EFFECTS OF ETIFOXINE ON LEARNING AND MEMORY OF INTACT RATS

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ABSTRACT

Etifoxine (ETX) is a non-benzodiazepine anxiolytic. There is no data available showing the effect of ETX on the cognitive functions of laboratory animals. **PURPOSE.** The aim of this study is to investigate the effects of ETX on the cognitive functions of intact rats. **METHODS.** Male Wistar rats (3 groups of 10) were treated intraperitoneally with: saline, ETX in doses of 50 and 100 mg/kg b.w., respectively. The influence on cognition was examined using automatic reflex conditioner (shuttle box). The following parameters were assessed: number of conditioned responses (avoidances), number of unconditioned responses (escapes), latency time and number of intertrial crossings. **RESULTS.** The animals receiving ETX in a dose of 50 mg/kg were found to improve their learning abilities and preserve their long-term memory compared to the controls. The animals treated with the higher dose ETX were found to decrease the number of avoidances, escapes and intertrial crossings compared to the control group. **CONCLUSIONS.** ETX at the lower dose has a better effect on learning and memory compared to the control. ETX at the higher dose of 100 mg/kg has a worse effect compared to the control. But no significant differences between the experimental groups and the control group were found.

Key words: etifoxine, anxiolytic, cognitive function, shuttle-box test, animals

INTRODUCTION

Anxiety is a recognized symptom of many psychiatric disorders, including generalized anxiety disorder (GAD), social anxiety disorder (SAD), obsessive-compulsive disorder (OCD), and posttraumatic stress disorder (PTSD) (1). The clinicians have several treatment approaches, but “the optimum anxiolytic compound” has not been developed yet. One approach is the use of antidepressants. Selective serotonin reuptake inhibitors (SSRI) and serotonin-norepinephrine reuptake inhibitors (SNRI) are generally considered as the first-line treatment option (2). These compounds lack tolerance development and abuse potential but take several weeks to work and also cause unwanted side effects. Another approach is the use of a benzodiazepine. By contrast, benzodiazepines (BZDs) have a quick onset of action but they are sedative drugs causing motor coordination deficits, memory impairment and their continuous use rather quickly induces tolerance effects, dependence, abuse and withdrawal symptoms, including the potential for rebound anxiety.

Etifoxine (ETX, Stresam[®]) is a non-benzodiazepine anxiolytic. The mechanism of ETX for anxiolysis is potentiation of GABA_A receptor function by a dual mode of action. It binds to $\beta 2$ and $\beta 3$ subunits of the GABA_A receptor complex, an allosteric site different from that of the classical BZDs (3). Flumazenil, the benzodiazepine specific antagonist, did not interfere with its anxiolytic effect (4). In addition, EXF binds to the 18 kDa translocator protein (TSPO) that control synthesis of neurosteroids like allopregnanolone, an allosteric positive modulator of GABA_A receptors (5-6). The intraperitoneal administration of ETX increased plasma and brain concentrations of pregnenolone, progesterone, 5 α -dihydroprogesterone and allopregnanolone by two- to four-fold independently from the adrenal glands (5). Neurosteroid biosynthesis inhibitors like finasteride, indomethacin and trilostane significantly blocked the effect of ETX (7). These data suggest that an enhancement of neurosteroidogenesis contributes to the anxiolytic effects of ETX. ETX has been shown to possess anxiolytic-like properties in rodents (8-9) and in humans without sedative, myorelaxant and

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amnesic side effects at anxiolytic doses (10-12). The most common adverse effect is drowsiness at the beginning of therapy. It does not usually cause any withdrawal syndromes (13). There is no data available showing the effect of ETX on the cognitive functions of laboratory animals.

PURPOSE

The aim of the current study is to define the effects of ETX on learning and memory of intact rats.

MATERIALS AND METHODS

All experiments were carried out according to the guidelines for the use of laboratory animals in EU and Bulgaria. Official permission for the study was obtained by Bulgarian Food Safety Agency No 87/9.01.2014.

Drug

Etifoxine - 2-ethylamino-6-chloro-4-methyl-4-phenyl-4H-3,1benzoxazine hydrochloride (Stresam[®], Biocodex, Gentilly, France).

Animals

In this study, 30 male Wistar rats with 170-220 g body weight were used, divided in 3 groups (n=10). The rats were kept under standard laboratory conditions in an 08:00-20:00 h light/dark cycle and provided with food and water *ad libitum*. The animals were treated once daily intraperitoneally respectively with: 1st group (control)- saline (0.1ml/100g); 2nd- ETX 50mg/kg and 3rd group- ETX 100mg/kg.

Behavioral tests

An automatic reflex conditioner for active avoidance "shuttle box" (Ugo Basile, Italy) was used in studying learning in rats. Training lasted 5 consecutive days with 30 training sessions daily, each consisting of a 6-second light and sound stimulus (670 Hz and 70 dB), and electrostimulation (0.4 mA) in the last 3 seconds delivered through the cage grid floor. Each session was followed by 12 seconds of rest. Seven days after this training session (day 12) retesting was done for one day with the same parameters to track the storage of memory traces. The following parameters were assessed: number of conditioned responses (avoidances), number of unconditioned responses (escapes), latency time and number of intertrial crossings.

