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Effects of a Combination of Transcranial DC Cerebellar Stimulation and Pioglitazone Administration on Pentylenetetrazole-Induced Seizures in Kindled Rats

L. S. Godlevsky,[1] O. B. Poshyvak,[2] M. P. Pervak,[1] K. A. Latypov,[1] K. O. Prybolovets,[1] and O. S. Yehorenko

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In rats with the kindling syndrome induced by i.p. injections of pentylenetetrazole (PTZ; 35 mg/kg daily, for three weeks), the averaged latency of seizures induced by a test injection of PTZ (35 mg/kg) was significantly greater (by 42.2%, $P < 0.05$ vs. control) after a combination of pioglitazone administration (100 mg/kg, i.p.) and transcranial DC stimulation (tDCS, 300 μA, 10 min, cathode on the skull surface) oriented toward the cerebellar cortex. Also, combined using of tDCS and pioglitazone prevented the occurrence of generalized tonico-clonic seizure attacks in 8 out of 10 rats of the respective group ($P < 0.05$), reduced the seizure severity by 31.3% on average ($P < 0.05$), and made shorter ictal discharges (by 45.0%; $P < 0.05$).

Keywords: transcranial DC stimulation (tDCS), cerebellum, pentylenetetrazole-induced seizures, kindling, peroxisomal proliferator-activated γ-receptors (PPARγ)

INTRODUCTION

Peroxisome proliferator-activated receptors-γ (PPARγ) play an important role in the antiseizure effects of cerebellar stimulation [1]. Blocking of PPARγ by a specific blocker, bisphenol A diglycidyl ether (2,2'-[(1-methylethylidene)bis(4,1-phenyleneoxymethylene)]bis-oxirane (BADGE) was found to abolish the antiseizure effects of cerebellar transcranial cathodic direct current stimulation (tDCS) in a model of pentylenenetrazole (PTZ)-induced epileptiform manifestations [1]. Agonists of PPARγ, pioglitazone in particular, caused suppression of the epileptiform activity in both acute and chronic models of epilepsy induced by PTZ [2, 3] and scopolamine [4] administration, and also suppressed febrile seizures [5]. Meanwhile, the combined effects of cerebellar tDCS and application of PPARγ agonists have not been investigated yet.

In our study, we aimed to determine the dynamics of the characteristics of seizures in rats with PTZ-induced kindling under conditions of cerebellar tDCS performed after administration of pioglitazone.

METHODS

Experiments were carried out on 35 male Wistar rats (body mass 180–250 g) kept under standard vivarium conditions. The kindling state was induced via i.p. injections of 35 mg/kg PTZ (Sigma Aldrich, USA) delivered every day for three weeks [1].

The readiness for generation of seizure activity in such animals was estimated by single test i.p. injections of PTZ in the above-mentioned dose (35 mg/kg). Kindled rats were subjected to implantation of nichrome electrodes (diameter 0.15 mm, insulated except for the tip) into the frontal cerebral cortex (FP = 1.5, L = 1.8) and ventral hippocampus of the left hemisphere (AP = = –4.3, L = 4.5, H = 8.0) according to the coordinates

[1] Odessa National Medical University, Ministry of Public Health of Ukraine,Odessa, Ukraine
[2] Danylo Halytsky Lviv National Medical University, Ministry of Public Health of Ukraine, Lviv, Ukraine
Correspondence should be addressed to L. S. Godlevsky: (e-mail: leonid.godlevskyj@onmedu.edu.ua).

of the atlas [7]. Implantation was performed under nembutal anesthesia (i.p., 35 mg/kg). The rats were taken into the experiment 7 to 10 days after surgery. The EEG activity was recorded monopolarly; a reference electrode was fixed in the nasal bone. A computerized electroencephalographic complex, "DX-4000-Practic" (DX-Systems, Ukraine), was used.

For tDCS, a round cathode (diameter 3.5 mm) was fixed with an adhesive tape on the skull midline caudally with respect to the lambda; this provided orientation of stimulation toward the cerebellum. The depilated skin below the electrode was preliminarily covered with a conductive gel [1]. A 40×45 mm anode was placed on the rat's abdominal surface. The stimulation current intensity (300 μA) was two times lower compared to that used earlier [1], considering the possibility of the addictive antiseizure effects of tDCS and pioglitazone. Stimulation was performed for 10 min; a modified generator, ETRANS, served as the source [1].

