INFLUENCE OF DIAZEPAM ON THE ESCAPE REACTION AND RABBITS’ BEHAVIOUR UNDER STRESS

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Abstract

The purpose of the study was to assess the influence of diazepam (DZ, administered iv. at the doses of 0.2 and 1.0 mg/kg) on the escape reaction and rabbit’s behaviour under stress evoked by repetitive electric stimulation of the ventromedial nucleus of the hypothalamus (VMH). Both DZ doses had no significant influence on the latent time of the escape reaction, but they changed the structure of rabbits’ behaviour, decreasing the tension and exploration phases and prolonging comfort. Changes were more potent during the first than the second hour of the experiment. The dose of 1.0 mg/kg had generally similar but a stronger effect on the behaviour than the dose of 0.2 mg/kg. DZ acted as an efficient anxiolytic and sedative drug, which eliminated symptoms of stress. Its effects in the reduction of rabbit’s fear and anxiety depended on the dose and time after its administration.

Key words: rabbits, diazepam, escape reaction, behaviour, stress, hypothalamus.

Aggression and escape reaction are usual forms of an organism response to acute stress. The rabbit is an animal that hardly shows aggression even in stress conditions. Therefore, the tension state and presence of the escape reaction are the main indicators of stress and anxiety (5).

Every stress disturbs the homeostasis of living organism, but if it is too excessive or acts too long, it contributes to increased morbidity and mortality. To decrease negative consequences of stress modern pharmacology has introduced a great number of chemical substances. One of them is diazepam (DZ) – the oldest derivative of 1,4 benzodiazepines (BD), which is characterized by the high therapeutic index and many properties such as: anxiolytic (4, 6, 13, 14, 17, 23, 24), myorelaxant (4, 6, 9, 19), sedative (6) and anticonvulsant. The pharmacological effects of DZ depend on the dose (6, 9, 12, 14), the way of administration, species (1, 7) sex and part of the body under influence. Small doses are used as anxiolytics and sedatives. In particular situations, if an overdose is taken, the drug can modify the structure of the behaviour and life functions (3). Diazepam is widely used in veterinary (e.g. in Poland as Relanimal) to calm aggressive and anxious animals. It provides great help during the transport, adaptation and gathering of animals in herds. DZ is used in premedication before invasive procedures as well.

The aim of the present study was to investigate the influence of diazepam on the escape reaction and behaviour of rabbits in experimental stress situation evoked by electric stimulation of the ventromedial nucleus of the hypothalamus.

Material and Methods

The experiment was performed on 30 male Chinchilla rabbits (mean body weight 3250 g) divided into three equal groups (two experimental receiving DZ and the control one without any treatment). Every rabbit was kept in a typical one-animal cage at the temperature of 20±2°C with proper air circulation and in natural light cycle at least two weeks prior to the experiment. Rabbits had free access to commercial feed (LSK, AGROPOL MOTYCZ, Poland) and tap water. One day before the experiment nickel-chrome bipolar electrodes were implanted into VMH (1 mm posterior to bregma, 1 mm lateral to medial raphe and 15.5-16 mm below the skull surface at the point of entry). All experiments were conducted between 9 a.m. and 2 p.m. They were made in accordance with ethical standards for the human treatment of animals and Polish legislation.

The rabbits were observed at least 24 h after the surgery manipulations in the calm and peaceful atmosphere of a soundproof room after one hour adaptation to the laboratory environment. On the initial - first day, the experiment was conducted without any injection. On the second day, DZ was administered in
experimental groups into the *vena marginalis* (0.2 mg/kg or 1 mg/kg, RELANUM SOL., POLFA, WARSZAWA, Poland), 30 min before the first stimulation and the start of observation. The control group did not receive any drug.

The experimental model of unavoidable stress conditions was an active defence, described as the escape reaction (5), which was evoked by electrical stimulation of the VMH (by current of 100 Hz frequency and 0.3 ms of the impulse width). The VMH was stimulated repeatedly at the beginning of every ten-minute period (5) during two hours for two subsequent days. The threshold voltage was adapted individually for every rabbit before the experiment and remained constant until the last day. The latency time of the escape reaction (LT), was measured from the beginning of the stimulation to the first symptoms of the motor reaction (5) with a digital time-meter (PFL-28A UNIVERSAL COUNTER, ZOPAM WARSZAWA, Poland) connected to the stimulator (SC-02-UW, COTM O/BIAŁYSTOK, Poland).

