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STUDY OF THE EFFECTS OF DIAZEPAM ON PASSIVE AVOIDANCE TEST AND LOCOMOTOR ACTIVITY IN RATS

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ABSTRACT

Diazepam is a classical benzodiazepine agonist with anxiolytic and anticonvulsant activity. It is known that benzodiazepines decrease the ability to induce memory traces and to recall them. The aim of our study was to compare the effects of three doses diazepam on learning and memory processes in rats using step-down passive avoidance test and activity cage test. The male Wistar rats were treated 60 minutes before testing: 1st Saline 0.1ml/100g (controls); 2nd Diazepam 1.0mg/kg; 3rd Diazepam 2.5mg/kg; 4th Diazepam 5.0mg/kg. All groups were trained in step-down passive avoidance test. Learning session was performed in 2 consecutive days, short memory retention (24-hours later) and long memory retention (7th day). A latency of reactions 60 seconds was used as a criterion for learning and retention. The effect of diazepam on exploratory activity of rats was studied in automatic set-up for horizontal and vertical activity "activity cage" with photo-sensors. The time spent in the apparatus was 5 minutes. In step-down passive avoidance test the rats with diazepam in dose 1.0 mg/kg did not change the latency of reactions compared to the same day controls. The group with 2.5 mg/kg diazepam increased the latency of reactions on the second day of learning, but did not keep it on the short and long memory retention tests compared to the respective day control group. The animals with 5.0 mg/kg diazepam increased the latency on two days learning and on the short and long memory tests compared to the same day saline group. In the locomotor activity test the control group showed the highest number of relative units compared to all studied groups on horizontal and vertical activity. The summarized data from our experiments allow us to conclude that the chronic administration of diazepam dose-dependently impaired learning capacity, memory function and vertical locomotor activity in rats.

Key Words: Diazepam, Amnesia, Step-down, Activity cage, Rats.

INTRODUCTION

Diazepam is classical benzodiazepine agonist with anxiolytic and anticonvulsant activity. It is known that benzodiazepines decrease the ability to induce memory traces and to recall them. They induce anterograde amnesia, the inability to remember events happened immediately after drug application [1]. Diazepam is known to be non-selective agonist for γ-aminobutyric acid-A ($GABA_A$) receptor subtypes [2]. Diazepam exerts anxiolytic effects through its well-known action on $GABA_A$ receptors [3]. Several studies have localized $GABA_A$ benzodiazepine receptors in areas critically related to memory process such as hippocampus, septum and amygdala [4, 5]. The $GABA_A$ receptor is a heteropentameric structure that consists of several subunits with GABA, benzodiazepine, alcohol, barbiturates and neurosteroid recognition sites [6]. Immunocytochemical studies demonstrated that brain regions exhibit different distribution of $GABA_A$ receptor subtypes. The strongest α5-subunit immune reactivity was present in forebrain regions such as hippocampus, olfactory bulb and hypothalamus [7]. Amnesia, produced by benzodiazepines is associated with a suppressing effect on long-term potentiation which is the possible cellular mechanism that may underline learning and memory [8]. Benzodiazepine diazepam binds equipotently to the omega-1 and omega-2 sites of $GABA_A$ receptors. Non-benzodiazepine zolpidem has low affinity for the omega-2 sites may accont for less

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memory impairments caused by zolpidem than by benzodiazepines. Therefore, only omega-2 sites mainly involved in modulation of hippocampal inhibitory mechanism and long-term potentiation in CA1 region of rat hippocampal slices [9].

$GABA_A$ receptors are considered the main target for clinically effective anxiolytic drugs. Several $GABA_A$ receptor antagonists such as bicuculine, flumazenil and picrotoxin, are currently used to identify the precise sites of action of drugs on the $GABA_A$ receptor [10]. Post-training intra-amigdala injection of flumazenil causes memory facilitation comparable to that found with systematic injections. The systematic injection of flumazenil before training attenuates the amnestic effects of post-training intra-amygdala injection of muscimol. Current studies show that stress and aging impair declarative memory in humans and more specifically memory processes involving the hippocampus and prefrontal cortex activity [11]. There is extensive evidence indicating a key role for GABA neurotransmission in the modulation in fearful defensive behavior. It has been found that systematic or intra-amygdala injections of GABA agonists reduce experimental fear and anxiety [12]. In addition to its anxiolytic properties, however, diazepam has various pharmacological effects including amnesia [13]. One of the most frequent and well investigated symptoms in depression is the reduced memory capacity [14]. Delgado et al. (2005) hypothesized that depression may affect the amygdale noradrenergic modulation of memory. The noradrenergic/GABAergic connection may modify the anterograde amnesic effect of benzodiazepines. Furthermore, the action of benzodiazepines may block the negative tendency on emotional memory tasks in depressed patients. Diazepam 10 mg given as once off dose did not affect memory in human [15].