Pioglitazone (Lilly S. A., Spain) was dissolved in 0.3–0.5 ml of DMSO solution and administered i.p. in a dose of 100.0 mg/kg 1 h before testing the kindling efficacy with a single i.p. injection of PTZ (35 mg/kg). Rats of the control group were subjected to all manipulations related to tDCS and DMSO introduction (0.3–0.5 ml, i.p.), except for passing the stimulation current (sham stimulation). Test injections of PTZ were performed 10 min after termination of tDCS. Stimulated and sham-stimulated rats were observed within the subsequent 30-min interval.

Statistical processing of values of the seizure latencies was carried out using ANOVA and the Newman–Keuls criterion. Estimates of the seizure severity and values of the duration of ictal epileptiform potentials were compared using the Mann–Whitney *U* criterion. The number of rats with generalized tonico-clonic seizure fits was compared using the z-criterion for comparing two proportions. Numerical results are presented below as means ± ± s.d.

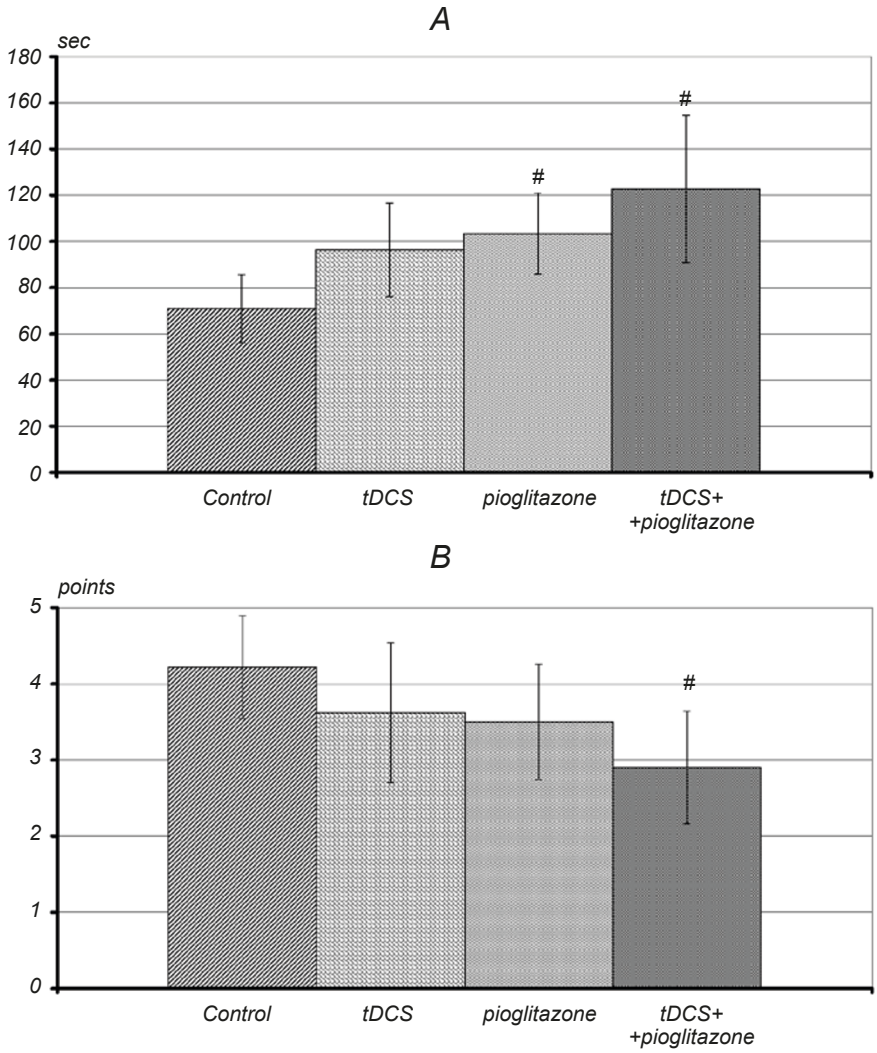
RESULTS

Administration of 35 mg/kg PTZ (i.p.) in control rats induced initial seizures with the mean latency of 70.9 ±14.2 sec (Fig. 1, A). A generalized tonico-clonic seizure pattern was observed in eight out of nine rats of this group, and repeated fits were observed in the three of them. The mean seizure severity in this group corresponded to 4.2± ±0.7 points (Fig.1, B).