The animals’ behaviour between stimulations was divided into several phases (changing each of them aperiodically): tension, exploration activity, passive observation, comfort, grooming, water and feed intake. Tension was manifested by the immobile standing position with the raised muscle tension and quickened breathing, whilst opposite it a comfort phase by resting horizontally without movement and with quiet breathing, often by napping or falling asleep. Examining environment was expressed during the exploration phase as increased motor activity with searching movements of all the body and/or sniffing at the experimental cage, water and feed bowls, whereas during passive observation it occurred in a lying or standing position by movements of head and/or ears. Grooming was body cleaning with the tongue and paws, sometimes rubbing against the cage walls. Water uptake was expressed in free quenching of rabbit’s thirst while feed uptake in eating and coprophagy. The duration of particular phases was recorded and estimated in seconds for every hour.

LT and duration of particular phases on the second day of the experiment were compared with those from the initial day. Statistical calculation was carried out by using *t*-Student test. Results were statistically different if *P*<0.05.

**Results**

The administration of diazepam did not change significantly the latency time of the escape reactions evoked by electrical stimulation of the ventromedial nucleus of the hypothalamus. The lower dose showed the tendency to reduce LT and the higher dose in contrast to increase it.

Both DZ doses changed the structure of rabbits’ behaviour in stress evoked by electrical stimulations of VMH (Figs 1 and 2). They reduced significantly the tension phase. The lower dose decreased its duration in the first hour of the experiment to 4.22% of the value from the initial day (from 1183.5±875.72s to 50±56.42s, *P*<0.01) and to 6.17% in the second one (from 413.3±302.04s to 10.79±21.27s, *P*<0.01). The dose of 1mg/kg reduced it almost completely to 0.96% (from 1562.5±1040.47s to 15±17.8s, *P*<0.001) and to 1.38% (from 904.5±621.36s to 12.5±19.61s, *P*<0.01), respectively.

![Fig 1. Structure of rabbits' behaviour under stress after administraton of DZ during the first hour of observation (in percentage of the values from the initial day).](image)

For 0.2 mg/kg **P<0.01. For 1 mg/kg ##P<0.01, ###P<0.001.
Both doses considerably prolonged the comfort phase. After administration of 0.2mg/kg duration of comfort rose during the first hour to 237.4% of the initial value (from 1007±791.01s to 2390.5±837.16s, P<0.01) and during the second one to 143.2% (from 1712±704.12s to 2451.5±863.08s, P<0.05). One mg/kg prolonged comfort to 381.85% (from 655.5±886.48s to 2514.5±779.47s, P<0.001) and to 204.08% (from 1250±876.45s to 2551±872.12s, P<0.01), respectively.

The duration of the exploration decreased, after administration of 0.2 mg/kg, in the first hour to 28.48% of the value from the initial day (from 553±283.6s to 157.5±108.56s, P<0.01) and to 47.73% in the second one (from 529±421.23s to 252.5±144.15s, P<0.05). The 1mg/kg dose reduced time of exploration to 15.89% (from 440.5±358.39s to 70±46.55s, P<0.01) and to 19.77% (from 470.5±433.16s to 93±67.63s, P<0.05), respectively.

Both doses of DZ doubled the water intake in the first hour but in the second one rabbits’ thirst decreased to 25.80% after administration of the higher dose and to 19.35% after the lower one. Nonetheless, the changes appeared in few animals; thus they had no statistical significance. The other phases i.e.: passive observation, grooming, and feed intake were not influenced significantly by the 0.2 mg/kg or 1 mg/kg dose.

In the control group no significant changes in the duration of LT and particular behavioural phases were observed.

**Discussion**

The ventromedial hypothalamic nucleus is not only known as an example of the emotionally negative zone of the brain (5) but is also responsible for many biochemical changes (18). VMH stimulation results in various behavioural and emotional changes as well: rats, for instance, reveal aggression; whilst rabbits react with an escape reaction (5). The experiment based on the repetitive stimulation of the VMH provided conditions of an unavoidable stress; therefore it was possible to assess the influence of particular doses of DZ on the escape reaction and rabbits’ behaviour in stressfull conditions. The amount of drug chosen was as in our previous investigations under spontaneous conditions (6), in which doses of 2 or 5 mg/kg, often used in experiments on rats, evoked intense drowsiness of rabbits, when lower than 0.2 mg/kg did not cause visible changes. Kawasaki (10) noticed a drowsy EEG pattern in rabbits after iv. DZ treatment at the doses of 1 to 2 mg/kg.

