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The influence of diazepam on the behaviour of rabbits in spontaneous conditions

Diazepam (DZ) is one of the many derivatives of 1,4 benzodiazepines (BD) and has a lot of properties such as anxiolytic, miorelaxant and sedative. It causes soporific and anticonvulsant effects (6, 10).

DZ is a fully synthetic drug and is not based on a natural archetype. Although there have been found some substances in human and animal organisms which have similar effects with BD, their chemical structures are different (11).

DZ exerts its pharmacological influence by a specific receptor (which is part of pentamer of $GABA_A$ receptor) present in the central nervous system (CNS). All its properties follow from hyperpolarisation of neurone's membrane caused by the connection of DZ to the part of $GABA_A$ receptor. This connection enables GABA to attach to the receptor and increases the frequency of opening Cl^- canal and slows down the velocity of nerve impulses conducting. According to Bloom, 30% of CNS nerve synapses works thereby (4).

Pharmacological effect of DZ depends on its dose, way of administration, species of animals (6), and also on the part of brain attached to DZ (5). Affinity and potency of the drug is high but its efficacy is connected with the period of administration and animals' behavioural conditions (6). DZ also acts on the peripheral receptors present in the liver, pancreas, urinary bladder and other organs (3).

The drug is easily attached to the blood albumins. Time of its pharmacological activity can be prolonged by oxazepam, which is one of the products of DZ metabolism. Then the drug and its remnants are eliminated by kidneys in 70 % and partly with feaces (6).

Usually small doses of diazepam are used by human beings as anxiolytics and sedatives but sometimes, if an overdose is taken or if a particular situation does not demand

any treatment, the drug consumption can modify their spontaneous behaviour and life functions (1, 7).

In order to characterize DZ influence on typical behaviour the experiment was carried out in spontaneous conditions without an external stimulation which could evoke stress. Small doses of the drug were administered to the rabbits.

MATERIAL AND METHODS

The experiment was performed using 30 randomised male Chinchilla rabbits (mean body weight 3,250 grams) were divided into three subclusters of 10 animal each and kept in typical one-animal cages at the temperature of 20±2 °C with proper air circulation and in natural light cycle at least two weeks prior to the experiment. The rabbits were given free access to commercial food (LSK, Motycz, Poland) and water. After one-hour adaptation to the new surroundings, the experiment was carried out in similar conditions in the laboratory. The animals' behaviour divided into several phases: tension, active observation, passive observation, comfort, grooming, water and food intake was observed. Duration of particular phases was recorded and estimated in seconds in 10-min periods for two hours.

Each rabbit was observed during 2 subsequent days between 9 a.m. and 2 p.m. in calm and peaceful atmosphere of the soundproof room. On the first day no injection of any chemical substance was made. On the second day, DZ (0.2 mg/kg and 1 mg/kg, Relanium sol. made by Polfa Poznań, N211197) was administered intravenously into *vena marginalis* on the rabbit's ear during the one-hour adaptation 30 min before the start of the observation. The control group did not receive any drug neither on the first nor on the second day.

Duration of the particular phase after DZ injection on the second day of experiment was compared with the same phase duration on the first (control) day. The statistical significance was checked with t-Student test.

RESULTS

The tension phase which is rare in spontaneous rabbits' behaviour was (12) reduced from 0.1% of all observation time to 0% after DZ at a 0.2 mg/kg dose and from 0.21 % to 0 % after the 1 mg/kg dose. However, the reduction was by 100% and any signs of tension were not observed during the whole experiment after the drug injection, the changes were not statistically significant due to the fact that tension phase occurred in few rabbits in spontaneous conditions.

The active observation phase decreased visibly after administration of 0.2 mg/kg dose of DZ ($p<0.05$) and it reached 45.9% of control value during the first hour (Fig. 1) and

