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COMPARISON OF THE EFFECTS OF DIFFERENT DOSES DIAZEPAM ON LEARNING AND MEMORY PROCESSES IN RATS USING ACTIVE AND PASSIVE AVOIDANCE TESTS

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ABSTRACT

Amnesia is one of the most common side effects of benzodiazepines. It is known that benzodiazepines decrease the abilities to induced memory traces. Amnesic effect of diazepam on a variety of memory tasks is well known. Benzodiazepines induce anterograde amnesia in both humans and animals. The aim of our study was to compare the effects of three doses diazepam on learning and memory processes using active and passive avoidance tests. The male Wistar rats (9 per group, with body weight 180-220g) treated intraperitoneally with: 1st Saline 0.1ml/100g (controls); 2nd Diazepam 1.0mg/kg; 3rd Diazepam 2.5mg/kg; 4th Diazepam 5.0mg/kg. The animals were trained in shuttle-box and step-through apparatus manufactured by UgoBasile, Italy. In shuttle-box active avoidance tests the following parameters were observed: number of conditioned stimuli responses (avoidances), number of unconditioned stimuli responses (escapes) and number of intertrial crossings. In step-through passive avoidance tests the latency of reaction was calculated in seconds (180 ± 2 s). The comparison between the groups was made by Instat computer program. In shuttle-box active avoidance test the rats with diazepam in all studied doses decreased the number of conditioned and unconditioned stimuli responses and intertrial crossings on learning and memory tests compared to the control group. In step-through passive avoidance test the animals treated with the highest dose of diazepam significantly decreased the latency of reaction on learning compared to the control group. All experimental groups with diazepam decreased latency of reactions on short memory test. Our results allow us to conclude that diazepam dose-dependently impaired learning and memory processes in rats.

Keywords: Diazepam, Amnesia and Rats.

INTRODUCTION

Memory is the ability of an individual to record sensory stimuli, events, etc., retain them over a certain period of time, and recall the same information at a later date when needed. Learning is the process of acquiring knowledge about the world and memory could be considered as the retention of the acquired knowledge, which can be recalled as and when needed [1]. Dementia is a mental disorder characterized by memory loss (initially of recent events), loss of executive function (such as the ability to make decisions or sequence complex tasks), other cognitive deficits, and changes in personality [2]. Dementia is multifunctional. Uncovering the mechanisms involved in the progression and development of Alzheimer's disease and other dementias has led to realization that several factors, genetic and environmental, influence its development and progression.

Pharmacological models have been very useful for evaluation of the role of various neurotransmitter systems in the learning and memory processes and determine how anti-dementia drug interact with cholinergic or glutamatergic systems. These types of rodent models provide a degree of predictive validity, as they have been a successful component of the drug discovery process as it is related to Alzheimer's disease [3]. In exteroceptive

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behavior models (elevated plus maze, Hebb-William maze, and Morris water maze), the stimulus lies outside the body, whereas in the case of interoceptive models (diazepam) it lies in the body. Diazepam-induced amnesia is used for studying the effects of different compounds on learning and memory processes [4]. Amnesic effect of benzodiazepines (especially diazepam) on a variety of memory tasks is well known [5]. Clinical data confirmed that diazepam selectively impaired anterograde episodic memory, attention and knowledge long-term memory [6].

The aim of our study was to compare the effects of three doses diazepam on learning and memory processes in rats using active (shuttle-box) and passive (step-through) avoidance tests. It is need to find the dose that impaired memory without inducing 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 METHODS

Ethical Statement

The present study was carried out in compliance with the European Convention for the protection of Vertebrate Animals used for Experimental and other Scientific Purposes. Official permission for the study was obtained by the Bulgarian Food Safety Agency №49/30.06.2011and the Ethics Committee of the Medical University Plovdiv №3/05.07.2012. Guidelines for proper laboratory animal care were fully implemented.

Drug

Diazepam (Sopharma, Sofia, Bulgaria) is 7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one.

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. The substances were applied intraperitoneally 60 minutes before testing.

Behavioral tests

Active avoidance test

An automatic reflex conditioner for active avoidance "Shuttle-box" (UgoBasile, Comerio-Varese, Italy) was used. A learning session of 5 consecutive days was performed. Each day consisted of 30 trials with the following parameters: 6s light and buzzer (670 Hz and 70 dB), 3s 0.4mA foot shock, 12s pause. A memory retention session with the same parameters without foot shock was performed 7 days later (12^{th} day). The following parameters were observed: number of conditioned stimuli responses (avoidances), number of unconditioned stimuli responses (escapes from food shock) and number of intertrial crossings.