Statistical analysis

All data are expressed as mean $\pm$ standard error of the mean (SEM). Statistical comparisons were done by One way ANOVA and Paired-samples T test of SPSS.19, after verifying the

normality of distribution by a Kolmogorof-Smirnov test. For data not normally distributed Two independent samples test and Two related samples test were used. Differences were considered to be statistically significant for $p \leq 0,05$.

RESULTS

In the shuttle-box active avoidance test, the control group showed a statistically significant increase of the number of avoidances on the 4th ($6,7 \pm 1,94$ v/s $2,4 \pm 0,69$, $p \leq 0,01$), 5th ($9,9 \pm 2,5$ v/s $2,4 \pm 0,69$, $p \leq 0,01$) and 12th days ($9,3 \pm 2,35$ v/s $2,4 \pm 0,69$, $p \leq 0,01$) compared to the number on the 1st day of experiment. The rats treated with ETX in a dose of 50 mg/kg showed a tendency of increase in the number of conditioned responses in comparison with the control on the 2nd, 3rd, 5th days of the learning session and in the retention session on 12th day. We found a reduction of the number of avoidances in the group treated with the higher dose ETX (100 mg/kg) compared to the control group during entire training period and in the study of long-term memory, but no statistically significant difference was found (**Figure 1**).

In the active avoidance test, the control group showed a significant decrease in the number of escapes on the 2nd ($p \leq 0,001$), 3rd, 4th, 5th and 12th days ($p \leq 0,05$) compared to the 1st day (**Figure 2**). The animals treated with ETX in a dose of 50 mg/kg had increased the number of escapes on days 4 and 5 of training session and in the memory retention test on day 12 compared with the controls. There was a significant reduction of unconditioned responses in animals treated with ETX in a dose of 100 mg/kg on days 1 and 3 of training session compared to the controls ($p \leq 0,05$).

Analyzing the latency time, the group treated with ETX in a dose of 100 mg/kg showed a tendency to increase this parameter compared to the control (**Figure 3**).

The animals treated with ETX in a dose of 50 mg/kg had increased the number of intertrial crossings with no significant difference on days 1, 2, 3, 4, 5 and 12 compared to the controls. The experimental group treated with ETX in a dose of 100 mg/kg had decreased the number of intertrial crossings on days 1, 2, 3, 4 and 12 compared to the controls, but no significant difference was found (**Figure 4**).

Figure 1. Effects of ETX on the number of conditioned responses (avoidances) in active avoidance test (shuttle box) in rats. $^{oo} p \leq 0,01$ compared to the 1st day control saline solution; $^{+} p \leq 0,05$ compared to the same day of animals treated with EFX 50 mg/kg.

Figure 2. Effects of ETX on the number of unconditioned responses (escapes) in active avoidance test (shuttle box) in rats. $^o p \leq 0,05$ and $^{ooo} p \leq 0,001$ compared to the 1st day control saline solution; $^{*} p \leq 0,05$ compared to the control saline solution for the respective day.

Figure 3. Effects of ETX in active avoidance test (shuttle box): latency time.

Figure 4. Effects of ETX in active avoidance test (shuttle box): number of intertrial crossings. + $p \leq 0,05$ and ++ $p \leq 0,01$ compared to the same day of animals treated with EFX 50 mg/kg.

DISCUSSION

In vivo, the i.p. administration of ETX (50 mg/kg) was associated with increased concentrations of pregnenolone, progesterone, 5 α - dihydroprogesterone and allopregnanolone in plasma and brain of sham-operated animals (5). Pregnenolone increase and

allopregnanolone impairs memory performances (14). There is no data available in the scientific literature showing the effect of ETX on the cognitive functions of laboratory animals. Clinical studies describe that ETX preserves attention, memory and psychomotor functions (11-12). The results of a double blind

parallel group placebo controlled comparison of sedative and amnesic effects of ETX and lorazepam suggest that 50 and 100 mg single dose of ETX do not induce amnesia and sedation as compared to lorazepam in humans (10). Data are consistent with our results obtained about the cognitive functions in rats. The animals receiving ETX in a dose of 50 mg/kg were found to improve their learning abilities and preserve their long-term memory compared to the controls with no significant difference. The animals treated with the higher dose ETX were found to decrease the number of avoidances, escapes and intertrial crossings compared to the control group, but no significant difference was observed. ETX has the tendency to increase the number of measured parameters in a dose of 50 mg/kg and to decrease them in a dose of 100 mg/kg in comparison to the controls. But no significant differences between the experimental groups and the control group were found.

CONCLUSIONS

ETX at the lower dose of 50 mg/kg has a better effect in the active avoidance test compared to the control and this can be an advantage to other anxiolytics. ETX at the higher dose of 100 mg/kg has a worse effect on learning and memory in the active avoidance test compared to the control.

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