

# The influence of cyclosporine A, cyclothiazide and luzindole on the electroconvulsive threshold in mice

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**Abstract:** The objective of this study was to determine the effect of cyclosporine A (CSA) (a widely used immunosuppressant drug), cyclothiazide (CTZ) (an inhibitor of desensitization of glutamatergic AMPA receptors) and luzindole (LZ) (an antagonist of melatonin MT2 receptors) on the threshold in the electroconvulsive test in mice. Cyclosporine A and luzindole (up to 50 mg/kg) did not affect this parameter. Cyclothiazide (5-20 mg/kg) dose-dependently lowered the threshold. Our results suggest that cyclothiazide should be avoided in epileptic patients. In contrast to chemically-evoked convulsions, cyclosporine A does not seem to affect electrically-induced convulsions. Finally, the protective action of melatonin is probably not mediated via MT2 receptors.

**Key words:** cyclosporine A, cyclothiazide, luzindole, electroconvulsions.

## INTRODUCTION

Cyclosporine A, a cyclic polypeptide, is widely used as an immunosuppressant drug after transplants, as well as in the treatment of autoimmune disorders, such as rheumatoid arthritis, psoriasis, Crohn disease, ulcerative colitis, or severe dermatoses [1]. Cyclosporine A enhances the GABA-ergic and serotonergic neurotransmission, and diminishes nitric oxide synthesis in the central nervous system [2]. Glutamatergic  $\alpha$ -amino-3-hydroxy-5-methylisoxazole-4- propionate (AMPA) receptors are responsible for most fast excitatory potentials in the brain. Cyclothiazide is a positive allosteric modulator of AMPA receptors, attenuating their desensitization [3]. Luzindole is a selective competitive antagonist of the brain melatonin MT2 receptors. Melatonin may be involved in the regulation of glutamate release from hippocampal neurons. This effect was reported to be biphasic. Initially, the hormone decreased, but subsequently it enhanced the glutamate concentration in the synaptic cleft [4]. It is of interest that the process described was reversed by luzindole.

Cyclosporine A is frequently used in clinical conditions. On the other hand, cyclothiazide may soon be introduced into the treatment of memory deficits, while luzindole is considered as a potential neuroprotective factor. Because the molecular actions of all 3 drugs justify their influence on a variety of central functions, we were motivated to evaluate the effect of cyclosporine A, cyclothiazide and luzindole on the electroconvulsive threshold.

## MATERIALS AND METHODS

**Animals.** All experiments were performed on male Swiss mice, kept in colony cages with free access to food and tap water, under standardized housing conditions. The animals

were randomly assigned to experimental groups consisting of 8 mice. Each mouse participated in only one experiment. All tests were performed between 09:00-14:00 to minimize confounding effects of circadian rhythms. All experimental procedures were approved by the Local Ethics Committee of the Medical University in Lublin.

**Drugs.** Cyclosporine A, cyclothiazide, and luzindole (all 3 drugs obtained from Tocris Ltd, UK) were suspended in a 1% solution of Tween 80 (Sigma St. Louis, MO, USA). All drugs were administered *i.p.*, cyclosporine 60 min and cyclothiazide and luzindole 30 min before the electroconvulsive threshold.

**Electroconvulsions.** Electroconvulsions were evoked with the use of alternating current (50 Hz) produced by a Rodent Shocker Generator (Ataner, Lublin, Poland), and delivered by ear-clip electrodes. The stimulus duration was 0.2 s. The electroconvulsive threshold ( $CS_{50}$ ) was the strength of current (in mA) necessary to induce tonic hind limb extension in 50% of the animals tested. In order to evaluate each  $CS_{50}$  at least 4 groups counting 8 mice were used. Subsequently, an intensity-response curve was constructed, based on the percentage of convulsing mice.

**Statistics.** Statistical analysis of the data obtained in the present study was undertaken by computer probit analysis, according to Litchfield and Wilcoxon [5].

## RESULTS

**Effect of cyclosporine A on electroconvulsive threshold.** Cyclosporine A (up to 50 mg/kg) did not affect the electroconvulsive threshold (Table 1).

**Effect of cyclothiazide on electroconvulsive threshold.** Cyclothiazide (applied at 5-20 mg/kg) significantly and dose-dependently lowered the electroconvulsive threshold in mice (Table 1, Fig. 1).

