Effect of Oleamide on Pentylenetetrazole-Induced Seizures in Rats

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Oleamide exhibits antiepileptic activity and significantly decreases the degree of pentylenetetrazole-induced seizures.

Key Words: *pentylenetetrazole; oleamide; seizures; signal systems*

Oleamide was isolated from the cerebrospinal fluid of sleep-deprived cats and received the name of sleep-inducing lipid [1]. Oleamide has unique pharmacological properties and modulates activity of three important signal systems: neuroreceptors (positive modulation of GABA-A receptors and inhibitory glycine receptors [6]), voltage-dependent Na^+ channels (blockade of function [14]), and gap junction-mediated communication between glial cells (selective inhibition of communication [5]). These systems mediate the effect of antiepileptic compounds. It was hypothesized that oleamide is an endogenous anticonvulsant [2].

Aquilegia vulgaris plants are used in folk medicine as an anticonvulsant drug [10]. Our previous studies showed that aqueous extract of *Aquilegia vulgaris* inhibits binding of ^{3}H-muscimol (GABA-A receptor agonist) to rat brain membranes, but stimulates binding of ^{3}H-flunitrazepam (specific ligand of the GABA-A receptor benzodiazepine site) [11]. The low-molecular-weight fraction of this extract modulates function of neuroreceptors and has the antiepileptic effect in Krushinskii-Molodkina rats with audiogenic seizures [11].

Gas chromatography/mass spectrometry revealed 2 active compounds of this fraction (oleamide and myo-inositol) [12]. Myo-inositol *in vitro* inhibits ^{3}H-muscimol binding to GABA-A receptors of rat brain. Oleamide stimulates ^{3}H-flunitrazepam binding in the same system [12]. Moreover, myo-inositol reduces the degree of seizures in rats after treatment with pentylenetetrazole (PTZ) and kainic acid [13]. There are no experimental data on antiepileptic activity of oleamide.

Here we studied the effect of oleamide on PTZ-induced seizures.

MATERIALS AND METHODS

Antiepileptic properties of the drug were studied with PTZ [3]. Oleamide (Sigma) was dissolved in dimethylsulfoxide (DMSO) [7]. The rats were divided into 3 groups. Oleamide in a dose of 10 mg/kg was injected intraperitoneally to group 1 animals. The drug in this dose does not modify the behavior of animals [4]. Group 2 and 3 animals received an equivalent volume of DMSO and physiological saline, respectively. PTZ in a dose of 60 mg/kg was administered to all rats after 30 min. Each animal was placed in an individual chamber. The behavior of animals was studied for 1 h. The severity of convulsions was estimated by a 6-point scale of Racine with modifications [8]: no motor seizures (0 points); immobility, closing of the eyes, twitching of the ears and whiskers, snorting, and facial clonus (1 point); head nodding due to severe facial clonus (2 points); bilateral forelimb clonus without vertical

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rearing posture (3 points); bilateral forelimb clonus and vertical rearing posture (4 points); fall on the side (no vertical rearing posture), absence of righting reflex, and generalized clonic seizures (5 points); and vertical rearing posture, fall on the side, and generalized clonic seizures (6 points). Apart from the severity of convulsions, we evaluated the latency and duration of PTZ-induced seizures in each animal. We calculated the percent of animals convulsions and mortality rate of rats.

The effect of oleamide on the severity of seizures was evaluated by Kruskal—Wallis test. Significant effects were compared by Mann—Whitney test. The effects of oleamide on animals without convulsions, mortality rate, and latency and duration of seizures were subjected to one-way analysis of variance. The animals dying during this study were excluded from the analysis. Student's *t* test was used for comparative analysis.

RESULTS

Oleamide produced a potent effect on the severity of seizures (p=0.04) and significantly decreased the degree of seizures as compared to physiological saline (p=0.04) and DMSO (p=0.02, Fig. 1).

Oleamide had no effect on the ratio of animals without convulsions (p=0.128). However, the number of animals with 0-point convulsions was much higher in the oleamide group.

Oleamide had a significant effect on the mortality rate of rats (p=0.007). The average mortality rate in oleamide-receiving animals was much lower compared to rats of other groups (Fig. 2). The mortality rate did not differ in the physiological saline and DMSO groups (p=0.695).

No intergroup differences were found in the latency (p=0.596) and duration of PTZ-induced seizures (p=0.319).

Our results indicate that oleamide has the antiepileptic effect during PTZ-induced seizures. Oleamide produces a specific effect on the degree of convulsions. Administration of oleamide is not accompanied by a decrease in the duration and latency of convulsions. Injection of oleamide in DMSO and treatment with DMSO significantly decrease the mortality rate after PTZ administration. These data show that the mortality rate decreases after injection of not only oleamide, but also of DMSO. Previous studies showed that DMSO has neuroprotective activity [7,9]. However, individual treatment with DMSO did not induce the anticonvulsant effect. The severity of seizures in DMSO-receiving animals did not differ from that in control animals. The difference between the animals receiving oleamide and DMSO was more significant compared to that in the oleamide and physiological saline groups (Fig. 1). We conclude that oleamide has the antiepileptic effect during PTZ-induced seizures.

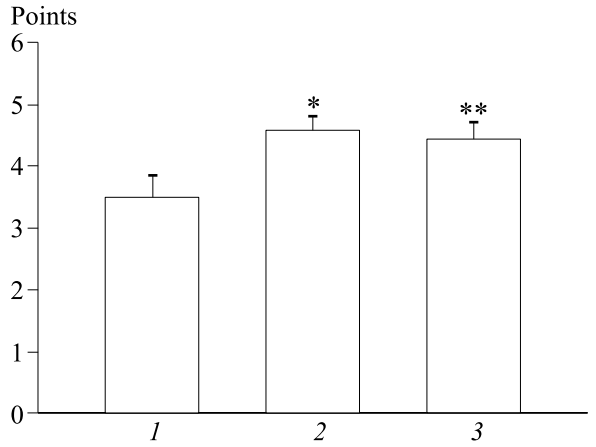

Fig. 1. Effect of oleamide on the severity of PTZ-induced seizures in rats. Each group consists of 10 animals (5 series of experiments). Here and in Fig. 2: oleamide-receiving animals (*1*); DMSO-receiving animals (*2*); and physiological saline-receiving animals (*3*). **p*=0.04 and ***p*=0.02 compared to oleamide.

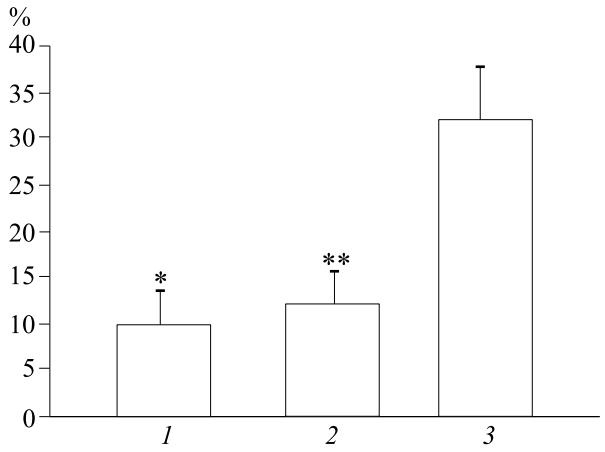

Fig. 2. Effect of oleamide on the mortality rate of animals with PTZ-induced seizures. Each group consists of 5 animals. **p*=0.06 and ***p*=0.028 compared to physiological saline.

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