Effect of Chronic Consumption of Sodium Valproate and Melatonin on Seizure Activity in Krushinskii—Molodkina Rats

T. A. Savina*, O. A. Balashova*,, and T. G. Shchipakina*,****

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 142, No. 11, pp. 549-552, November, 2006
Original article submitted April 6, 2006

Experiments on Krushinskii—Molodkina rats with hereditary predisposition to audiogenic seizures showed that chronic consumption of aqueous solution of melatonin (50 mg/liter) had no effect on the pattern of seizures induced by 20-fold acoustic stimulation. Sodium valproate (50 mg/liter) insignificantly decreased the seizure response. Combined treatment with sodium valproate and melatonin produced a potent anticonvulsant effect, *i.e.* increased the latency and decreased the severity of audiogenic seizures. However, myoclonus in animals receiving combined treatment with these drugs developed much more rapidly compared to rats receiving melatonin or sodium valproate monotherapy.

Key Words: *melatonin; sodium valproate; seizures; hereditary epilepsy*

Valproic (diprylacetic) acid and its salts are used in clinical practice as the gold standard for antiepileptic therapy. High effectiveness of valproate in the therapy of various seizures is determined by its effect on several targets controlling the balance between excitation and inhibition in the central nervous system. Binding of valproate to potential-dependent sodium channels is followed by a decrease in the frequency of action potential generation [8] and GABA accumulation in the brain due to changes in activity of enzymes for its synthesis (glutamate decarboxylase) and degradation (GABA aminotransferase) [6]. The anticonvulsant effect of pineal gland melatonin results from initiation of GABAergic inhibition through G-protein-coupled membrane receptors for this hormone (Mel1) [13, 14]. Previous studies showed that modulation of brain excitability under the influence of melatonin is closely related to the phase of the circadian cycle. In the nighttime this hormone decreases seizure threshold and produces a proconvulsant effect [13]. Combined treatment with melatonin and anticonvulsants carbamazepine, phenytoin, and phenobarbital potentiated the effectiveness of these drugs [1,5].

Here we compared the anticonvulsant effects of chronic treatment with sodium valproate and melatonin in low doses in rats with hereditary audiogenic seizures.

MATERIALS AND METHODS

Experiments were performed on 3-month-old Krushinskii—Molodkina (KM) rats obtained from the Laboratory of Behavioral Physiology and Genetics (M. V. Lomonosov Moscow State University). The animals were maintained under standard conditions and 12:12-h light/dark cycle and had free access to water and food.

Some rats received aqueous solution of sodium valproate (50 mg/liter, Sigma-Aldrich) or melatonin (50 mg/liter, Sigma-Aldrich). Other animals received both anticonvulsants (50 mg/liter each). These

*Laboratory of Experimental Neurobiology, Institute of Experimental and Theoretical Biophysics, Russian Academy of Sciences; **Pushchino State University. ***Address for correspondence:*** neurochem@mail.ru. T. A. Savina

0007-4888/06/1425-0601

agents were dissolved in tap water (pH 5.0) and the solutions were replaced 2 times a week (sodium valproate) or daily (melatonin). Control rats received water. The drugs were given after the first acoustic stimulation.

The rats were exposed to daily acoustic stimulation (80 dB, 12-15 kHz, 6 days a week) for 20 days until the development of persistent myoclonic seizures (audiogenic kindling). The severity of seizures was scored as follows: 0, no response to sound for 90 sec; 1, running around the chamber; 2, start of clonic seizures, fall on the abdomen; 3, clonic seizures of forelimbs and hindlimbs on the side; 4, tonic forelimb seizures, hindlimb clonus; 5, tonic seizures of forelimbs and hindlimbs. Acoustic stimulation was stopped after the start of clonic seizures and fall on the floor. Non-responding animals were exposed to acoustic stimulation for 90 sec. The total evaluation of seizures was performed by the severity of seizures (score), latency of the 1st and 2nd stage of seizures (sec), and appearance of an additional stage in seizure (tic-like contractions of muscles, myoclonus). Parameters of seizures were recorded at 18.00-20.00.

The results were analyzed by ANOVA for multiple comparisons (Scheffe test) and Newman—Keuls test. Intergroup differences in the seizure response to audiogenic kindling were evaluated by regression analysis. Intergroup differences were significant at $p<0.05$. The data are presented as means and standard errors.

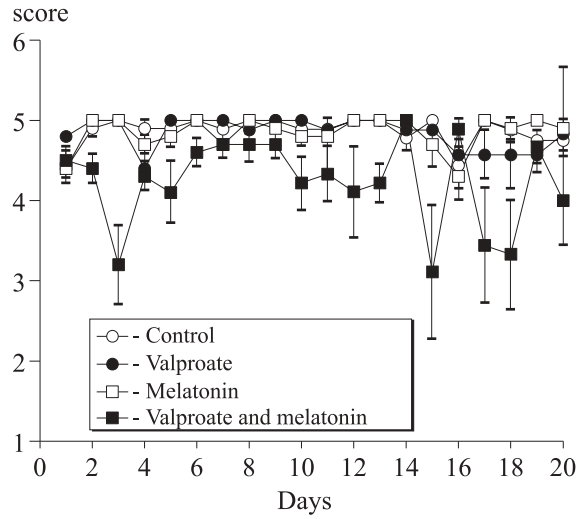

Fig. 1. Severity of seizures in KM rats after chronic consumption of melatonin and/or sodium valproate under conditions of audiogenic kindling.