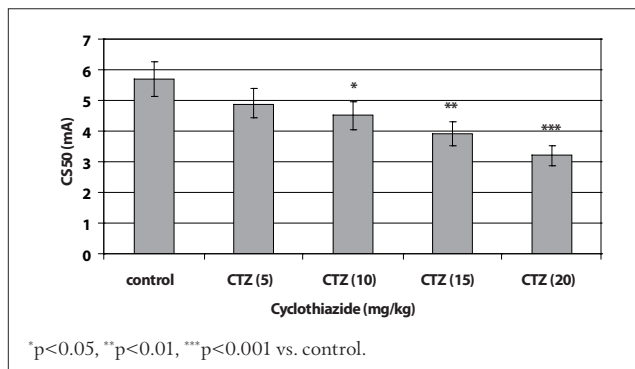
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**Table 1** Effect of cyclosporine A (CSA), cyclothiazide (CTZ) and luzindole (LZ) on the electroconvulsive threshold in mice.  $CS_{50}$  (in mA) is the strength of current which produced convulsions in 50% of animals. Cyclosporine A was administered 60 min, and cyclothiazide and luzindole 30 min before the electroconvulsive test

Treatment (mg/kg)	$CS_{50}$ (mA)
Control	5.5 (4.7 - 6.3)
CSA (10)	6.5 (5.5 - 7.6)
CSA (20)	5.8 (5.1 - 6.5)
CSA (30)	4.8 (4.3 - 5.4)
VIN (50)	5.0 (4.6 - 5.5)
CTZ (5)	4.9 (4.1 - 5.8)
CTZ (10)	4.5 (4.1 - 4.9)*
CTZ (15)	3.9 (3.2 - 4.8)**
CTZ (20)	3.2 (2.4 - 4.2)***
LZ (10)	5.1 (4.4 - 5.9)
LZ (30)	4.9 (4.1 - 5.8)
LZ (50)	5.0 (4.2 - 5.8)

\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  vs. control



**Figure 1** Influence of cyclothiazide (CTZ) on the electroconvulsive threshold in mice.  $CS_{50}$  (in mA) is strength of current producing tonic convulsions in 50% of animals.

#### Effect of luzindole on electroconvulsive threshold.

Luzindole (up to 50 mg/kg) did not affect the threshold in mice (Table 1).

## DISCUSSION

The results demonstrate that cyclothiazide, a positive modulator of AMPA receptors, lowered the electroconvulsive threshold in the tested animals, while both cyclosporine and luzindole remained without effect in this respect.

In previous studies, acute treatment with cyclosporine A was reported to enhance bicuculline [9] and pentylenetetrazole-induced convulsions [6]. This effect was more evident after chronic administration of the drug. Both proconvulsants are known to attenuate inhibitory GABA-ergic neurotransmission. Therefore the action of cyclosporine A also impairing GABA-ergic transmission was not surprising. In clinical studies, a plasma concentration of cyclosporine A higher than 1  $\mu\text{mol/ml}$  was associated with seizures [7]. The therapeutic concentration of the drug ranges from 0.33-0.88  $\mu\text{mol/ml}$  [9]. Our results suggest that acute cyclosporine A therapy is not associated with increased susceptibility to electrically-evoked seizures. Nevertheless, further investigations, including chronic

administration of cyclosporine A, are necessary to draw any reliable conclusions.

Cyclothiazide inhibits the desensitization of glutamatergic AMPA receptors. In the same way as conventional AMPA receptor agonists [8], the tested substance significantly and dose-dependently lowered the threshold for electroconvulsions. In light of evidence, it is interesting that cyclothiazide is employed in clinical trials with patients suffering from neurodegenerative diseases accompanied by memory deficits [3].

Melatonin was reported to trigger excitatory potentials in seizure-related brain areas [9]. In contrast, in other experimental studies, melatonin (50 mg/kg) significantly increased the electroconvulsive threshold in mice [10]. Since luzindole did not alter this parameter, one may speculate that MT2 receptors are not crucial for this effect.

## CONCLUSIONS

1. Cyclosporine A did not enhance the electrically-evoked convulsions in mice.
2. Cyclothiazide showed proconvulsant action lowered electroconvulsive threshold, which indicates that the drug should be avoided in epileptic patients.
3. Melatonin MT2 receptors are probably not involved in the anticonvulsant action of melatonin.

## ACKNOWLEDGMENTS

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