Passive avoidance test

An automatic set-up for a passive avoidance "Step-through" (UgoBasile, Italy) was used in a wire cage with separate light and dark compartments. The test parameters were as follow: door delay for 6s, door opened to allow entry into darkened chamber for 12s, 0.4mA foot shock 9 sec later. Learning session was performed over 2 consecutive days, a short memory retention session was performed 24 hours later (3^{rd} day) and long memory retention was performed on the 10^{th} day. Memory retention tests were performed using the same parameters, with the exception of the foot shock. Sessions consisted of 3 trials separated by 30 minute intervals. The learning criterion was a latency of reaction of 180 ± 2 seconds in the light chamber.

Statistic

It was used Instat computer program for analysis of variance. The Mean and Standard error of mean (±SEM) for each group was calculated. A two-way ANOVA for repeated measurements was used to compare the experimental groups with the corresponding control group. A p-value of $P<0.05$ was considered representative of a statistically significant.

RESULTS

Effects of treatment on active avoidance testing

The control group showed increased number of conditioned stimuli responses (avoidances) on the 2^{nd} ($p<0.05$), 3^{rd}, 4^{th} and 5^{th} days ($p<0.01$) of learning session in comparison with the 1^{st} day learning (Fig. 1). The animals treated with 1.0mg/kg diazepam decreased the number of avoidances on the 4^{th} and 5^{th} day learning ($p<0.01$) compared to the respective day control group. The rats with 2.5mg/kg diazepam decreased the number of conditioned stimuli responses on the 2^{nd} and 3^{rd} days ($p<0.05$) and the 4^{th} and 5^{th} days ($p<0.01$) compared to the respective day saline group. The group with 5.0 mg/kg diazepam decreased the number of avoidances on the 2^{nd} ($p<0.05$), 3^{rd}, 4^{th} and 5^{th} ($p<0.01$) days learning when compared to the respective day controls (Fig. 1).On memory retention test the control group significantly increased the number of conditioned stimuli responses ($p<0.01$) compared to the 1^{st} day saline group. The three experimental groups with diazepam decreased the number of avoidances ($p<0.01$) compared to the respective day control group (Fig. 1).

The control group did not change the number of unconditioned stimuli responses (escapes) on learning and memory tests (Fig. 2). The experimental group with diazepam at dose 1.0 mg/kg significantly decreased the

number of escapes on five days learning the 1st, 2nd, 3rd and 5th day ($p<0.05$), 4th day ($p<0.01$) compared to the respective day control group (Fig. 2). The rats treated with 2.5 mg/kg diazepam decreased the number of escapes on the 1st, 4th and 5th days ($p<0.05$) and on the 2nd and 3rd days ($p<0.01$) learning when compared to the respective day saline group. The animals with diazepam at 5.0 mg/kg significantly decreased the number of escapes in all days learning ($p<0.01$) compared to the respective day control group (Fig. 2).

On memory retention test diazepam in the three studied doses significantly ($p<0.01$) decreased the number of unconditioned stimuli responses compared to the 12th day saline group (Fig. 2).

In active avoidance test the control rats increased the number of intertrial crossings on the 3rd, 4th and 5th days learning ($p<0.05$), but did not keep it in the memory retention compared to the 1st day control group (Fig. 3). The group with diazepam 1.0 mg/kg decreased the number of intertrial crossings on the 3rd, 4th and 5th day learning ($p<0.05$) compared to the respective day control group. The rats with diazepam at dose 2.5 mg/kg decreased the number of intertrial crossings on the 2nd, 3rd and 4th day ($p<0.05$) and on 5th day ($p<0.01$) compared to the respective day saline group. The animals with 5.0 mg/kg diazepam significantly decreased the number of intertrial crossings on the 2nd, 3rd, 4th and 5th day ($p<0.01$) of learning session when compared to the respective day control group (Fig. 3).

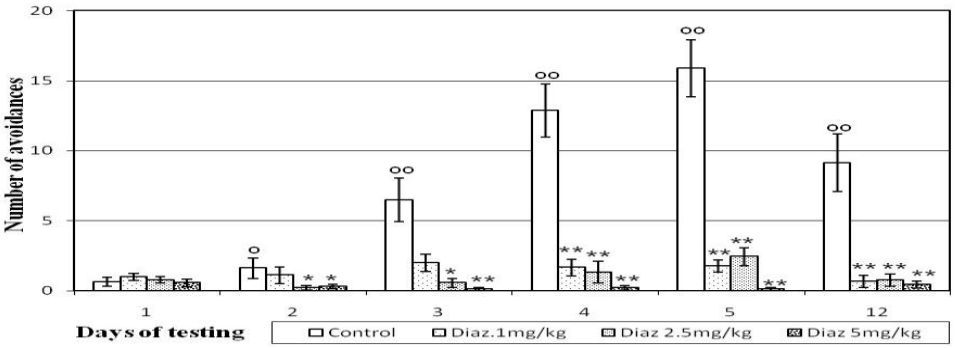
On memory retention test the experimental groups treated with the lowest and the highest dose of diazepam decreased the latency of reaction ($p<0.05$) compared to the same day control group. The group with 2.5 mg/kg diazepam did not change the number of intertrial crossings (Fig. 3).