The latency time of the escape reaction reflected animals’ awareness. Both doses of DZ did not change LT significantly, that could indicate they did not influence the reactivity of the VMH to the electrical stimulation. Fukuda (9) observed the dose-dependent marked elevation in the threshold for direct attack evoked by electrical stimulation of the medial hypothalamus in cats after DZ treatment. Kawasaki (10) reported the suppression of EEG arousal to auditory stimulation and to electrical stimulation of the posterior hypothalamus in rabbits after iv. administration of DZ at the doses of 1 to 2 mg/kg.

The tension state, which is rare for rabbits under spontaneous conditions but typical in stress (5), was reduced by more than 90% after the administration of both doses of DZ. It undeniably confirmed anxiolytic properties of DZ. Many researchers noticed significant reduction of anxiety after diazepam administration in mice e.g. in four plates test (4) and in rats in elevated plus-maze (24), in open field (17), in tail-swing test (23), in the operant procedure and footshocks received in the drinking test (13). Naito (14) observed the attenuation of the ultrasonic vocalisation responses elicited by air-puff stimuli and electric foot-shock in rats after DZ.
The comfort phase, characteristic for the relaxation and often connected with somnolence, is in stress much shorter than under spontaneous conditions because of the presence of a much longer tension phase (5). After DZ treatment in stressful conditions evoked by electrical stimulation of the VMH comfort was prolonged visibly, especially at the higher dose. The rate of the phase in the structure of rabbits’ behaviour comprised more than two thirds of the whole investigation time, which confirmed the sedative activity of DZ. Silakov (20) reported a tranquillising effect of DZ in rhesus monkeys and baboons.

The state of the orientation-searching activity depends on rabbits’ awareness and is much longer in stress caused by electrical stimulation of the VMH than under spontaneous conditions (5). In this investigation, the dose-dependent and significant depletion of exploration and simultaneously prolongation of comfort in stress by both doses of DZ could be the result of drug sedative activity. In rats diazepam administration leads to the intensification of exploratory activity (19, 22).

In this study the absence of significant changes in the duration of passive observation and grooming did not show the animals’ somnolence and myorelaxant effect of DZ in both used doses. Except premedication, the myorelaxation caused by DZ is considered as side effect of the drug. Fukuda (9) observed in cats dose-dependent muscle relaxant activity of DZ in the reactions evoked by electrical stimulation of the medial hypothalamus. Bourin (4) recorded the myorelaxant influence of DZ in mice. Sheriff (19) noticed the decreasing of rat’s motor activity after DZ administration. Kumar (12) reported a dose-dependent increase in self-grooming in unrestrained rhesus monkeys living in social colonies after DZ treatment.

DZ did not change significantly water and feed uptake in rabbits in stressful conditions. Both doses showed tendencies to the prolongation of rabbits’ drinking in the first hour and its shortening in the second one, whilst only a higher dose to a reduction in eating at the end of the experiment. The observed changes were not authoritative due to big deviations among particular animals. Undoubtedly the stimulation of the VMH has a considerable effect on feeding (21), causing hunger suppression in rats (18), monkeys and rabbits. Also diazepam influences eating. It facilitates feeding (2) and often induces hyperphagia in mice (16), and rats (15). Foltin (8) noticed the increase of maximal feed intake in adult male baboons after DZ. The dose-dependent increase in feeding after diazepam treatment was observed in sated grey wolves (11) and in unrestrained rhesus monkeys living in social colonies (12).

In conclusion DZ did not influence the latency time of the escape reaction, evoked by electric stimulation of the VMH, but changed significantly rabbits’ behaviour under stress. It decreased the tension and exploration phases and prolonged comfort. The 1mg/kg dose of DZ had generally a stronger anxiolytic and sedative activity than 0.2 mg/kg. Changes were more potent during the first than the second hour of the experiment.

References

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