The aim of our study was to compare the effects of three doses diazepam on learning and memory processes and on locomotor activity in rats using step-down passive avoidance test and locomotor activity test. It is need to find dose that impaired memory without including muscle relaxation in experimental animals. This dose may be used like a pharmacological model of amnesia to compare the effects of cholinesterase inhibitors and NMDA-antagonist on learning and memory in rats.

MATERIAL AND METHOD

Ethical Statement

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 №49/30.06.2011 and Ethics Committee of the Medical University Plovdiv №3/05.07.2012.

Drug

Diazepam (Sopharma, Sofia, Bulgaria) is 7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzo diaze pin-2-one, was used in this study.

Animals

Male Wistar rats weighting 180-220 g kept under standard laboratory conditions (08.00-20.00 light, food and water at libitum) are used. The animals were divided into the following experimental groups (n=9): A: Control – Saline 0.1 ml/100g body weight; B: Diazepam 1.0 mg/kg; C: Diazepam 2.5 mg/kg; D: Diazepam 5.0 mg/kg. It was performed pre-treatment of the animals with saline and diazepam in the indicated doses for respective groups for two weeks once daily prior to testing in the apparatus. The substances were applied intraperitoneally 60 minutes before testing every day.

Behavioral tests

Step-down passive avoidance test

An automatic set-up for a passive avoidance Step-down" test (Ugo Basile, Italy) was used in a wire-floor cage with round central plastic platform. Learning sessions consisted of 2 trials separated by a 60 minute interval. During each trial, electronic stimulation (0.4mA) was delivered to the wire floor for duration of 10s. Learning sessions were performed over 2 consecutive days, a short memory retention session was performed 24 hours later (3^{rd} day), and a long memory retention session was performed on the 7^{th} day. The memory retention tests were performed using the same parameters, but with the absence of a foot shock. A latency of reaction of 60 s (the rat was required to remain on the platform for more than 60 s) was used as a criterion for learning and retention.

Locomotor activity test (Activity cage)

An automatic set-up for exploratory activity "Activity cage" (Ugo Basile, Italy) was used to study horizontal and vertical locomotor activity in rats. The animals were placed in a plastic cage with photo-sensors for 5 minutes. The movements of the animal were counted and recorder by the electronic unit and printed in digital form. The locomotor measurements were performed between 08:00 and 12:00 in a quiet room under normal laboratory lighting. The animals which underwent the test sessions were placed in the testing room approximately 2 hours before the drug administration. The rats were treated with either saline or diazepam and then temporarily returned to their home cage. Activity testing was initiated 60 minutes after the substances injection.

Statistical evaluation

The means ± SEM for each group of rats were calculated using Instat computer program. A two-way ANOVA for repeated measurements was used to compare different groups with the respective controls with the Turkey-Kramer multiple comparison test. A p-value of

P<0.05 was considered representative of a significant difference.

RESULTS

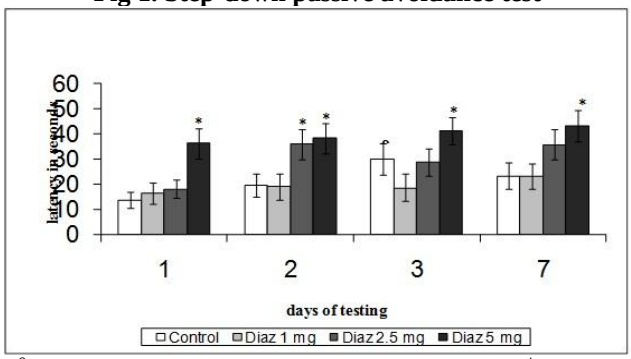
Effects of diazepam on step-down passive avoidance test

In the step-down passive avoidance test the control rats did not change the latency of reactions (the time spent on the plastic platform apparatus) on learning session and long memory retention, but significantly increased it on short memory retention test (p<0.05), compared to the 1st day (Figure 1). The group with 1mg/kg diazepam did not change the latency of reactions on learning session and on long memory test, compared to the same days control group. On short memory test this group lightly decreased the latency compared to the respective controls. The animals with 2.5mg/kg diazepam statistically significant increased the latency of reactions (p<0.05) on 2nd day learning, compared to the same day control group. The group with 1mg/kg diazepam did not change the latency of reactions on two memory tests. The rats with 5mg/kg diazepam significantly increased the latency of reactions on two days learning (p<0.05), short and long memory retention (p<0.05), compared to the same days control group (Figure 1).