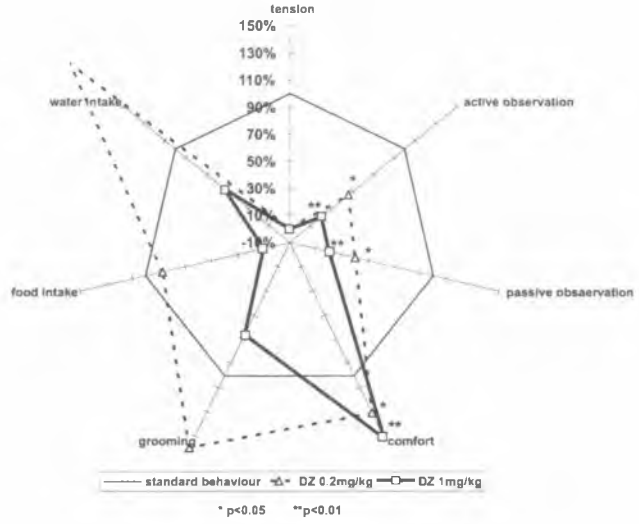

Fig. 1. Structure of rabbits' behaviour after administration of diazepam during the first hour of obserwation (in percentage relation to control results of each dose)

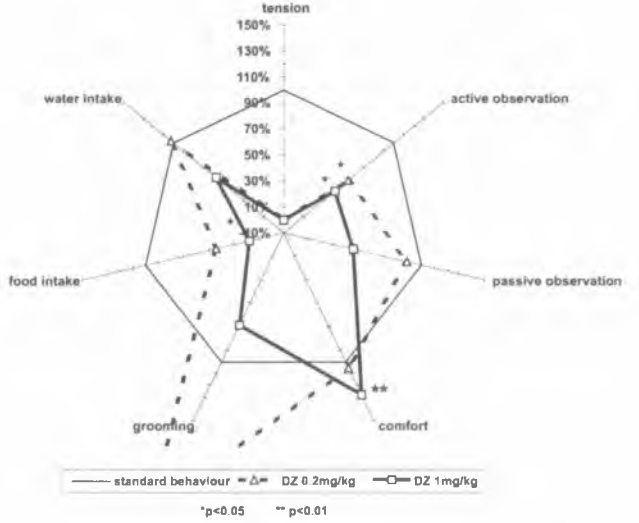

Fig. 2. Structure of rabbits' behaviour after administration of diazepam during the second hour of observation (in percentage relation to control results of each dose)

54.14% during the second hour (Fig. 2). The 1 mg/kg dose reduced time of active observation by 79.25% and by 59% ($p<0.05$) respectively.

Passive observation after the smaller dose got shorter and achieved 40.1% ($p<0.05$) of the control value during the first and 88.8% in the second hour. The higher dose clearly shortened passive observation to 20.14% ($p<0.05$) and 45.12 % respectively.

Although observation time decreased, duration of comfort phase extended and reached 130.83% ($p<0.05$) in the first and 105% in the second hour after injection of 0.2 mg/kg dose. Administration of 1 mg/kg of DZ increased the comfort phase significantly to 151.72% and 127.8% respectively ($p<0.01$).

We did not observe comparable values of grooming after the injection of both doses of the examined drug. The lower dose increased this phase to 159.67% during the first hour and to 224% ($p<0.05$) during the second. Changes in the first hour were not authoritative due to big deviations among individual rabbits. The higher dose slightly decreased grooming reaction and its value achieved 66.08% and 68.47%, these changes were different from the trend of the lower dose. The dose of 0.2 mg/kg DZ decreased food intake during two hours of observation to 86.97% and 43.79%, respectively. The higher dose acted in the same way and the ratio reached 25% and 54.16%. The examined substance in the lower dose prolonged the time of water intake to 202.11% and 102.6% of the initial value. On the contrary, the higher dose of DZ decreased water intake to 53.42% and 57.88%, but neither the smaller nor the higher one produced statistically significant results. That was because duration of food and water intake phases varied greatly and caused a big standard deviation.

In the control group, there were not observed any statistical changes in the rabbits' behaviour.

DISCUSSION

The experiment was carried out with 0.2 and 1 mg/kg doses of diazepam (also known as Relanium) because the doses higher than 1 mg/kg, e.g. 2 or 5 mg/kg which are often used in experiment on rats (2), produced a profound sedation in rabbits. Low doses i.e 0.2 mg/kg caused changes with "p" value smaller than 5% only during the first hour of the experiment. Even though the results during the second hour were not statistically significant, they showed well the trend how DZ affects the spontaneous rabbit's behaviour. Visible effects were observed after the administration of 1 mg/kg dose, which was regarded as the most appropriate for analysing DZ features and its effects on rabbits' behaviour.

