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Anti-Seizure Effects of Fluoxetine on Rats with Susceptibility to Audiogenic Seizures

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ABSTRACT

Background: Seizures formed in response to a sound in rats allows studying the mechanisms of both occurrence of pathological seizure activity and anti-seizure effects of new medications. Contemporary antidepressants are targeted at enhancing the level of the main neurotransmitters in the brain. One of the important biochemical factors forming mixed anxiety-depressive disorders is serotonin (5-HT). Deficiency of 5-HT leads to impairment of synaptic transmission in neurons of the CNS and forms depressive states. Among the drugs that affect 5-HT synaptic transmission, the leading role is given to selective serotonin reuptake inhibitors, such as fluoxetine. The present paper explores the effects of fluoxetine on seizures in Wistar rats with enhanced sensitivity to audiogenic stress.

Methods: The Wistar rats (body mass of 250-300 g) were preliminarily tested for tolerance to audiogenic seizures. The difference in innate susceptibility to seizures allowed dividing the animals into 2 types: Seizure-Sensitive (SS) and Seizure-Tolerant (ST) rats. The SS rats were tested for whether they exhibited all the phases of seizures, as well as the latent period of the beginning of motor activity. 1 h prior to the experiment, the experimental animals were orally administered with fluoxetine at a dose of 25mg/kg, while the control rats were administered with the diluent - distilled water - in the equal volume.

Results: The control SS animals exposed to audiogenic stimulus exhibited wild running around in circles and jumping as some of the signs of seizure responses that evolved into tonic-clonic seizures, while, in the experimental rats, seizure manifestation decreased. The effects of fluoxetine were manifested in increase in the latent period of motor activity in comparison with the control group of animals. we utilized the scale in a seizure susceptibility study of a transgenic mouse model. We demonstrated that the maximum severity scores obtained with the Racine scale highly correlated .

Conclusion: Decrease in seizures manifestation in the SS Wistar rats under the effects of fluoxetine are supposed to be associated with its effects on innate peculiarities of serotoninergic activity of the brain structures.

INTRODUCTION

Epilepsy and other seizure disorders are some of the most common diseases of the Central Nervous System (CNS). Seizures caused by a loud sound in laboratory animals (audiogenic epilepsy) are considered as one of the appropriate experimental models of human epilepsy due to the "non-invasive" method of their inducing [1]. Stable (99% of all cases) and intensive tonic-clonic epileptiform seizures formed in response to a

sound in rats allows studying the mechanisms of both occurrence of pathological seizure activity and anti-seizure effects of new medications. On the other hand, it is known that the monoaminergic systems of the brain play a key role in the pathogenesis of the convulsive response induced by a sound [2]. Contemporary antidepressants are aimed at the monoaminergic transmission and, moreover, all of them are targeted at enhancing the level of the main neurotransmitters in the brain and thereby decreasing the symptoms of depression [3]. One of the important biochemical factors forming mixed anxiety-depressive disorders is serotonin (5-HT) [4]. In recent years, great importance has been attached to the role of the central serotonergic activity in the pathogenesis of depression. Deficiency of 5-HT leads to impairment of synaptic transmission in neurons of the CNS and forms depressive states. Consequently, many of anxiolytics and antidepressants applied in medical practice are targeted at enhancing serotonin transmission. In this regard, antidepressants attract attention as the drugs that have the ability to activate the central serotonergic processes.

Of particular interest in treatment of depressive disorders in the patients with epilepsy are the second-generation antidepressants – Selective Serotonin Reuptake Inhibitors (SSRIs). Among the drugs that affect 5-HT synaptic transmission, the leading role is given to selective serotonin reuptake inhibitors, such as fluoxetine [5]. The drug, binding to a specific protein-serotonin transporter, selectively prevents the reuptake of 5-HT into the presynaptic nerve endings, which leads to an increase in the concentration of this neurotransmitter in the synaptic cleft, as well as strengthens and prolongs its effect on the postsynaptic receptors [1] h after administration of fluoxetine, the level of extracellular 5-HT already increases in many of the brain structures [6]. A number of works [7] showed that this blockade by fluoxetine resulted in significant decrease in the brain content of the metabolite - 5-HIAA (5-hydroxyindoleacetic acid).

On the other hand, at present, there are several strains of laboratory animals that develop generalized seizures in response to sound stimulus with sufficient constancy: KM, WAG/Rij, DBA/2J [8]. However, other strains of rats used in neurophysiological and biochemical experiments also contain certain percentage of animals with audiogenic seizures [9]. In particular, only 20% of Wistar rats develop tonic-clonic seizures accompanied sometimes by vocalization in response to a loud sound. Considering the aforementioned, of particular interest is to study the effects of selective serotonin reuptake inhibitor – fluoxetine – on seizures in Wistar rats with high sensitivity to audiogenic stress. Audiogenic seizure activity of rats is used as a model for analyzing the physiological and biochemical mechanisms of epilepsy and for searching for the ways to prevent and treat that disease [10].