Effects of treatment on step-through passive avoidance testing

The control group of rats showed a prolonged latency of reaction ($p<0.05$) on the 2nd day learning, short (on the 3rd day) and long (on the 10th day) memory tests ($p<0.05$) compared to the 1st day (Fig. 4). The experimental rats with 5.0 mg/kg diazepam significantly decreased the latency of reactions on the 2nd day learning ($p<0.05$) when compared with the respective day control rats (Fig. 4).

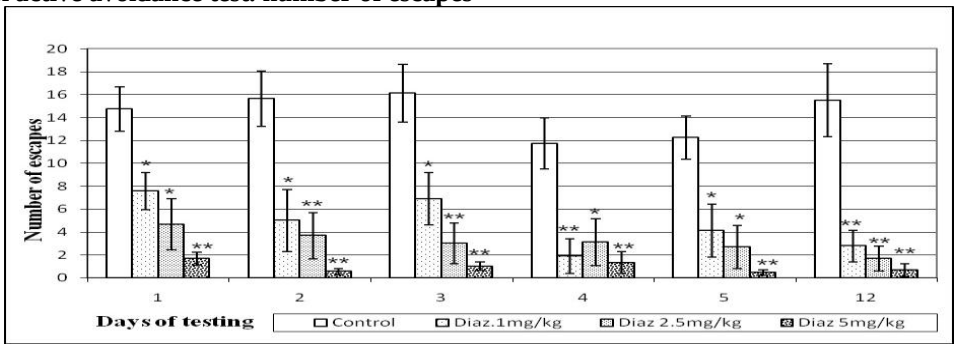
On short memory retention test all experimental groups treated with diazepam decreased the time spent in the light chamber of the apparatus - ($p<0.05$) for groups with diazepam 1.0 mg/kg and 2.5 mg/kg and ($p<0.01$) for group with diazepam 5.0 mg/kg when compared to the respective day saline group (Fig. 4). None of the experimental groups showed significant change in the latency of reaction on the long memory test compared to the same day control group (Fig. 4).

Fig 1. Shuttle-box active avoidance test. Number of avoidances



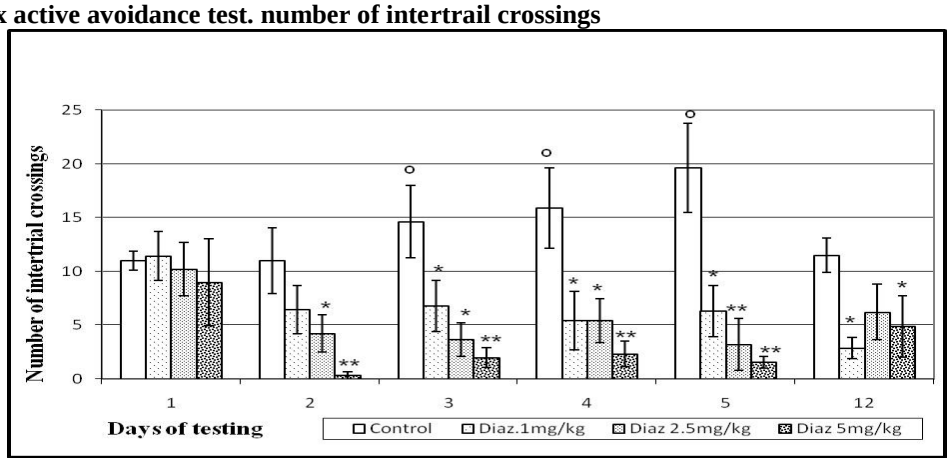
$^{0}p<0.05$ and $^{00}p<0.01$ compared to the first day control group; *$p<0.05$ and **$p<0.01$ compared to the same day control group.