Rabbits display a low level of aggression, tension, and even when threatened they get calm quite quickly, so the fact that the tension phase after both doses of DZ treatment was reduced completely to 0 value (even statistically insignificant) can be regarded as

confirmation of sedative, anxiolytic and soporific properties of this 1,4 benzodiazepine derivative and it is consistent with pharmacological reports (8).

Administration of the examined drug prolonged the comfort phase which was a main phase in rabbits' spontaneous behaviour and it took over a half of all the experimental time. The increase was very important and proved most of DZ properties.

The decrease in the active observation phase after DZ treatment was noticed. During this phase rabbits were very lively, they were looking around, sniffing the cage, their food, water, and often tried to get out of the cage. Both doses of DZ decreased significantly this phase ($p<0.05$), though during the second hour after 0.2 mg/kg, there was no statistical significance.

Passive observation is a phase when rabbits are staying relaxed in one place of the cage but they are not asleep. They observe surroundings and are able to react to the external stimuli. This activity is typical of a relaxed animal and should not be shortened after the administration of selective anxiolytic drugs. Both doses of DZ reduced the passive observation phase visibly. The obtained results confirmed sedative and soporific DZ's properties because in that case observation turned into the comfort phase.

CONCLUSIONS

1. DZ acts on the spontaneous behaviour of rabbits and its influence depends on the drug dose and time elapsed after its injection.
2. The dose of 1 mg/kg reduced all phases of spontaneous behaviour except the comfort phase which increased visibly instead. It had strong soporific and miorelaxant properties.
3. Effects of DZ at the dose of 1 mg/kg on the rabbits' behaviour were stronger in the first than in the second hour of observation.
4. DZ at the dose of 0.2 mg/kg was able to decrease most phases of behaviour except for comfort, grooming and water intake but the dose was not effective enough to cause its pharmacological effects longer than one hour.
5. During the second hour of the experiment after the administration of DZ at 1 mg/kg dose effects were very similar to the results, which were noticed in the first hour of the experiment with 0.2 mg/kg dose.

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SUMMARY

The study dealed with a popular drug diazepam also known as Relanium. Two doses of the substance were tested: 0.2 mg/kg and 1 mg/kg. The experiment was carried out on 10-rabbit groups for two hours. The animals were observed in peaceful environment of the laboratory after one hour of adaptation. There were distinguished a few phases of behaviour: active and passive observation, comfort, tension, grooming, food and water intake.

Both doses eliminated completely the tension phase. Changes in other phases were similar to each other but depending on the dose there were differences in strength and duration.

The smaller dose caused significant prolongation of the comfort phase at the expense of active and passive observation time decrease during the first hour of observation. The second hour trends were not changed but lost their statistical relevance. The changes caused by the bigger dose were almost identical and they were statistically significant during both hours of the experiment, but their amplitude was stronger than that caused by the lower dose.

The outcome of the experiment confirms anxiolytic, sedative and miorelaxant properties of diazepam.

Wpływ diazepamu na zachowanie królików w warunkach spontanicznych

Praca dotyczyła popularnie używanego leku – diazepamu, znanego także pod nazwą handlową Relanium. Testowano dwie dawki leku: 0.2 mg/kg oraz 1 mg/kg przez okres dwóch godzin na grupach królików liczących po dziesięć zwierząt. Obserwacja prowadzona była w spokojnym środowisku laboratorium po uprzednim jednogodzinnym okresie adaptacji. W strukturze zachowania wyróżniono szereg faz: obserwację czynną, obserwację bierną, komfort, naprężenie, gruming, jedzenie i picie. Obie dawki diazepamu wyeliminowały całkowicie fazę naprężenia oraz wywołały podobne zmiany w innych fazach zachowania, jednak ich czas i siła działania były różne. Dawka mniejsza w pierwszej godzinie w statystycznie istotny sposób wydłużyła fazę komfortu kosztem skrócenia czasu obserwacji czynnej i biernej. W drugiej godzinie zachowała te same tendencje, chociaż bez statystycznej istotności. Dawka większa spowodowała podobne zmiany zachowania, istotne statystycznie zarówno w pierwszej, jak i drugiej godzinie, ale ich natężenie było silniejsze niż po mniejszej dawce. Zaobserwowane zmiany zachowania królików w warunkach spontanicznych potwierdzają przeciwlękowe, uspokajające oraz zwiotczające właściwości diazepamu.