COMPARISON OF THE INFLUENCE OF DIAZEPAM AND NEW BENZODIAZEPINE DERIVATIVE (BD-1158) ON THE RABBITS’ BEHAVIOUR AFTER ELECTRICAL STIMULATION OF THE VENTROMEDIAL HYPOTHALAMIC NUCLEUS

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Abstract

The aim of the present study was to compare the influence of diazepam and new benzodiazepine derivative BD-1158 on different forms of rabbits’ behaviour after ventromedial hypothalamic nucleus (Vmh) stimulation. The experiments were performed in 2 groups of animals. In the first group, diazepam was injected intravenously at a dose of 1 mg/kg. In the second group, BD-1158 was administered intraperitoneally at a dose of 10 mg/kg, diluted in 1% starch solution up to 1 ml. The substances were administered 35-40 min before the behaviour recording. Each daily session lasted 2 h. It was divided into 10-minute intervals. Electrical stimulation of the Vmh was induced at the beginning of each 10 min interval for the whole time of the experiment until the escape reaction occurred. It was found that both agents exert anxiolytic and sedative effects, they do not prove to be factors in modulating grooming, eating and drinking in experimental stress situation. Diazepam influence on orientation-searching reactions is more stronger than BD-1158 influence.

Key words: rabbits, benzodiazepines, ventromedial hypothalamic nucleus, stress, behaviour.

The benzodiazepines (BDZs) represent one of the most frequently used drug classes in the world by virtue of their anxiolytic, anticonvulsant, muscle relaxant and sedative effects (3, 5, 19). They are also used in veterinary practice to relax aggressive or anxious animals, as premedication before invasive procedures. They exert their behavioural effects by facilitating coupling of GABA, the inhibitory transmitter, to its receptor, which is the main element of a macromolecular GABA/benzodiazepine receptor complex (BZR) (9, 22, 29, 30). Little et al. (13) propose a continuous spectrum of action of ligands for the BZR, ranging from full agonists through neutral antagonists to full inverse agonists. Occupation of the receptor by the agonist, such as diazepam, enhances GABA induced increases in chloride ion conductance by increasing the frequency of ion channel openings. BZR inverse agonists affect chloride conductance by decreasing the time that ion channels remain opened. They have pharmacological effects opposite to those of the full agonists, they are convulsant, anxiogenic and heighten arousal. The benzodiazepine receptor antagonists are neutral and block the effects of both agonists and inverse agonists, do not influence on GABAergic function (29). There are also partial agonists and partial inverse agonists that produce milder effects than the full agonists and the full inverse agonists and have a lower efficacy. They also block the effects of high-efficacy compounds (6, 9, 30).

In the present experiments, we compared the influence of BZR agonists: diazepam (a frequently used reference compound) and new benzodiazepine derivative – BD-1158, on different forms of the rabbits’ behaviour under experimental stress conditions, evoked by electrical stimulation of the ventromedial hypothalamic nucleus (Vmh). The electrical stimulation of the Vmh or the medial hypothalamus is a procedure in which an active defence or avoidance behaviour is induced in cats and rabbits (4, 8, 27) and appetite is suppressed (20).

Material and Methods

Animals. Twenty male Chinchilla rabbits of mean body weight of 3250 g, divided into 2 equal groups were used. The animals had free access to feed and water. Room temperature was 20±2°C. The animals
were brought to the experimental room, placed in the experimental cage and acclimatized to the surrounding conditions 1 h before starting any experiment.

**Substances.** The following substances were used: 1 ml of 1% starch solution, diazepam (Relanium solution, Polfa Poznań, N211197) at a dose of 1 mg/kg of body weight and BD-1158 at a dose of 10 mg/kg, diluted in 1% starch solution up to 1 ml. The starch solution was administered intraperitoneally, diazepam was injected intravenously (vena marginalis), BD-1158 was dissolved in the solution immediately prior to use and injected intraperitoneally. The compounds were given 35-40 min before experimental sessions.

**Procedures.** Six forms of rabbits’ behaviour: tension, orientation-searching reactions, comfort, grooming, water and feed intake were distinguished with the purpose of the estimation of the substances’ influence on different animals’ reactions. 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 the change in motor activity including exploratory behaviour. The comfort phase was the relaxation of the animal, sleepiness and decrease in the muscle tension. Grooming was the nursing activity. Duration of each phase was measured in seconds with a stopwatch. Each daily session of behaviour observation lasted 2 h. 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 until the escape reaction occurred.

On the first day of the experiment, the nickel-chrome bipolar electrode was implanted into the Vmh in both groups. 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 median 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 (28). On the second day, in both groups the behaviour of the rabbits under stress conditions, evoked by electrical stimulation of the Vmh, was tested. Electrical stimulation of the Vmh was performed by the current of 100 Hz frequency, 0.3 ms the impulse width and 3-6 V voltage according to the excitability of the center. On the third day, group 1 received injections of diazepam and group 2 - injections of 1% starch solution. On the fourth day, BD-1158 was administered to rabbits of the group 2.

**Statistics.** The significance of solvent and substance influence on the duration of the phases was analysed with *t*-Student test. Results were statistically different if *P* < 0.05.

**Results**

The reaction of the dissolvent was excluded before testing the influence of BD-1158. The duration of each phase was expressed in %, as the share of a given phase in 2-h observation time.