RESULTS

The imbalance between GABAergic and glutamatergic transmission in the inferior collicular nuclei of the quadrigeminal plate and other structures of the forebrain in KM rats leads to neuronal hyperexcitability in response to acoustic stimulation [3,12]. Brainstem structures play a key role in the induction of audiogenic seizures. Repeated acoustic stimulation over 15-35 sec results in the spread of epileptiform activity in forebrain structures (neo-

TABLE 1. Persistent Myoclonus (%) in KM Rats after Chronic Consumption of an Aqueous Solution with Melatonin and/or Sodium Valproate

Parameter	Group			
	control (n=10)	melatonin (n=10)	valproate (n=10)	valproate+melatonin (n=10)
Days of treatment				
1-12	0	0	0	0
13	0	0	0	11
14	13	10	0	67
15	38	40	14	67
16	75	70	29	78
17	88	90	57	78
18	100	90	86	78
19	100	100	86	89
20	100	100	86	89
Mortality (number of animals)	2	0	3	1
Number of animals with myoclonus (survived animals in group)	8	10	6	8

Note. n, initial number of animals in group.

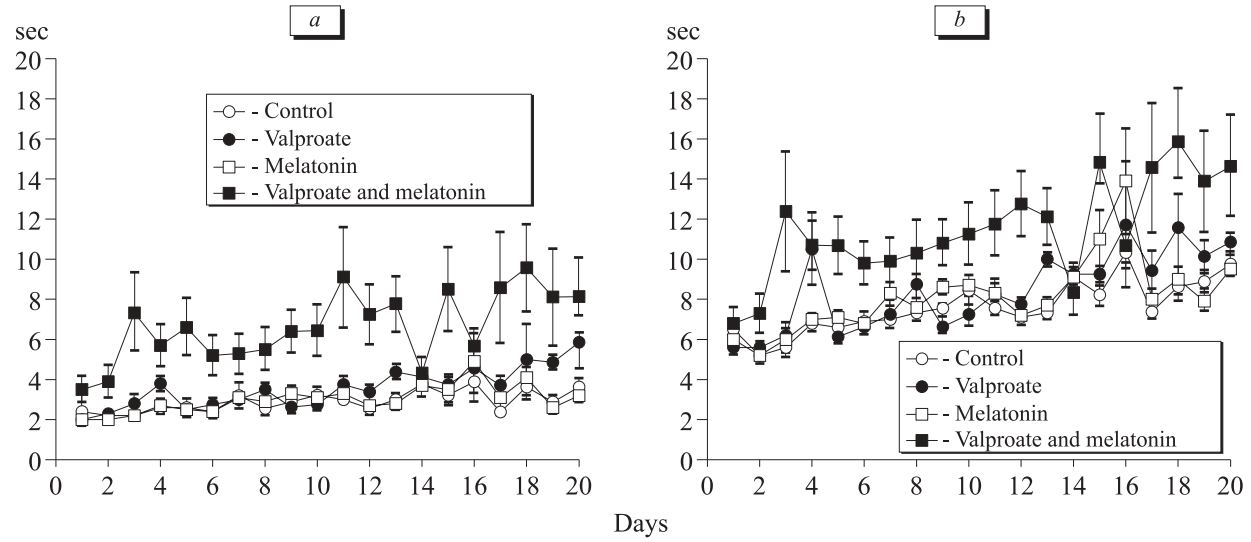

Fig. 2. Latency of seizures in KM rats after chronic consumption of melatonin and/or sodium valproate under conditions of audiogenic kindling. (*a*) Latency of stage 1; significant differences between groups of "valproate" and "control" (F=15.66, $p<0.01$), "valproate+melatonin" and "control" (F=73.29, $p<0.01$), and "valproate" and " valproate+melatonin" (F=45.30, $p<0.01$). (*b*) Latency of stage 2; significant differences between groups of "valproate" and "control" (F=4.36, $p<0.05$), "valproate+melatonin" and "control" (F=44.13, $p<0.01$), and "valproate" and " valproate+melatonin" (F=20.60, $p<0.01$).

cortex and hippocampus), which is accompanied by myoclonic seizures. They are similar to face and forelimb seizures during electrical kindling [2]. Comparative study of audiogenic seizures in KM rats showed that sodium valproate has no effect on the severity of this disorder during electrical kindling (Fig. 1), but we revealed a minor, but statistically significant increase in the latency of running (F=15.66, $p<0.01$) and clonic seizures (F=4.34, $p<0.05$, Fig. 2). Chronic consumption of melatonin did not modulate the severity and latency of seizures (Figs. 1 and 2).

