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Differences in diazepam influence on the behaviour of rabbits under spontaneous conditions, and after electrical stimulation of the ventromedial hypothalamic nucleus

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Abstract: The purpose of the present study was to compare the influence of diazepam on different forms of rabbits' behaviour under stress and spontaneodial hypothalamic nucleus, escape reactionus conditions. The experimental model of a stress situation was induced by electrical stimulation of the ventromedial hypothalamic nucleus. Diazepam was injected intravenously at a dose of 1mg/kg 40 min before the behaviour recording. Diazepam reduced the tension phase and orientation-searching reactions, and prolonged the comfort phase in both situations. Grooming was not influenced by diazepam, but eating and drinking were inhibited. Only the reduction in food intake in normal conditions was a statistically significant change.

Key words: diazepam, ventrome

INTRODUCTION

Diazepam is a widely prescribed psychotropic drug that has a variety of effects, including anxiolytic, sedative, anticonvulsant, and muscle relaxant activity [1]. It has been established that diazepam binds to at least two classes of sites – the central and peripheral type benzodiazepine binding sites (CBR and PBR, respectively). A specific central benzodiazepine receptor is associated in a macromolecular complex with a site for GABA and a chloride ion channel. CBR is located within the central nervous system. CBR mainly mediates the effects of Diazepam. To date, the functional role of PBR has not been defined. The peripheral binding sites are distributed in the brain and in several peripheral tissues. They differ from the central ones in their lack of coupling to GABA receptors [2].

The primary aim of the present study was to examine the influence of diazepam on different forms of rabbits' behaviour under stress and normal conditions, and compare the results. The experimental, animal model of a stress situation was an active defence, described as the escape reaction, induced by electrical stimulation of the ventromedial hypothalamic nucleus (Vmh) [3, 4].

MATERIAL AND METHODS

The experiments were performed on 20 male Chinchilla rabbits, mean body weight 3,250g, divided into 2 equal groups. The animals had free access to food and water. Room temperature was 20±2° C. The animals were brought to the laboratory, placed in the experimental cage and acclimatized to the surrounding conditions 1 h before starting any experiment. Then 2-hourly recording of of the rabbits' behaviour was initiated. The time of observation was divided into 10-min intervals. At the beginning of each 10-min interval. for the whole time of the recording, electrical stimulation of the Vmh was induced in Group 1 until the escape reaction occurred. Six forms of behaviour were distinguished and estimated: the tension and comfort phases, orientation-searching reactions, grooming, water and food uptake. The duration of each phase was measured in seconds with a stopwatch. The tension phase was manifested by the tension posture, immobility of the animal, acceleration of breathing and increase in tension of skeletal muscles. The orientation-searching reactions meant change in motor activity and exploratory behaviour. The comfort phase was the relaxation of the rabbit, decrease in muscle tension, and sleepiness. Grooming was the nursing activity.

The experiment was carried out for 3 subsequent days. On the first day of the experiment, the nickel-chrome bipolar electrode was implanted into the Vmh of Group 1. At first, 10 ml of 1% Polocain (Polfa) was injected subcutaneously into the frontoparietal area of the head. Then, after uncovering the tectum of the cranium, a cannula was located 1 mm posterior to bregma, 1 mm lateral to the medial raphe and 15.5 mm below the skull surface at the point of entry, according to co-ordinates in the stereotactic atlas (Cvietkova I.P.1987) The electrode was inserted through the cannula. On the second day, behaviour was tested, in Group 1 – under stress conditions, and in Group 2 – in a spontaneous situation. Stress was evoked by electrical stimulation of the Vmh [3, 4] (100 Hz frequency, 0.3 ms impulse width, 3-6 V voltage). On the third day, diazepam (Relanium solution, Polfa Poznań,

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N211197) was injected intravenously (vena marginalis) at a dose of 1 mg/kg of body weight, 40 min before experimental sessions in both groups.

Statistical analysis was performed. The values of studied features were characterized by evaluation of the statistical parameter: the arithmetic mean, median, standard deviation and the quartiles, probability of differences between the mean values (p). The W Shapiro-Wilk's test was used for checking the normal distribution and the U Manna-Whitney's test was used to compare the 2 independent groups. Results were statistically different if $p<0.05$. The StatSoft Statistica 8.0 Software was used to analyze all the data.