METHODS

The study was performed on male Wistar rats (body mass of 250-300 g). The animals were preliminarily tested for tolerance to acoustic startle stimulus. To that end, each animal was exposed to a sound of an electric alarm bell (90-110 dB) for 2 min in the soundproof box. The bell was switched off immediately when the seizures began. Such restriction of duration of the acoustic startle stimulus prevents animals from death, as well as development of large subdural hematoma [11]. Most animals exhibited just short orienting responses to the stimulus, or their behavior did not change significantly, but the other animals had all stages of seizures (1 - increased motor activity, wild running around in circles and jumping; 2– clonic seizures while lying on the belly; 3-tonic-clonic seizures followed by falling on the side; 4-generalized tonic seizures) [12]. In our experiments, the 2nd, 3rd, and 4th phases of seizures were generalized and defined as tonic-clonic seizures accompanied sometimes with vocalization. Only the rats exhibiting the same acoustic startle responses 3-4 times in a row were selected for the experiments. The difference in innate susceptibility to seizures allowed dividing the animals into 2 types: Seizure-Sensitive (SS) and Seizure-Tolerant (ST) rats.

From the total number (111) of the rats, 29 ST and 27 SS rats were selected. Both types of the animals were divided into the experimental and control animals. 1 h prior to the experiment, the experimental animals (ST (n=15), SS (n=14)) were orally administered with fluoxetine (Pharmascience, Montreal, Canada) at a dose of 25mg/kg, while the control rats (ST (n=14), SS (n=13) were administered with the diluent - distilled water - in the equal volume. During 2 days prior to the main experiments, the animals were handled for 5 min per day in order to equalize their responses to this stimulus.

Anti-Seizure Effects of Fluoxetine on Rats with Susceptibility to Audiogenic Seizures. Neurological Disorders & Epilepsy Journal. 2020; 3(1):131.

The rats were housed in standard cages (6-7 rats per cage) at the vivarium of the Institute of Physiology, Azerbaijan National Academy of Sciences (ANAS). They had free access to food and drinking water. All the experimental procedures were carried out in accordance with the international and national standards for the care and use of laboratory animals and approved by the appropriate committee of the Institute of Physiology, ANAS.

The results of the study were processed with application of a nonparametric Mann–Whitney U test and Student's t-test. Mathematical calculations were performed using an analytics software package – STATISTICA.

Seizures using the scale of Racine following were studied: Stage 0: no response, Step 1: hyperactivity, tremors, twitching, stage 2: nod, convulsions, muscle head and a myoclonic jerk, step 3: seizure muscular unilateral foreleg , Stage 4: Complications with bilateral musculoskeletal musculoskeletal disorders, Stage 5: Colonic tunic syndrome.

RESULTS

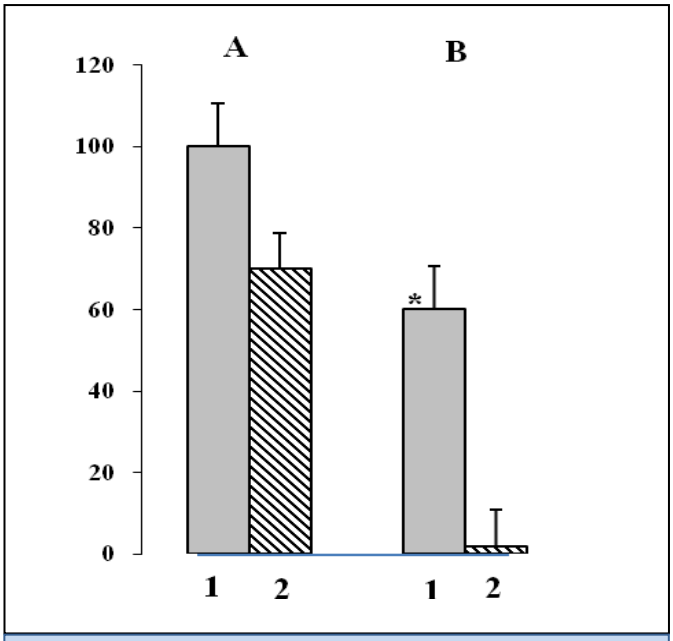

Figure 1: Manifestation (%) of the phases of seizures in the SS rats under the effects of fluoxetine. A – control animals, B – experimental animals; 1 – running around in circles + jumping, 2 – tonic-clonic seizures. The reliability of the differences between the experimental and control animals was evaluated using Student's t-test: *p <0.05.