The test drugs were administered in extremely low doses. The mean daily consumption of water in rats is 15 ml. Hence, the doses of the test drugs are incompatible with those used in systemic treatment to stop seizures. As distinct from melatonin, sodium valproate had a minor anticonvulsant effect. These differences are probably related to various half-life periods of sodium valproate and melatonin (2.5 h and 40 min, respectively) [7,14].

Combined treatment with sodium valproate and melatonin had a potent anticonvulsant effect, which manifested in longer seizure latency during electrical kindling. The severity of audiogenic seizures in these rats was lower compared to animals of the sodium valproate group (Figs. 1 and 2). However, myoclonus in these rats developed more rapidly compared to animals of other groups (Table 1). On day 14 of acoustic stimulation, persistent myoclonus was observed in 67% animals receiving both drugs. Myoclonus developed much less frequently in controls and animals receiving melatonin or sodium valproate.

Published data show that 24-h incubation of glioma C6 cells with 3-5 mM sodium valproate increased the synthesis of melatonin Mel1 receptor mRNA. These changes are accompanied by an increase in the concentration of receptor protein [10]. There are 2 subtypes of membrane Mel1 receptors for melatonin, Mel1a and Mel1b receptors. Both subtypes of receptors are coupled to G-proteins, but have various effects on function of the $GABA_A$ receptor [15]. There is no general agreement about melatonin signal transduction through these subtypes of Mel1 receptors. The ratio of Mel1 receptors in various brain structures remains unknown. Administration of luzindole (antagonist of Mel1a and Mel1b receptors) abolishes the anticonvulsant effect of melatonin [9,13]. Therefore, these subtypes of receptors play a key role in the anticonvulsant effect of melatonin. It can be hypothesized that sodium valproate-induced expression of Mel1 receptors contributes to the anticonvulsant effect of melatonin in low dose. Experiments on mice with acute seizures induced by electroshock showed that the anticonvulsant effect is more pronounced after long-tern treatment with drugs. For example, single systemic administration of melatonin (25 mg/kg) in combination with sodium valproate (250 mg/kg) did not potentiate the anticonvulsant effect of these drugs [1].

Myoclonic seizures during audiogenic kindling are accompanied by an increase in hippocampal

excitability and appearance of epileptiform activity [11]. Myoclonus develops more rapidly in animals receiving sodium valproate and melatonin, which is associated with the ability of valproates to induce synthesis of melatonin Mel1 receptors. Administration of 4-phenyl-2-propionmidotetralin (antagonist of melatonin Mel1b receptors) in the hippocampus has a strong anticonvulsant effect on rats only in the night phase of the circadian cycle [13]. In our study seizure parameters were recorded in the nighttime, when melatonin exhibits the highest anticonvulsant activity [4]. Probably, binding of melatonin to Mel1b receptors during the night phase of the circadian cycle contributes to the increased spread of epileptiform activity from the stem to forebrain structures. These changes are accompanied by rapid development of myoclonus in animals receiving the mixture of anticonvulsants.

We conclude that a great deal of attention should be paid during replacement therapy and combination therapy of epileptiform seizures with sodium valproate and melatonin.

This work was partially supported by the Russian Foundation for Basic Research (grant No. 03-04-48787-a).

REFERENCES

1. K. Borowicz, R. Kaminski, M. Gasior, *et al*., *Eur. Neuropsychopharmacol*., **9**, No. 3, 185-190 (1999).
2. J. B. Eells, R. W. Clough, R. A. Browning, *et al*., *Neuroscience*, **123**, No. 1, 279-292 (2004).
3. C. L. Faingold, *Hear Res*., **168**, No. 12, 223-237 (2002).
4. D. A. Golombek, D. D. Fernandez, M. G. Brito Sanchez, *et al*., *Eur. J. Pharmacol*., **210**, 253-258 (1992).
5. Y. K. Gupta, M. Gupta, G. Chaudhary, *et al*., *Methods Find. Exp. Clin. Pharmacol.*, **26**, No. 2, 99-102 (2004).
6. C. U. Johannessen, *Neurochem. Int*., **37**, 103-110 (2000).
7. W. Loscher, *Eur. J. Pharmacol*., **342**, 1-13 (1998).
8. M. J. McLean and R. L. Macdonald, *J. Pharmacol. Exp. Ther*., **237**, 1001-1011 (1986).
9. M. Ray, P. K. Mediratta, K. Reeta, *et al*., *Methods Find. Exp. Clin. Pharmacol.*, **26**, 177-181 (2004).
10. L. M. Rincon Castro, M. Gallant, and L. P. Niles, *J. Neurochem*., **95**, No. 5, 1227-1236 (2005).
11. R. N. Romcy-Pereira and N. Garcia-Cairasco, *Neuroscience*, **119**, 533-546 (2003).
12. K. C. Ross and J. R. Coleman, *Neurosci. Biobehav. Rev.*, **24**, No. 6, 639-653 (2000).
13. L. S. Stewart and L. S. Leung, *Epilepsia*, **46**, 473-480 (2005).
14. J. Vanecek, *Physiol. Rev*., **78**, No. 3, 687-721 (1998).
15. Q. Wan, H. Y. Man, F. Liu, *et al*., *Nat. Neurosci*., **2**, 401-403 (1999).