Rats subjected to cerebellar tDCS demonstrated a greater, on average, latency of the first seizures by 26.3% when compared with the control value ($P > 0.05$, i.e., the difference was statistically insignificant; Fig. 1, A). Generalized seizures were prevented in three out of eight DCS-subjected rats, and the average severity of seizures corresponded to 3.6±0.9 points ($P > 0.05$; Fig.1, B). The latency of PTZ- induced seizures after isolated pioglitazone administration (100 mg/kg, i.p.) exceeded the control value by 31.4%, on average, and was equal to 103.4±17.1 sec ($P < 0.05$; Fig. 1, A). Generalized PTZ-provoked test-seizures were observed in five out of eight rats. The mean seizure severity in these animals corresponded to 3.5±0.8 points ($P > 0.05$ in comparison to the control; Fig. 1, B).

After combined application of pioglitazone and tDCS, the mean latency of the first seizures was 122.7±31.8 sec, which significantly exceeded the respective value in the control group by 42.2% ($P < 0.05$; Fig. 1, A). Generalized PTZ-provoked seizures were registered in this group only in two out of ten kindled rats, and the mean seizure severity was equal to 2.9±0.7 points, which was less than in the control by 31.3% ($P < 0.05$; Fig. 1, A).

The mean duration of ictal discharges in the brain cortex of control kindled rats was 27.9±11.7 sec (Fig. 2, A). All animals subjected to isolated cerebellar tDCS displayed PTZ-induced ictal discharges, and the average duration of the latter was 23.9±11.6 sec; the difference from the respective value in the control group did not reach the significance level ($P > 0.05$; Fig.2, B). Ictal discharges were registered in seven out of eight rats treated with pioglitazone, and the mean duration of such discharges was 21.7±8.7 sec; the difference from the control was also insignificant ($P > 0.05$; Fig. 2, C). The combined use of cerebellar tDCS and pioglitazone treatment prevented manifestations of ictal discharges in seven out of ten rats of the respective group. The mean duration of ictal discharges in the remaining three rats was equal to 15.9±9.7 sec, which was less than in the control group by 45.0%, on average ($P < 0.05$; Fig.2, D).