The present paper explores the effects of fluoxetine on seizure occurrence in the rats with high sensitivity to audiogenic stress. All the rats were tested for whether they exhibited all the phases of seizures, as well as the latent period of the beginning of motor activity. It was shown that in 100% of the cases, the control SS animals exposed to audiogenic stimulus exhibited wild running around in circles and jumping as some of the signs of seizure responses that evolved into tonic-clonic seizures in 70% of the cases (Figure 1). 1 h after acute administration of fluoxetine, the pattern of seizures changed dramatically. Thus, seizure manifestation decreased in the experimental animals, and only in 60% of the cases ($p<0.05$) they exhibited running around in circles and no tonic-clonic seizures at all.

Moreover, the effects of fluoxetine were manifested in increase in the latent period of motor activity in comparison with the control group of animals (93.8±12.3 and 49.6±9.2 sec, respectfully) ($p<0.5$) (Table 1).

Table 1: The latent period of the beginning of motor activity (sec) in the SS rats under the effects of fluoxetine.

Control rats	Experimental rats
49.6	93.8*

1 – control animals (n=13); 2 - experimental animals (n=14). The reliability of the differences between the experimental and control animals was evaluated using Student's t-test: * $p<0.05$.

DISCUSSION

Acute administration of fluoxetine to the rats with susceptibility to seizure activity induces marked decrease in seizure manifestation. According to some experimental data, increase in the level of 5-HT induced by fluoxetine led to decrease in the frequency of seizures and reduction of the symptoms of depression.3 Moreover, the clinical trials of that SSRI showed that seizures occurred more seldom in the depressed patients treated with that medication, compared to those receiving placebo [13]. Perhaps, that is associated with the fact that seizures are one of the symptoms of psychological disorders treated with antidepressants. In the previous experiments, the biochemical analysis of the primary content of monoamines in the brain revealed high level of Noradrenaline (NA) and low level of 5-HT in the ST rats (non-prone to audiogenic seizures) compared to the SS rats characterized by high susceptibility to

Anti-Seizure Effects of Fluoxetine on Rats with Susceptibility to Audiogenic Seizures. Neurological Disorders & Epilepsy Journal. 2020; 3(1):131.

audiogenic seizures and higher level of 5-HT and Dopamine (DA). That indicates an important role of balance between 5-HT- and CA-systems in maintaining normal behavior of animals, as well as the correlation between some innate peculiarities of behavior and disbalance in activities of those systems [14]. Comparing the nature of the seizure with the level of biogenic amines in the brain of the SS rats indicated that they have a deficit of NA, accompanied by increased metabolic rate of 5-HT in contrast to the animals that are tolerant to a sound stimulus. There is evidence that the cause of convulsive epileptiform responses in animals may be a deficiency in the central noradrenalinergic transmission [15]. Hence, the initial content of biogenic amines in the brain structures determines the nature of the stress response [16], which is due to the genetic functional organization of the CNS [17]. Thus, it was shown that epileptic seizures can be induced by deficiency of 5-HT in the brain [18]. But our experiments demonstrate that the level of 5-HT in the brains of the SS rats is not high enough to prevent development of seizures, and artificial stimulation of serotonin by fluoxetine reduces seizure activity. In our view, high level of 5-HT in the brains of the SS rats is compensatory in nature and facilitates reducing of seizure activity in the SS rats, which do not apparently have genes determining tolerance to stress stimuli. The existence of such genes determining stress tolerance is indirectly indicated in the results of the studies of Ungar [19], who identified the low-molecular peptide in the brains of the rats – amelitin enhancing tolerance of the animals (reducing of seizure activity) to the effects of audiogenic stress signals. The ST rats characterized by initially low level of 5-HT in the brain do not have epilepsy, perhaps, due to the fact that non-audiogenic (non-prone to audiogenic seizures) rats compared to audiogenic ones have enhanced activity of the genetic apparatus of neurons containing high concentration of ribonucleic acid molecules [20]. Pronounced anti-seizure activity of fluoxetine, in view of a lot of investigators [21], indicates that activation of the serotonin receptor - 5-HT1A -induces membrane hyperpolarization response caused by enhanced Na+ conduction and exerting anti-seizure effect on freely moving rats. The anti-panic effect of fluoxetine is considered to be based on its capacity to selectively block 5-HT reuptake via presynaptic membrane, which leads to increase in the level of the transmitter in the synaptic cleft and to enhancing of serotonergic activity responsible for development of the anti-seizure effect.

