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Research report

Midazolam impairs acquisition and retrieval, but not consolidation of reference memory in the Morris water maze

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HIGHLIGHTS

- Benzodiazepines cause anterograde rather than retrograde amnesia.
- We studied the effects of midazolam on the water maze behavior in rats.
- It impaired acquisition and retention, but not consolidation of spatial learning.
- Midazolam administered before the probe test impaired retrieval of reference memory.
- There is a possibility of retrograde amnesia when midazolam is clinically used.

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ABSTRACT

Amnesia is one of the most discussed properties of the benzodiazepine class of drugs. The effects of benzodiazepines on human memory are usually anterograde, while changes in retrograde memory functions were seldom reported. Such inconsistent findings have prompted numerous animal studies investigating the influences of these positive modulators of inhibitory neurotransmission on different stages of memory. Among the benzodiazepines, memory effects of midazolam are of special interest due to its many and varied clinical applications. The present Morris water maze study in adult male Wistar rats was performed in three experiments in which midazolam was administered at doses of 0.5, 1 and 2 mg/kg intraperitoneally, before or immediately after each of five daily learning sessions, with two trials in a session, as well as before the probe test. Midazolam impaired acquisition and subsequent retention of spatial learning of the position of the hidden platform even at a pre-training dose of 0.5 mg/kg. This low dose was not associated with impairment of the procedural component of learning, manifested by increased time spent in the periphery of the pool. The lack of midazolam effect on consolidation has not been confounded by the observed below-chance performance of the control group since our additional experiment using diazepam also administered immediately after each of five learning sessions has revealed a similar pattern of results. Finally, midazolam administered before the probe test impaired retrieval of reference memory at all tested doses. Hence, induction of retrograde, besides anterograde amnesia should be kept in mind as a possibility when midazolam is used in clinical settings.

1. Introduction

Gamma-aminobutyric acid (GABA) is the principal inhibitory neurotransmitter of the brain and its main actions are mediated by fast-acting $GABA_A$ receptors. Binding of benzodiazepines at the benzodiazepine site of $GABA_A$ receptors results in potentiation of inhibitory neurotransmission, with consequent behavioral changes. Their capacity to elicit sedative, anxiolytic, myorelaxant, hypnotic and antiepileptic effects has made benzodiazepines the drugs widely used in clinical practice. However, they may also exert some effects, such as amnesia, usually thought of as an adverse effect [1,2].

Regarding the possible effects on learning and memory processes, benzodiazepines have probably been more extensively investigated than any other therapeutic class. In humans, amnesic effects were firstly recognized in 1960s by anesthesiologists using benzodiazepines as pre-medication and the finding was repeatedly corroborated and elaborated (reviewed in [3]). Nonetheless, it was noted that most patients taking benzodiazepines do not complain of memory problems [4]. It is generally suggested that the effects of benzodiazepines on human memory are anterograde, while the retrograde memory is usually not affected [2]. Although

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commonly considered to be unwanted, anterograde amnesia is desirable in situations such as perioperative periods in surgery or during procedures like endoscopies [3]; the anti-anxiety actions of benzodiazepines may modulate their disruptive effects on memory processes in emotionally-arousing situations [5].

In animals, learning and memory cannot be measured directly, but only inferred from behavior [6]. Since pharmacological treatments can be administered and eliminated from the organism within a relatively short time window, they provide an experimentally feasible way for dissecting three stages of memory: acquisition, consolidation, and retrieval [7]. If trying to generalize findings from animal memory tests with benzodiazepines, among which the passive avoidance paradigm was most frequently published, one can conclude that these ligands elicit an acquisition-impairing effect as a rule, a consolidation-impairing effect as an exemption [8,9], while data on the influence on retrieval are contradictory to a certain extent (reviewed in [10–12]). At the cellular level, it has been repeatedly observed that benzodiazepines inhibited induction of long-term potentiation (LTP), a proposed electrophysiological correlate of learning and memory, in rat hippocampal slices [13,14]. While this effect may be a part of the mechanism by which benzodiazepines affect memory, it is noticeable that such finding was not reproduced with all drugs from this class; for example, it was the case with midazolam, but not clonazepam [15]. The latter raises the issue of generalizability of data collected in memory studies with different benzodiazepines.

When looking at the Morris water maze, a highly used test of spatial learning and memory [16], there are surprisingly few studies investigating in parallel the influences of benzodiazepines on different stages of memory. In the delayed-matching-to-position paradigm, which represents one of the working memory versions of the water maze task, it was shown that chlordiazepoxide impairs performance when given during the acquisition and retrieval, but not consolidation phase [17]. In regard to the water-maze reference memory task, there is only a consistent finding that benzodiazepines impair acquisition and subsequent retention of spatial learning in rodents [18–21], while data on possible influences on consolidation and retrieval are rare. It was shown that single administration of triazolobenzodiazepines brotizolam and triazolam, but not diazepam, impairs retrieval in the probe test in mice treated only with solvent during four days of the learning phase [20]; the lack of effect of diazepam on retrieval replicated the finding obtained in rats [19].

Memory effects of midazolam are of considerable clinical importance, since it is widely used as a sedative and anxiolytic in ambulatory care settings and intensive care units, as well as a premedicant or an anesthetic induction agent in anesthesiology [2,22]. In the present study, we investigated in rats the effects of midazolam (0.5, 1 and 2 mg/kg) on spatial learning, as well as on acquisition, consolidation and retrieval stages of reference memory, assessed in a probe test 24 h after five days of learning the position of the hidden platform during learning sessions in the Morris water maze. It is generally cited that midazolam has the fastest onset of action and the shortest duration of effect of all benzodiazepines in both humans and rodents, with elimination half-life in rats equaling less than 0.5 h [23–25]. Such a pharmacokinetic profile of midazolam is particularly beneficial if one wants to attribute the possible cognitive effects of treatment given before or after learning sessions to the specific changes in acquisition or consolidation of memory, respectively [7].