Fig 2. Shuttle box active avoidance test. number of escapes



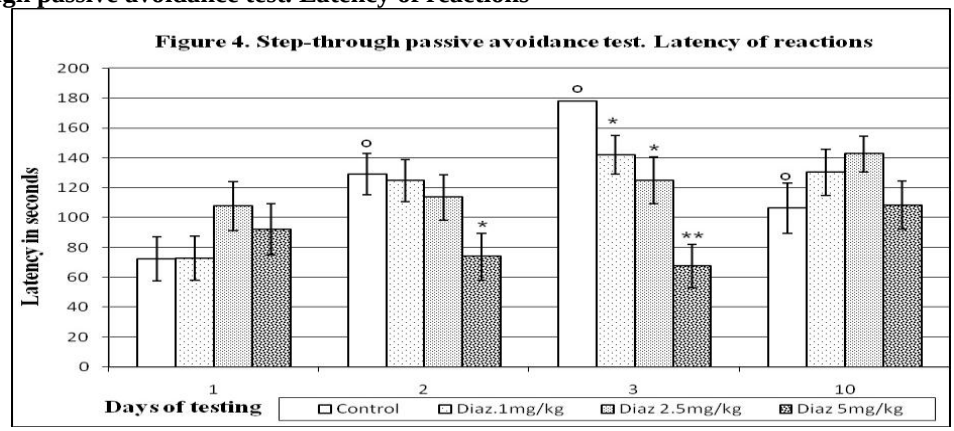
*$p<0.05$ and **$p<0.01$ compared to the same day control group

Fig 3. Shuttle box active avoidance test. number of intertrail crossings



^{0}p<0.05 compared to the first day control group; *p<0.05 and **p<0.01 compared to the same day control group.

Fig 4. Step through passive avoidance test. Latency of reactions



^{0}p<0.05 compared to the first day control group;*p<0.05 and **p<0.01 compared to the same day control group.

DISCUSSION

Our results showed that diazepam dose-dependently impaired learning, short and memory processes in rats during active and passive avoidance test. In active avoidance test diazepam has more significant effect on learning and long-term memory, whereas in passive avoidance test it has better effect on short-term memory. We found that the effect of diazepam on locomotor activity also is dose-depended during the learning session, but it does not retain in memory test.

Our results support the hypothesis [7] that benzodiazepine agonist diazepam affecting acquisition process and impair learning and memory performance of animals in avoidance learning task [8]. Thiebot (1985) published data for benzodiazepines induced anterograde amnesia in both humans and animals twenty years ago. Findings by Izquierdo and colleagues (1990) suggest that benzodiazepine impaired memory involves GABAergic type A receptors in the amygdala. Post-training intra-amygdala injection of flumazenil causes memory facilitation comparable to that found with systematic injections, and systematic injection of flumazenyl before training attenuates the amnestic effects of post-training intra-amygdala injection of muscimol.

Benzodiazepine effects are mediated through the GABA (A) complex by enhancing GABA-induced synaptic inhibition [9]. Studies examining the memory-modulating effects of drug treatments have provided evidence that memory can be modulated by systematic as well as intra-amygdala GABAergic compounds. When administered shortly after training GABAergic agonists (eg, muscimol and baclofen) impair memory retention, while GABAergic antagonists (eg, picrotoxin and bicuculine) enhance retention [10, 11]. Furthermore, lesions of the amygdale attenuate the anti-anxiety as well as the memory-modulating effects of GABAergic drugs [12, 13].

A study with magnetic resonance spectroscopy revealed low GABAergic levels in the occipital cortex of depressed patients, but in vivo GABA (A)-receptor binding activity with benzodiazepine radioligand was not altered [14]. Cortical benzodiazepine binding to GABA (A) receptors has been measured with ^{123}I-labeled flumazenil and single photon emission computed tomography in unmedicated patients with major depression and healthy

volunteers [15]. It is proved that single administration of 10 mg diazepam did not provide memory disturbances in humans (healthy volunteers and depressed patient). Consequently, the action of diazepam on the amygdala, which has been proposed to be the basis of its anxiolytic action, might be altered, modifying the modulation of memory in patients [16].

Another factor in interpreting findings from rodent models of dementia illness lies in the behavioral paradigm employed to model human cognition [17]. Experiments of Beracochea and collegues (2011) illustrate how stress and benzodiazepines could modulate cognitive functions of middle-aged mice depending on hippocampus activity. The investigators present data for the impact of benzodiazepine administration on hypocampal glucocorticosteroids concentration and its consequence on memory in a hypocampal-dependent contextual memory task in stressed middle-aged mice.

Animal models are an invaluable tool for characterizing the mechanisms underlying disease and exploring possible therapeutic approaches. Combining information from complementary animal models should provide a more detailed understanding of the potential benefits of new anti-dementia drugs [17].

CONCLUSION

Diazepam applied chronically impaired learning capacities and memory function of the animals and decreased their locomotor activity. Our results allow us to conclude that the doseof 2.5 mg diazepam is appropriate for pharmacological model of amnesia. It induces learning and memory impairments without muscle relaxation in rats.

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