Effects of diazepam on locomotor activity test (Activity cage)

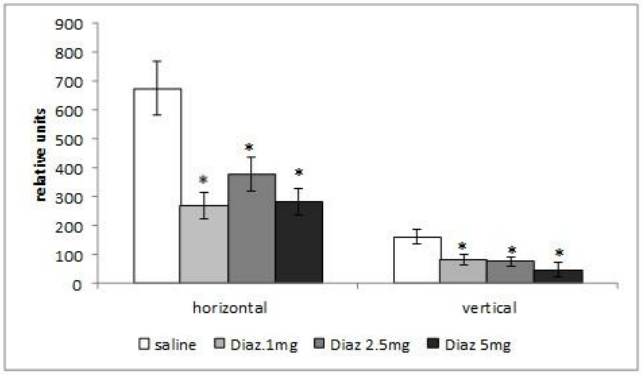
In locomotor activity test control rats showed higher number of relative units on horizontal and vertical movements compared to all studied groups. The groups with three doses of diazepam statistically significant (p<0.05) decreased the number of relative units on horizontal and vertical locmotor activity in comparison with control rats (Figure 2).

Fig 1. Step-down passive avoidance test



0 p<0.05 compared to the 1st day control group; * p<0.05 compared to the same day control group.

Fig 2. Locomotor activity test (Activity cage)



* p<0.05 compared to the control group

DISCUSSION

In step-down passive avoidance test the controls increased the latency of reactions in the learning and short-memory test. Our observations and experience show that the rats treated with different doses of diazepam increased latency of reaction in step-down test for passive learning but it cannot be accepted as a criterion for a memorized task. We suppose that the prolonged time spent on the platform of the apparatus is due to the suppressive effect of the diazepam on the CNS and its muscle-relaxant effect. We recognize the fact that we have two weeks pre-treatment with diazepam and the accumulation of its negative effects.

Our hypothesis is supported by the data of the locomotor activity test in activity cage apparatus. In this test we also observed that diazepam dose-dependently decreased the vertical movements in rats. The horizontal motor activity was the least affected by the middle dose of diazepam. All doses of diazepam decreased the horizontal and vertical movements of the animals.Other scientists found that diazepam given acutely in doses 0 . 3, 1 and 3 mg/kg reduced the lococmotion [16]. Lynch III *et al*. (2011) suggest that a change in spontaneous locomotor activity is an excellent preclinical indicator for the effects of different compounds on the central nervous system [17].

Chakravarthi *et al*. (2013) used 7 mg/kg diazepam intraperitoneally in 1-month-old Wistar rats as a model of amnesia. Using this model they tested plants with probable memory enhancing properties. The hippocampus plays an important role in spatial navigation and long term memory. It is located inside the medial temporal lobe beneath the cortical surface. Stressful conditions are often associated with loss of memory and cognitive functions, which may lead to threats of schizophrenia and Alzheimer's disease [18].

Data from other authors showed that the benzodiazepine agonist diazepam impaired learning and memory performance of animals in avoidance learning task [19]. The diazepam significantly decreased the spontaneous alternation performance in the Y-maze [20] and impaired acquisition of spatial memory in the Morris

water maze test in rats [21]. Comparison between the effects of ethanol and diazepam on spatial working memory in the rat found that both produced dose-dependent increases in working memory errors and are consistent with the hypothesis probably connected with potentiation of GABA at $GABA_A$ [22]. The scientists suggest that effects of diazepam on retention of inhibitory avoidance and shuttle avoidance tasks in rats are age-related. Diazepam impaired the retention of step-down inhibitory avoidance in 30 days-old rats at the three doses used (0.2, 1.0 and 5.0 mg/kg). In the 60-70 days-old animals, diazepam at the dose of 0.2 mg/kg was facilitated in step-down test, while doses of 1.0 mg/kg and 5 mg/kg impaired retention of both tasks [23].

Diazepam produced a reliable impairment in the performance of the passive avoidance. Such deficits are frequently interpreted as drug-induced amnesia. Diazepam and lorazepam did alter the acquisition of the passive response, but did produce a dose-dependent impairment of retention [24]. Other authors measured the emotional memory on the elevated T-maze and found that diazepam did not affect acquisition in performance but induced a dose-dependent impairment of the inhibitory avoidance in the memory test. They supported hypothesis that disruptive effects of diazepam on processes involved in long-storage of information [25].

CONCLUSION

The summarized data from our experiments allow us to conclude that the chronic administration of diazepam dose-dependently impaired learning capacity, memory function and vertical locomotor activity in rats. Based on results of this study we think that the dose of 2.5 mg/kg diazepam is suitable for pharmacological model of amnesia because impair learning and memory but least affected horizontal motor activity.

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