2. Materials and methods

2.1. Behavioral experiments

Experiments were carried out on eight weeks old outbred Wistar albino male rats (120 in total), weighing 200–250 g and supplied by Military Farm, Serbia. Rats were housed in Makrolon type III cages (42 cm × 26.5 cm × 18 cm) in groups of six and had free access to food and water. The temperature of the animal room was 22 ± 1 °C, relative humidity 40–70%, illumination 120 lx, with a 12-h light/dark cycle (lights on at 06:00 h). All experiments took place during the light phase of the diurnal cycle (09:00–15:00 h). The behavior was recorded by a ceiling-mounted camera and analyzed by ANY-maze Video Tracking System software (Stoelting Co., USA). Midazolam and diazepam were obtained from Galenika (Belgrade, Serbia) and both substances were suspended/dissolved with the aid of sonication in the same solvent (85% distilled water, 14% propylene glycol, and 1% Tween 80). Different doses of midazolam (0.5, 1 and 2 mg/kg), diazepam (2, 5 and 10 mg/kg) or solvent were administered intraperitoneally (i.p.) in a total volume of 2 ml/kg, 20 min before or immediately after behavioral experiments, as specified below. All procedures in the study conformed to EEC Directive 86/609 and were approved by the Ethical Committee on Animal Experimentation of the Faculty of Pharmacy in Belgrade.

2.2. Morris water maze

The water maze consisted of a black cylindrical pool (diameter 200 cm, height 60 cm), with a uniform inner surface. The pool was filled with water at 22 °C (±1 °C) to a height of 30 cm. The escape platform of black plastic (15 cm × 10 cm) was submerged 2 cm below the water surface. The platform was the same color as the pool wall so it was invisible to rats [26]. There were many distal cues in the testing room (doors, pipes on the walls and the ceiling, and cupboards). An indirect illumination in the experimental room was provided by white neon tubes fixed on the walls.

The study, as originally designed, consisted of three experiments: Experiment 1 (the influence of treatment on acquisition), Experiment 2 (the influence of treatment on consolidation) and Experiment 3 (the influence of treatment on retrieval). Each experiment comprised of five swimming blocks during the learning phase (five consecutive days, two trials per day lasting a maximum time of 120 s) and a 60 s probe test with the platform omitted given 24 h after the completion of the learning phase. In Experiments 1 and 2, separate groups of randomly assigned rats received the appropriate treatment (0.5, 1 and 2 mg/kg midazolam or solvent) 20 min before or immediately after the swimming block, respectively. There were no injections prior to the probe test. In Experiment 3, rats received an injection of solvent on each day of training 20 min before the swim. After the fifth learning day, the rats were ranked according to their average latencies to find the platform on that day, and then appropriately assigned to homogenous treatment groups, which received 0.5, 1 and 2 mg/kg midazolam or solvent 20 min prior to the probe test. In order to validate the results of Experiment 2, it was decided to replicate the procedure using diazepam (2, 5 and 10 mg/kg), as a standard benzodiazepine (Experiment 4). The overall experimental procedure is depicted in Fig. 1.

For each trial the rat was placed in the water facing the pool at one of four pseudo-randomly determined starting positions. Since the platform was hidden in the middle of the NE quadrant during training sessions, the four distal start locations were: S, W, NW and SE. Once the rat has found and mounted the escape platform it was permitted to remain on the platform for 15 s. The rat was guided to the platform by the experimenter if it failed to locate it within 120 s. In order to ensure that any spatial bias is a consequence of the spatial memory of escape location, rather than of a specific swim strategy, the probe test was started from the novel, most distant SW location [27]. The tracking software virtually divided the pool into four quadrants, three concentric annuli and a target region consisting of the intersection of the platform quadrant and the platform (middle) annulus, as graphically represented in Figs. 2A and 3A. The platform annulus equaled 40%, the target region was set up to 10% of the whole area, whereas the area of the peripheral annulus was 50% of the whole [21].

Dependent variables chosen for tracking during the learning phase of Experiments 1, 2 and 4 were: the latency to find the platform (time from start to goal), the total distance traveled (the path length), the path efficiency (the ratio of the shortest possible path length to actual path length) and the percentage of time spent in periphery (the peripheral annulus). As regards the probe test in all experiments, the latency and the path efficiency to first entry to the target region were selected parameters.

2.3. Statistical analysis

All numerical data shown in Fig.s were given as the mean ± SEM. In order to assess the influence of treatment during the learning phase of Experiments 1, 2 and 4, we used two-way ANOVA (factors: Treatment and Days) with Days as the repeated measure. For parameters measured in the water maze during each day of these experiments, the mean value was calculated for each rat (total data/total number of trials). Prior to ANOVA, data sets were checked for homogeneity of variance and normality. If the two-way ANOVA was significant, Student–Newman–Keuls's (SNK) test was used. In the case of significant interaction, separate one-way ANOVAs (factor Treatment) were conducted to assess the influence of treatment within individual levels of factor Days (dependant variable consisted of the means for each rat for the respective day). In the probe test, a one-way ANOVA with Dunnett's *post hoc* test was used to assess the significance of difference between midazolam- or diazepam-treated and solvent-treated rats in each experiment. To further investigate the influence of treatment in all three experiments, we used a two-way ANOVA (factors: Treatment and Experiment) with *post hoc* SNK's test. Statistical analyses were