CONCLUSION

Considering the aforementioned, we can suppose that decreased manifestation of seizures in the SS rats under acute administration of fluoxetine is probably associated with different natures of the effect of increased level of 5-HT on the serotonin receptors with different levels of expression in neurons of the SS and ST rats: increased level – in the ST rats and decreased one – in the SS ones. Such nature of expression of the serotonin receptors is due to the significant innate difference in the levels of 5-HT in neurons of those types of animals.

Thus, the anti-seizure effect of fluoxetine in Wistar rats is supposed to be associated with its action on the innate peculiarities of serotoninergic activity of the brain structures.

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CONFLICT OF INTERESTS

The author claims that there is no conflict of interests.

REFERENCES

1. Vorobyova OV. (2013). Drug treatment of depression in epilepsy. Effective pharmacotherapy. 32: 38-44.
2. Malikova LA, Narkevich VB, Klodt PM, Kudrin VS, Raevskii KS, et al. (2008). Effects of the novel anticonvulsant levetiracetam on the content of monoamines and their main metabolites in the brain structures of rats of the Krushinskii-Molodkina strain. Neurochemical Journal. 2: 289-92.
3. .Hovorka J, Herman E, Nemcova I. (2000). Treatment of interictal depression with citalopram in patients with epilepsy. Epilepsy Behav. 1: 444-447.
4. Vein AM, Dukova GM, Vorobyova OV, Danilov AB. (2004). Panic attacks. Guidelines for physicians. Eydos Media. 408p.

5. Wong DT, Perry KW, Bymaster FP. (2005). Case history: the discovery of fluoxetine hydrochloride (Prozac). Nat. Rev. Drug Discov. 4: 764-774.
6. Shishkina GT. (2007). Neuroadaptive changes in the brain under the effects of the serotonin reuptake blockers. Russian Journal of Physiology. 93: 1245-1251.
7. Harkin A, Shanahan E, Kelly JP, Connor TJ. (2003). Methylenendioxyamphetamine produces serotonin nerve terminal loss and diminished behavioural and neurochemical responses to the antidepressant fluoxetine. Eur. J. Neurosci. 18:1021-1027.
8. Ross KS, Coleman JR. (2000). Developmental and genetic audiogenic seizure models: behavior and biological substrates. Neurosci. Biobehav. Rev. 24: 639-653.
9. Jobe PC, Mishra PK, Ludvig N, Dailey JW. (1993). Genetic models of the epilepsies. Epilepsy: models, mechanisms, and concepts (ed. Schwartzkroin PA). Cambridge: University Press. 16: 94-140.
10. Kuznezova GD. (1998). Audiogenic seizures in the different genetic strains of rats. Pavlov Journal of Higher Nervous Activity. 48: 143-152.
11. Meerson FZ, Mamalyga LM. (1994). The protective action of adaptation to hypoxia in audiogenic epilepsy and its prolongation by using pharmacologic agents. Bulletin of Experimental Biology and Medicine. 117: 120-123.
12. Laird HE, Jobe PC. (1987). The genetically epilepsy-prone rat. Neurotransmitters and epilepsy. (ed. Jobe PC & Laird HE). Clifton NJ.: Humana Press. 57-94.
13. Lebedeva AV. (2007). Peculiarities of depression in patients with epilepsy. Medical Journal of the Russian Federation. 10: 892.
14. IsmayilovaKhYu, Aghayev TM, Semenova TP. (2007). Individual peculiarities of behavior (monoaminergic mechanisms). Nurlan Press. 228p.
15. Yan QS, Dailey JW, Steenbergen JL, JobePC. (1998). Anticonvulsant effect of enhancement of noradrenergic transmission in the superior colliculus in Genetically Epilepsy-Prone Rats (GEPRs): amicroinjection study. Brain Res. 780: 199-209.
16. Gorbunova AV. (2000). The autonomic nervous system and resistance of the cardio-vascular functions to emotional stress. Neurochemistry. 17: 163-184.
17. Dovedova EL, Monakov MY. (2000). Metabolism of neurotransmitters in cortical and subcortical brain structures in rats with different behavioral characteristics. Bulletin of Experimental Biology and Medicine. 130: 861-863.
18. Jobe PC. (2003). Common pathogenetic mechanisms between depression and epilepsy: an experimental perspective. Epilepsy Behav. 4: 14-24.
19. Ungar G. (1975). Is there a chemical memory trace? Isr. J. Chem. 1975;14:160-176.
20. Kulagin DA, Bolondinski VK. (1986). Neurochemical aspects of the emotional reactivity and motor activity of rats in new circumstances. Frontiers of Physiology Sciences. 17: 92-109.
21. Okuhara DY, Beck SG. (1994). 5-HT1A receptor linked to inward-rectifying potassium current in hippocampal CA3 pyramidal cells. J Neurophysiol. 71: 2161-2167.