The experiments were conducted in accordance with the ethical standards for the humane treatment of animals and Polish legislation concerning animal experimentation.

RESULTS

There was no significant difference in the duration of the tension phase before and after diazepam administration under spontaneous conditions, (p=0.45), probably due to low initial values, while the changes between the control group and and the group treated with diazepam in a stress condition were statistically significant, (p=0.0002), (62.08 vs. 0.00) (Table 1).

Table 1 Tension

Group	Mean	Standard Deviation	Q25	Median	Q75
Control	0.42	0.90	0.00	0.00	0.00
Diazepam	0.00	0.00	0.00	0.00	0.00
		Z=0.7; p=0.45			
Control, Vmh Stimulation	150.75	173.56	4.33	62.08	186.67
Diazepam, Vmh Stimulation	2.08	3.27	0.00	0.00	5.00
		Z=-3.78; p=0.0002*			

The orientation searching reactions were shortened significantly under spontaneous and stress conditions (changes from 113.33-45.00, p=0.02 and from 184.17-101.25, p=0.05, respectively) (Table 2).

Table 2 Orientation – searching reactions

Group	Mean	Standard Deviation	Q25	Median	Q75
Control	129.17	77.39	67.50	113.33	152.50
Diazepam	56.00	47.97	18.33	45.00	94.17
		Z=2.42; p=0.02*			
Control, Vmh Stimulation	193.42	97.68	130.83	184.17	263.33
Diazepam, Vmh Stimulation	132.33	149.09	65.83	101.25	117.50
		Z=-1.97; p=0.05*			

The substance prolonged comfort under normal conditions (407.92 vs. 491.67 p=0.008) and understress (248.33 vs.443.75, p=0.01), (Table 3).

Table 3 Comfort

Group	Mean	Standard Deviation	Q25	Median	Q75
Control	395.25	89.55	308.33	407.92	481.67
Diazepam	504.25	54.16	458.33	491.67	555.83
		Z=-2.65; p=0.008*			
Control, Vmh Stimulation	208.33	146.08	43.33	248.33	305.00
Diazepam Vmh Stimulation	372.58	185.41	329.17	443.75	500.83
		Z=2.49; p=0.01*			

Grooming was not influenced by diazepam (a slight reduction from 21.25-10.83, p=0.52 under spontaneous conditions, and from 20.83-6.25, p=0.27 under stress conditions) (Table 4).

Table 4 Grooming

Group	Mean	Standard Deviation	Q 25	Median	Q75
Control	30.42	34.39	6.67	21.25	36.67
Diazepam	20.83	24.32	5.83	10.83	25.83
		Z=0.64; p=0.52			
Control, Vmh Stimulation	23.58	19.74	4.17	20.83	41.67
Diazepam, Vmh Stimulation	13.00	15.39	4.17	6.25	19.17
		Z=-1.09; p=0.27			

A statistically significant decrease in food intake under spontaneous conditions was observed (p=0.04), while the changes of eating under stress were not significant (p=0.41) (Table 5). Diazepam did not alter drinking in spontaneous conditions (6.25 vs.1.25, p=0.17) or after Vmh stimulation (p=0.29) (Table 6).

Table 5 Food intake

Group	Mean	Standard deviation	Q25	Median	Q75
Control	38.83	35.91	0.00	44.17	50.83
Diazepam	6.83	21.03	0.00	0.00	0.00
		Z=2.04; p=0.04*			
Control, Vmh Stimulation	19.42	29.41	0.00	0.00	33.33
Diazepam, Vmh Stimulation	6.33	13.44	0.00	0.00	0.00
		Z=-0.83; p=0.41			

Table 6 Water intake

Group	Mean	Standard deviation	Q25	Median	Q75
Control	8,50	6,93	2,50	6,25	15,00
Diazepam	5.28	8.03	0.00	1.25	6.67
		Z=1.36; p=0.17			
Control, Vmh Stimalation	5.17	10.95	0.00	0.00	5.83
Diazepam, Vmh Stimulation	1.00	3.16	0.00	0.00	0.00
		Z=-1.06; p=0.29			

DISCUSSION

In our experiments, the behaviour of the rabbits under spontaneous and stress conditions was examined following acute administration of diazepam. An electrical stimulation of the Vmh produced a strong anxiety stimulus [3, 4]. Sudakow called the Vmh a hypothalamic centre of anxiety [4].