As might be expected, both substances significantly decreased the tension phase in stressful state, BD-1158 from 18.97% to 1.44% and diazepam from 34.26% to 0.38% (*P* <0.05). The comfort phase was prolonged after BD-1158 administration from 58.24% to 84.36% and after diazepam injection from 26.47% to 70.35%. These changes were also statistically significant (*P* < 0.05). The orientation-searching reactions were reduced after BD-1158 and diazepam administration from 10.21% to 8.13% and from 30.42% to 15.28%, respectively. Diazepam exerted more stronger influence than BD-1158 on locomotion and only its suppressive effect was a statistically significant change (*P* <0.05). Grooming was not influenced by both pharmacological agents ( slight lengthening by BD-1158 from 1.98% to 2.88% and slight reduction by diazepam from 3.27% to 1.22%). Both substances did not prove to be significant factors in modulating eating and drinking. BD-1158 decreased feed intake from 8.77% to 2.26% and water intake from 1.48% to 0.95%. Diazepam also inhibited eating and drinking (changes from 3.25% to 0.38% and from 0.56% to 0.1%, respectively).

**Discussion**

In the present experiments an electrical stimulation of the Vmh, which is anxiety-producing stimulus (4, 8, 27), was applied. Sudakow (26) calls the Vmh a hypothalamic center of anxiety. We measured the duration of various reactions of rabbits before and after anxiolytic agent administration. We assessed sensitivity to these agents and compared BD-1158 to diazepam effects because diazepam has been frequently used as a reference compound.

Present data indicated that both substances reduced the tension phase, which resulted from their anxiolytic effect. Defense and escape reactions are particularly sensitive to benzodiazepines, for example defensive reactions induced by pain and isolation - induced by aggressive behaviour (30). Benzodiazepines exert anticonflict effects but minimum effective dose of diazepam in rats according to Agmo et al. (1) is 1 mg/kg. Diazepam tested on the animal models of anxiety significantly increases the percentage of time spent on the open areas, frequency of head dips over the edge of the platform, decreases the frequency of stretched attend postures (23), and increases the entries into and the time spent in the open arms (15, 18).

Benzodiazepines have also been reported as sedative compounds. It has been hypothesized that benzodiazepine-mediated sedation requires almost 100% occupancy of the BZR (29). Sedative effects are often associated with the conflict reduction effects (21,
In our experiments both diazepam and BD-1158 prolonged the comfort phase significantly.

Motor activity is depressed for a prolonged period following stressor exposure, for example inescapable shock. Locomotion in response to an eliciting stimulus recovers in 2-3 d after exposure to inescapable shock. Adinazolam which acts similarly to the classic BDZs, exerts little effect on the activity of inescapably shocked subjects at high doses, although there is a tendency to have a sedative influence. The low doses of adinazolam reverse the activity reduction and eventually produce hyperactivity in previously shocked rats (14). We found that orientation – searching reactions under stress conditions were reduced significantly after diazepam administration. The results also suggest, that doses of the used compounds are strongly sedating but they are too low to increase the activity reduced by stress. Agmo et al. (1) indicate that BDZs produce motor deficiencies in doses larger than those required for anticonflict effects. Rex et al. (18) say that a dose of 10 mg/kg induces hyperactivity. According to Sherif et al. (24) diazepam administration increases exploratory activity in rats resulting from anxious-like effects of the drug. Lister et al. (12) also note that diazepam increases exploratory behaviour in mice.

Both substances had no effect on the grooming phase. Time spent grooming reduction after diazepam administration is described by Stout and Weiss (25).

Benzodiazepines have been found to exert the craving effects that are independent of their anxiolytic activity (25). They induce hyperphagia in laboratory animals (10). This effect is similar to that of feed-deprived animals (7). Hunt et al. (10) note that hunger group exhibits significant increase in eating highly palatable feed as compared to low palatable ones. The benzodiazepine treated rats show an equivalent increase in feed-intake regardless of whether the feed is of high or low palatability. These data suggest that the stimulatory effect of BDZs on feed consumption does not interact with appetite (10). Electrical stimulation of different areas of the hypothalamus can produce a diversity of motivational effects. For example electrical stimulation of the lateral hypothalamus induces hunger in feed-satiated rats. Diazepam facilitates this reaction (2). Benzodiazepine - induced facilitation of feed intake in rats, while they are hungry or satiated. This was confirmed by Lett et al. (11). Rex et al. (18) reported that in their feed consumption test in home cages and in open field diazepam (doses from 0.35 to 6.00 mg/kg) did not increase the appetite of rats. Whilst the highest doses (12mg/kg) cause a decrease in the amount of feed eaten. It is suggested that hyperphagia induced by diazepam is mediated by dopamine (17) and histamine receptors (16).

Animals injected with diazepam drink more frequently than do animals given physiological saline. Stout et al. (25) do not attribute this effects to influence on thirst or drinking because diazepam does not alter the amount of water consumed in the home cage, where anxiety is not a frequent factor. Increasing doses of diazepam produce increases the frequency of drinking in the open field, but this time is greatly reduced after the fearfulness produced by shock. Other measures including number of approaches to the water tube, amount of water consumed are decreased, probably as a result of sedative activity of diazepam (25). In the present experiments both agents reduced eating and drinking, but not significantly. Additionally, eating was inhibited by electrical stimulation of the VMh and neither diazepam nor BD-1158 reversed the decrease in feed intake.

References