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Effects of fluoxetine on the anticonvulsant action of valproate and ethosuximide in mouse model of myoclonic convulsions

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Abstract

Depression is becoming a growing problem in rural areas. This psychiatric disorder often accompanies epilepsy. The aim of this study was to assess the influence of fluoxetine (FXT), a commonly used antidepressant, on the protective action of two conventional antiepileptic drugs: ethosuximide (ETX) and valproate (VPA), against pentylenetetrazole (PTZ)-induced convulsions in mice. Motor coordination and long-term memory deficits induced by FXT, antiepileptic drugs alone and in combinations with FXT were assessed in the chimney test and passive-avoidance task, respectively. Brain concentrations of ETX and VPA were measured by immunofluorescence. Obtained results indicate that FXT at the dose of 15 mg/kg (*ip*, 30 min before the test) significantly increased the threshold for clonic convulsions. The antidepressant drug at lower doses remained ineffective in this respect. Moreover, FXT at the highest subprotective dose (10 mg/kg, *ip*) markedly enhanced the anticonvulsant effects of VPA, but not of ETX, against PTZ-induced seizures. The interaction between FXT and VPA seems to be pharmacodynamic because the antidepressant drug did not alter the brain concentration of VPA. With regard to adverse effects, FXT, VPA, ETX, and the combinations of FXT with antiepileptic drugs, did not impair motor coordination and long-term memory in mice. In conclusion, the combination of FXT with VPA may be advantageous in the treatment of myoclonic epilepsy, and therefore it should be recommended for further study in clinical conditions.

Key words

fluoxetine, valproate, ethosuximide, antiepileptic drugs, drug interactions, pentylenetrazole-induced seizures

INTRODUCTION

The incidence of depression in rural areas of Poland reaches up to 49.2%, whereas only 20.2% of patients (vs. 48.7% in cities) have contact with a general practitioner [1]. Depression is the most frequent co-morbid psychiatric disorder in epilepsy [2, 3]. The prevalence of mood disorders varies from 30-60%, depending on the diagnostic criteria used [4, 5]. Co-existing depression deteriorates the quality of life of people with epilepsy. Antidepressants may display not only anticonvulsant, but also proconvulsant action. Therefore, the treatment of both disturbances requires the selection of appropriate drugs. According to several reports, selective serotonin re-uptake inhibitors (SSRIs) exhibit the lowest risk of inducing seizures [6]. On the other hand, numerous preclinical and clinical studies suggest that serotoninergic and noradrenergic neurotransmission systems play a crucial role in the pathogenesis of both epilepsy and depression [7, 8]. Fluoxetine (FXT) was reported to exhibit unequivocal anticonvulsant [9, 10, 11] or proconvulsant [12, 13] action in several animal models of seizures. The influence of FXT on the action of antiepileptic drugs (AEDs) were examined only in the mouse maximal electroshock test. This prompted us to investigate the effect of FXT alone, and in combinations with two conventional AEDs in the pentylenetetrazole (PTZ) seizure model in mice.

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MATERIALS AND METHODS

Animals and experimental conditions. All experiments were performed on adult male Swiss mice weighing 22-26 g. The animals were kept in cages with free access to food and tap water, and in standardized housing conditions (experimental temperature 21 ± 1°C, natural light-dark cycle). The experimental groups, consisting of 8 (in the convulsive test) or 10 animals (in the chimney test and passive-avoidance task) were randomly assigned to experimental groups. After 3 days of adaptation to laboratory conditions, each mouse was used only once. All experiments were conducted between 08:00 – 14:00 to minimize confounding effects of circadian rhythms. The experimental procedures employed in this study were in agreement with rules approved by the Ethical Committee in Lublin.

Drugs. The following drugs were used in this study: fluoxetine (FXT; Anpharm S.A., Poland), ethosuximide (ETX; Sigma, St. Louis, MO, USA), valproate (VPA; magnesium salt – ICN-Polfa S.A., Rzeszów, Poland). FXT and ETX were suspended in a 1% solution of Tween 80 (Sigma, St. Louis, MO, USA), while VPA was dissolved in a sterile saline. Pentylenetetrazole (PTZ; Sigma, St. Louis, MO, USA), dissolved in a sterile saline, was administered *ip* in a volume of 5 ml/kg body weight. All drugs were administered *ip* as a

single injection, in a volume of 5 ml/kg body weight. Drugs were administered as follows: FXT and VPA – 30 min; ETX – 45 min before initiation of PTZ-induced seizures.

PTZ-induced seizures in mice. The 50% convulsive dose of PTZ was determined in control mice by *ip* administration of PTZ at doses ranging from 50-100 mg/kg. Following the injection, the mice were placed separately into transparent Plexiglas cages (25 × 15 × 10 cm) and observed for 30 min for the occurrence of clonic seizures. Clonic seizure activity was defined as clonus of the whole body lasting over 3 s. The influence of FXT (administered at doses 5-15 mg/kg) on the CD_{50} value of PTZ was determined as in the control animals. At least 4 groups of animals (8 mice per group) were used to estimate each CD_{50} value for the combination of FXT and PTZ. The anticonvulsant activity of AEDs administered alone and in combination with FXT was estimated as ED_{50} (the dose of an AED protecting 50% of mice against convulsions) values. To determine each ED_{50}, the mice were administered with PTZ at its CD_{97} (the dose inducing clonic seizures in 97% of animals; 102.2 mg/kg), and simultaneously, with a respective AED at various doses. Subsequently, ED_{50} values were calculated according to an intensity-response curve based on the percentage of mice with clonic convulsions.