Diazepam has been reported to show anxiolytic effects, and is active in most animal tests of anxiolytic activity. Using the elevated 'zero-maze', which is a modification of the elevated plus-maze model of anxiety, diazepam (0.125-0.5 mg/kg) significantly increases the percentage of time spent in the open quadrants, the frequency of head dips over the edge of the platform, and reduces the frequency of stretched attend postures from the close to open quadrants. [5]. Diazepam shows dose-dependent increases in both number of entries into the open arms of the plus-maze and in the time spent in the open arms (doses from 0.6mg/kg) [6]. This is considered to be indicative of anxiolytic drug action. This effect was similar to the findings of Mechan et al. who observed the increase of open-arm behaviour following diazepam [7]. In the present experiments, there was the expected effect of treatment with diazepam, the substance reduced significantly the tension phase in a stress situation.

Diazepam extended the comfort phase in both conditions, which resulted from its sedative properties. Diazepam exerts its anxiolytic and sedative effects by facilitating coupling of the inhibitory transmitter GABA to its receptor, via the benzodiazepine-GABA-receptor complex (BZR). Sedative effects occur at almost 100% benzodiazepine receptor occupation [8]. Defence and escape behaviours are particularly sensitive to diazepam, for example, defensive reactions induced by pain and isolation-induced aggressive behaviour. The aggression reducing effects are often associated with the sedative activity of benzodiazepines [9]. A dose of 1mg/kg transiently reduces aggression between 0.5-1.5 h, but only the highest dose of diazepam (5 mg/kg) produces marked and sustained suppression [8].

In our experiments, orientation-searching reactions were depressed after diazepam administration. Perhaps this compound considerably reduced the interest of the environment. Agmo et al. indicate that diazepam produces motor deficiencies in doses larger than those required for anticonflict effects [10]. According to Rex et al., diazepam produces a slight hyperactivity after a dose of 10 mg/kg. Other doses did not alter locomotor activity significantly [6]. Sherif et al. noted the intensification of exploratory activity in rats in a new environment after diazepam administration which, in their opinion, may account for the anxiolytic effect of the drug. Simultaneously, there is a decrease of motor activity in animals [11].

Present data shows that diazepam minimally reduces the grooming phase. It has been reported that diazepam-treated rats perform less grooming behaviour than vehicle-treated controls [7].

Several benzodiazepines have been shown to the intake of food and water under various test conditions [10, 12]. The stimulatory actions of diazepam on eating has been reported in a stressful environment [10]. Chronic stress increases the appetite for sweet food, independently of hunger, and diazepam is able to reverse this behaviour [13]. Chlordiazepoxide increases food intake in rats whether they are hungry or satiated. It also enhances feeding in rats subjected to activity-induced anorexia. There is evidence that benzodiazepines do not result in an increase in body weight of rats [14]. According to Rex et al., diazepam shows a dose-dependent increase in the ratio of rats feeding to rats not feeding. But in the food consumption test in the home cages and in the food consumption in the open field the drug does not increase the feeding of animals [6]. There have been other studies suggesting that the stimulatory effects on food consumption do not interact with appetitive, motivational factors associated with food palatability [15]. Animals given diazepam drink significantly more times than animals injected with physiological saline. But this compound does not significantly induce the amount of water consumed and does not reduce latency to begin drinking. Those observations suggest that the behaviour of animals injected with diazepam was associated with the sedative effects of the drug [16].

Our experiments indicated that diazepam significantly decreased food intake in spontaneous conditions. Other behaviours, including drinking in both situations and eating in the stressful environment, were not influenced by this compound. Although feeding was inhibited by electrical stimulation of the Vmh, our observations allowed the suggestion that diazepam minimized this effect.

Recent studies have revealed that BZR is a modulatory site on GABA A receptor, and that there are at least 15 different subunits of these receptors [9]. Alterations to the density, affinity, regional brain distribution of BZR, and arrangements of the subunits, influence on different behavioural reactions after diazepam and other benzodiazepines administration. Additionally, it has been found that the ability to decrease 5-HT synthesis, release and turnover, could be the mechanism by which diazepam exerts its anxiolytic effect [17].

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