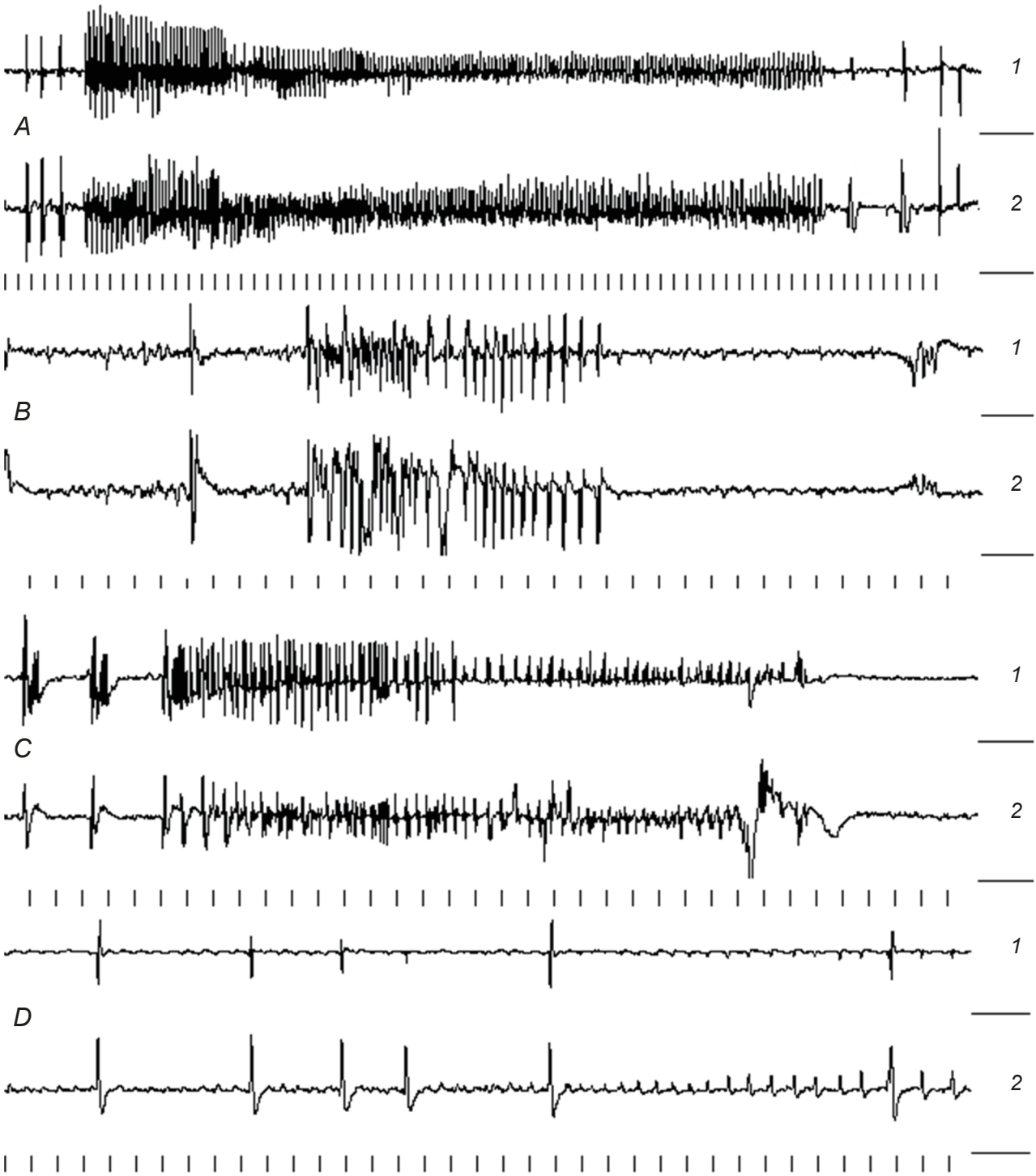


F i g. 1. Effects of cerebellum-oriented tDCS and pioglitazone administration on test seizure activity in rats with pentylenetetrazole (PTZ)-induced kindling.
A) Latencies of the first test seizure, sec; B) seizure severity, points; means ± s.d. are shown. # $P < 0.05$ in comparison with the control (sham-stimulated kindled rats).

DISCUSSION

Thus, the data obtained in our study confirm the conclusion on the existence of considerable antiseizure effects provided by cathodal tDCS oriented toward the cerebellum cortex of the rats with the pre-formed kindling. Such effects were expressed as greater values of the latent period of the first test seizures and a reduction of their severity. At the same time, it should be mentioned that the observed obvious differences of both above indices from those in the control group did not reach the level of statistical significance (mostly because of the high variability of the individual analyzed values). In general, these data are in concordance with those obtained earlier [1]. A nearly analogous situation exists with the effects of isolated applications of pioglitazone in another group of the experimental rats.

Combined applications of cerebellar tDCS and pioglitazone resulted in noticeably more intense reduction of seizure manifestations in the respective experimental group. In the latter, the latency of the first test PTZ-induced seizures and the mean duration of ictal discharges in the hippocampus were more than 40% smaller than the respective values in the control group ($P< 0.05$). tDCS+pioglitazone-induced suppression of the ictal discharges in the brain cortex of kindled rats was somewhat less intense but nonetheless also quite obvious.



F i g. 2. Effects of tDCS and pioglitazone on electrographic manifestations of PTZ-induced kindling. 1) ECoG samples recorded from the sensorimotor cortex; 2) hippocampograms recorded from the ventral hippocampus. A) 19.5 min after test PTZ administration (35 mg/kg, i.p.); B) 32.0 min after application of tDCS (cathode on the skull, 300 μA for 10 min); C) 75 min after pioglitazone administration; D) 72.5 min after pioglitazone administration and 25.5 min after tDCS. Intervals between consecutive parts of the records, sec, are indicated by vertical lines.

Nearly the same can be commented with respect to other characteristics of epileptiform activities, in particular, the relative number of animals with generalized tonico-clonic seizures and average indices of the intensity of seizure activity.

Significant intensification of the GABA-ergic inhibitory control of cerebellar cortical neurons is the probable mechanism responsible for mutual strengthening of the effects of tDCS and pioglitazone in kindled rats. Such effects are known as inhibitory cerebellar-neocortical influences [8, 9]. The above-mentioned increased GABA-ergic inhibitory neuronal control might be, to a significant extent, the consequence of activation of the PPARγ link of the GABA-ergic system [10, 11].

Thus, the presented data together with the abolishment of antiseizure effects by PPARγ blocking under conditions of tDCS of the paleocerebellar cortex [1] suggest that the latter receptors (PPARγ) play a rather significant role in the realization of cerebellar antiseizure effects.

All experiments were carried in accordance to the National Institute of Health Guidelines for the care and use of laboratory animals and the European Council Directive on 24 November 1986 for Care and Use of Laboratory Animals (86/609/EEC) and approved by Odessa National Medical University Bioethics Committee (UBC) (approval No. 3 dated 14/03/2016) before the study.

The authors, L. S. Godlevsky, O. B. Poshyvak, M. P. Pervak, K. A. Latypov, K. O. Ptybolovets, and O. S. Yehorenko, declare that the research was conducted in the absence of any conflicts with respect to commercial or financial relationships and those between the co-authors.

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