Chimney test. The effects of FXT and AEDs on motor performance were determined in the chimney test [14], in which the animals had to climb backward up a plastic tube (3 cm inner diameter, 25 cm length). Motor impairment was indicated by the inability of mice to climb backward up the tube within 60 s.

Passive avoidance task. According to Venault et al. [15], the step-through passive-avoidance task may be recognized as a measure of long-term memory. Each mouse was administered an AED alone or in combinations with FXT on the first day before training. Subsequently, the animals were placed in an illuminated box (10 × 13 × 15 cm) connected to a larger dark box (25 × 20 × 15 cm) equipped with an electric grid floor. Entry of the animals into the dark box was punished by an electric foot shock (0.6 mA for 2 s; facilitation of acquisition). The animals that did not enter the dark compartment within 60 s were excluded from the experiment. Twenty four hours later, the pre-trained animals were placed again into the illuminated box and observed for up to 180 s. Mice that avoided the dark compartment for 180 s were considered to remember the task. The control (vehicle-treated animals) did not enter the dark box within the observation time limit. The time that the mice took to enter the dark box was noted and the median latencies (retention times) with 25th and 75th percentiles were calculated.

Estimation of the brain concentrations. The animals were administered with VPA or VPA with FXT. Mice were killed by decapitation at times scheduled for the convulsive test. Brains of mice were homogenized in original Abbott dilution (Abbott, Irving, TX, USA) buffer in Eppendorf tubes. Samples of brain homogenates were centrifuged at 10,000 rpm. (Abbott centrifuge; Irving, TX, USA) for 3 min. Brain supernatant samples of 70 μl were transferred to Abbott system cartridges. Brain concentrations of the drugs were estimated by immunofluorescence, by using an Abbott TDx analyzer (Abbott). Data were expressed in micrograms per gram of wet brain tissue as means ± SD of at least 8 determinations.

Statistics. Both the CD_{50} and ED_{50} values with their 95% confidence limits were calculated by computer log-probit analysis, according to Litchfield and Wilcoxon [16]. Subsequently, standard error of the mean values were calculated on the basis of confidence limits [17]. The statistical evaluation of respective ED_{50} vs. control values was performed with Student's *t* test. Statistical analysis of data from the chimney test was performed by using the Fisher's exact probability test, while the data from the passive avoidance task were analyzed the non-parametric Kruskal-Wallis ANOVA test followed by Dunn's post-hoc test. Differences among the values were considered statistically significant if $p<0.05$. Total brain concentrations of VPA administered alone or in combinations with FXT were statistically analyzed using the unpaired Student's *t*-test.

RESULTS

Influence of FXT on PTZ-induced convulsions in mice. FXT, applied at the dose of 15 mg/kg, significantly increased the CD_{50} value of PTZ ($p<0.05$). The antidepressant at lower doses remained ineffective in this respect (Tab. 1).

Table 1. Effects of fluoxetine on pentylenetetrazole-induced convulsions in mice

Treatment (mg/kg)	CD_{50} (mg/kg) ± SEM
Vehicle + PTZ	76.5 ± 3.0832
FXT (5) + PTZ	83.7 ± 3.2069
FXT (10) + PTZ	84.1 ± 4.0190
FXT (15) + PTZ	87.3 ± 4.4697*

Data are presented as median convulsive doses of PTZ. Values of CD_{50} (in mg/kg, with SEM) estimated using computer log-probit analysis, according to Litchfield and Wilcoxon [16]. PTZ and FXT were administered *ip,* 30 min before the seizure test.
PTZ, pentylenetetrazole; FXT, fluoxetine; *p < 0.05 vs. control group.

Effects of FXT on protective action of conventional AEDs against PTZ-induced clonic convulsions in mice. FXT, administered at its highest subprotective dose (10 mg/kg), significantly enhanced the anticonvulsive action of VPA ($p<0.01$), but not that of ETX (Tab. 2).

Table 2. Effects of fluoxetine, ethosuximide, valproate alone and in combination on the anticonvulsant activity against PTZ-induced seizures in mice

Treatment (mg/kg)	ED_{50} (mg/kg) ± SEM
FXT	13.6 ± 0.6361
ETX	137.7 ± 6.4409
ETX + FXT (10)	122.1 ± 5.7112
VPA	117.2 ± 5.4820
VPA + FXT (10)	78.2 ± 3.6578**

Results are expressed as median effective doses (ED_{50} in mg/kg SEM) of drugs that protected 50% of animals against PTZ-induced seizures. Statistical analysis was calculated according to the log-probit method elaborated by Litchfield and Wilcoxon [16]. All drugs were administrated *ip*, as follows: FXT and VPA – 30 min; EXT – 45 min prior to the test. PTZ, pentetrazole; FXT, fluoxetine; ETX – ethosuximide; VPA – valproate. ** p<0.01 vs. control group.

