared with the controls.

**Figure 2. Anticonvulsive effect of LTG on PTZ-induced seizures in mice.**

**DISCUSSION**

There are a number of animal models of generalized type of seizures in rodents described in the literature, including PTZ and BIC-induced. All of them share behavioral and EEG similarity to human seizures and show pharmacologic specificity for GABA-ergic mechanism of antiepileptic drugs. These models are predictable, reproducible, and easy to standardize. They are useful in studying the mechanisms of pathogenesis of clonic seizures and in screening for anticonvulsant effect of potential...
antiepileptic drugs. In our experiments, we used both PTZ and BIC-induced seizures as models of generalized seizures on mice. It is well known that PTZ and BIC inhibit the specific binding of GABA to GABA$_A$-receptor Cl$^-$-ion channel complex on neuronal membrane and decrease the influx of Cl$^-$ ions in the membrane.

It is now generally accepted that PTZ acts at the picrotoxin site of the GABA$_A$ – receptors/Cl$^-$-ionophore complex. When activated, the Cl$^-$-channel of the receptor opens, leading to an influx of Cl$^-$ and neuronal hyperpolarization.

BIC is a competitive antagonist of GABA$_A$-receptors, which are ligand-gated ion channels concerned chiefly with the passing of chloride ions across the cell membrane, thus promoting an inhibitory influence on the target neuron. BIC acts competitively by blocking the action of the GABA$_A$-receptors. In addition to being a potent GABA$_A$-receptor antagonist, BIC can be used to block Ca$^{2+}$-activated potassium channels.

In our experiment control mice from both groups (BIC and PTZ-treated) showed well expressed clonic seizures, probably due to blockade of GABA$_A$-receptors in the brain.

The main mechanism of action of LTG is use-dependent blockade of sodium channels, but additional mechanism on calcium and potassium channels or secondary effects on neurotransmitter systems were also demonstrated. Sodium channel
A Study of the Effects of Lamotrigine on Mice Using Two Convulsive Tests

inhibitors as LTG act in a state-dependent manner; a characteristic that is suggested to arise from the ability of the compounds to stabilize an inactivated conformation of the channels. Once activated by depolarization, sodium channels rapidly transition into the inactivated state. Recovery from inactivation to the closed state requires repolarization of the plasma membrane and occurs relatively slow.13 During a burst of action potentials, sodium channels gradually accumulate in the inactivated state, naturally terminating the burst. Sodium channel blockers as LTG accelerate this process.14

Others have found that systemic application of kainic acid induced Na\(^+\)-K\(^+\)-ATPase activity inhibition in the rat hippocampus and cortex and LTG pretreatment showed a partially protective effect on the enzyme activity.

Chen et al. suggests that LTG treatments significantly inhibited seizure-induced proliferation of neural progenitors in the rat hippocampus.

Other researchers have found that LTG suppresses postsynaptic AMPA receptors and reduces glutamate release in granule cells of dentate gyrus, a gateway that regulate seizure activity in the rat hippocampus. The postsynaptic effect can be one of the underlying mechanism LTG’s anticonvulsive action.3

**CONCLUSIONS**

Our results allowed us to suggest that LTG suppressed convulsions induced by PTZ or BIC through decreasing seizure intensity, increasing the latency to PTZ seizures and decreasing tonic seizures and mortality in BIC model.

On the basis of our results and the fact that LTG mainly influenced glutamate-ergic neurons in the CNS by blocking sodium channels and the convulsants BIC and PTZ blocked GABA\(_A\)-receptors and controlled chloride channels in the brain, we suggest that anticonvulsive effect of LTG is due to disturbed interactions between excitatory (glutamate) and inhibitory (GABA) transmitter systems in the brain areas responsible for seizure activity and propagation to the motoneurons.

**REFERENCES**


Изучение эффектов ламотригина на мышь с применением двух судорожных моделей

Д. Гетова, А. Михайлова

Резюме

Цель: Исследование ставит себе целью изучить эффекты ламотригина на бикукулинную и пентилентетразоловую модели эпилепсии.

Материал и методы: В целях работы использованы белые мыши, разделенные на 8 групп (по 6 в каждой группе). За 30 мин. до введения пентилентетразола (50 мг/кг) или бикукулина (1 мг/кг) грызуны инъецированы физиологическим раствором 0.1мл/10гр.м.т. или ламотригином 10мг/кг, 15 мг/кг и 20 мг/кг. Интенсивность судорог и латентный период до появления первой судороги наблюдали в течение 60 мин. после инъекции пентилентетразола или бикукулина. Использована следующая шкала оценки интенсивности судорог:

Результаты: Контрольные группы, получившие бикукулин, показали интенсивность судорог - 5. Более высокие дозы ламотригина снижали интенсивность судорог (p < 0.05). Ламотригин во всех исследуемых дозах не показал изменения в латентном периоде до появления первой бикукулиновой судороги по сравнению с контрольной группой (p < 0.05). Контрольные животные, получившие пентилентетразол, показали интенсивность судорог - 4. Более высокие дозы ламотригина снижали интенсивность судорог (p < 0.05), как при бикукулинотесте. Ламотригин во всех примененных дозах удлинил латентный период до появления первой судороги по сравнению с контрольной группой (p < 0.05). В оба конвульсанта оказывают влияние на ГАМК-эргическую медиаторную систему мозга, конкурентно блокируя ГАМКА рецепторы.

Ламотригин ингибирует глутаматную медицию и натриевые каналы. Обе медиаторные системы – глутаматная и ГАМК – тесно связаны с контролем судорог.

Заключение: Ламотригин показывает противоэпилептический эффект на обе судорожные модели, подавляя интенсивность судорог и удлиняя латентный период до появления первой судороги.