Results obtained from passive-avoidance task and chimney test. Conventional AEDs applied alone (at doses

equal to their ED_{50} values) or in combinations with FXT (10 mg/kg) did not produce significant motor long-term memory deficits (Tab. 3).

Table 3. Effect of fluoxetine and conventional antiepileptic drugs alone and in combination on motor performance and long-term memory in mice

Treatment (mg/kg)	Animals impaired (%)	Retention time (s)
FXT (10)	0	180 (180,180)
ETX (137.7) – ED_{50}	0	180 (180,180)
ETX (137,7) + FXT (10)	10	180 (180,180)
VPA (117.2) – ED_{50}	0	180 (180,180)
VPA (117.2) + FXT (10)	20	180 (180,180)

Results are expressed as percentage of animals that failed to perform the chimney test, and as median retention time (with 25th and 75th percentiles) during which the animals avoided the dark compartment in the step-through passive avoidance task. AEDs in monotherapy were administered at doses equal to their ED_{50} doses. Statistical analysis of data from the chimney test was performed by using the Fisher's exact probability test, whereas the results from the step-through passive avoidance task were analyzed using the non-parametric Kruskal-Wallis ANOVA test followed by Dunn's post-hoc test. PTZ, pentylenetetrazole; FXT, fluoxetine; ETX – ethosuximide; VPA – valproate; ED_{50} – median effective doses.

Influence of FXT on brain concentrations of VPA. FXT (10 mg/kg) did not affect brain concentrations of VPA (90.4 mg/kg) (Tab. 4).

Table 4. Influence of fluoxetine upon total brain concentrations of valproate in mice

Treatment (mg/kg)	Brain concentration (μg/ml) ± SD
VPA (90.4) + vehicle	2.720 ± 0.756
VPA (90.4) + FXT (10)	3.364 ± 0.784

Data are presented as means ± SD of at least 8 determinations. Statistical analysis of brain AED concentrations was performed using the unpaired Student's *t* test. FXT, fluoxetine; VPA, valproate.

DISCUSSION

The results obtained in the presented study demonstrate that fluoxetine (15 mg/kg) exhibited significant anti-convulsive action against PTZ-induced clonic convulsions in mice. Importantly, according to Zienowicz et al. [13], FXT (20 mg/kg) pretreatment did not change PTZ concentration in brain tissue. This suggests that the anticonvulsive effect of FXT was not due to pharmacokinetic events. Our results support the previous report that FXT protected mice against PTZ-induced convulsions. The maximum anticonvulsive effect was observed when FXT was administered at the dose of 20 mg/kg [18]. In other seizure models, acute and repeated FXT treatment (20 mg/kg) showed anti-convulsive properties, enhancing doses of picrotoxin producing convulsions in mice [19]. Moreover, acute FXT (15-25 mg/kg) significantly increased the electroconvulsive threshold in mice, and potentiated (at sub-protective doses) the anticonvulsive action of VPA, carbamazepine (CBZ), phenobarbital (PB), and phenytoin (PHT) against maximal electroshock-induced seizures [10]. In chronic treatment, FXT (up to 20 mg/kg) did not affect the threshold for electroconvulsions, but enhanced the action of VPA, CBZ, and PHT [9].

Quite different results were found in rats subjected to PTZ-induced convulsions – a single injection of FXT (10 mg/kg) did not affect the seizure threshold, while chronic (7-day, 10 mg/kg) administration of the antidepressant even diminished this parameter [12]. Also, according to Ceyhan et al. [20], FXT (2.5-20 mg/kg) did not produce any significant difference in latency and intensity of the PTZ-induced seizures in rats. Nevertheless, other researchers found that FXT given at the same dose of 10 mg/kg enhanced the pro-convulsive action of PTZ in rats [13, 21]. In our opinion, all discrepancies may result from the experimental protocol: in the two former studies the seizure score was the main criterion, while in the latter, seizure occurrence was taken into consideration. These findings confirm the observation that the effects of the drugs may vary, depending on the treatment schedule (i.e. acute or chronic), animal species and the model of seizures.

The anti-convulsant properties of FXT could be explained by an increase of synaptic serotonin due to the blocking (through $5\text{-}HT_1$ receptors) of the negative feedback system at somatodendritic level [22]. FXT was also reported to enhance $GABA_A$ receptor activity and to inhibit excitatory NMDA receptors [20]. Finally, the antidepressant inhibits Ca^{2+} channels [23].

CONCLUSIONS

The obtained results suggest that FXT may be a beneficial antidepressant drug in the treatment of epileptic patients with depression. The combination of FXT with VPA demonstrated greater efficacy than VPA alone in the animal model of myoclonic seizures, and therefore it should be recommended for further study in clinical conditions. The presented results may improve the treatment of depression co-existing with epilepsy, and reduce the occurrence of the two diseases in rural areas.

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