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Effects of midazolam on acquisition and extinction of conditioned taste aversion memory in rats

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ABSTRACT

Some intravenous anesthetic agents such as midazolam are known to induce anterograde and retrograde amnesia. We analyzed the effect of midazolam by the conditioned taste aversion (CTA) acquisition and retention. After the rats were offered 0.1% sodium saccharin (Sac) as conditioned stimulus (CS), an intraperitoneal (i.p.) injection of several concentrations (5–30 mg/kg) of midazolam was followed by an i.p. injection of 0.15 M LiCl (2% of body weight) as unconditioned stimulus (US). The rats, which acquired CTA by every CS–US paradigm, strongly avoided Sac on the 1st test day after conditioning and maintained the avoidance for 3 days. We have already reported that Sac intake abruptly increased on the 2nd test day and the almost complete extinction occurred on the 3rd test day after conditioning by injection of subhypnotic dose of propofol before LiCl-injection. In contrast, we found that subhypnotic dose of midazolam suppressed not only CTA acquisition, but also CTA retention. On the other hand, an α2-adrenergic blocker, yohimbin (1 mg/kg) suppressed only the CTA retention. These results suggest that the subhypnotic doses of midazolam firstly affect the acquisition mechanism of the CTA memory (CTAM), resulting the suppression of the retention of CTAM.

Intravenous anesthetics such as midazolam, one of benzodiazepine anesthetic agents, which possess both γ-aminobutylic acid (GABA)-like [13,29], are known to induce anterograde and retrograde amnesia in human [16,17] and rodents [24,26]. In rodents, it has been reported that anterograde amnesia of an avoidance task was elicited by subhypnotic doses of the intravenous anesthetics, but retrograde amnesia was induced merely by hypnotic doses of these anesthetics [24,25,27].

Previous works using a conditioned taste aversion (CTA) paradigm, where CTA memory (CTAM) related to malaise is formed when an animal consumes a novel taste such as saccharin (conditioned stimulus, CS) and then experiences the symptoms of poisoning such as LiCl (unconditioned stimulus, US) [9], show that the strong CTA is established rapidly by novel taste stimuli in a single learning procedure [5,32]. The association between the CS and the US can proceed under deep anesthesia induced by pentobarbital or ketamine when hypnotic doses of these anesthetics are administrated after CS-presentation, or subhypnotic doses of them, which do not impair drinking, was applied before CS-presentation [6,33].

However, subhypnotic doses of the intravenous anesthetics are often used for disabled patients even in light operation such as extraction of teeth to sedate their hyperactivity [22,31]. We often encounter patients, who have lost the memory during operation after administration of sedative dose of these intravenous anesthetics while they preserved their consciousness during operation [22]. In the previous paper [15], we reported that subhypnotic but not hypnotic doses of propofol, one of non-benzodiazepine anesthetic agents, which has GABA-potentiating properties [12,23,25] in mammalian central neurons, accelerate only the extinction of the long-term memory formed by the CTA. Recently, Yasoshima and Yamamoto have reported that conditioned animals did not rejected the CS transiently by a systemic injection of midazolam (1.5 mg/kg) before retention, although midazolam injection before CS did not affect acquisition and retention of CTA memory [34]. Their results shows that benzodiazepine such as midazolam applied before CS increases palatability [34].

In the present study, we examined the effect of anesthetics applied between CS- and US-presentations on the long-term CTAM using several dosages of benzodiazepine agent, midazolam

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and compared the effects of midazolam with those of non-benzodiazepine, propofol. Furthermore, noradrenergic system has been reported to play an important role in the memory retention [7,18,20]. Therefore, we examined the effect of yohimbin, an α2 adrenoceptor antagonist on acquisition and retention of CTAM.

We purchased adult male Wistar rats, weighing 250–320 g at the beginning of experiment from an animal-supplying company. They were housed in individual wire mesh cages (285 mm × 450 mm × 210 mm) in a temperature (23 °C)-controlled room. They were allowed to ad lib access to food (Dry pellets, MF, Oriental Yeast, Osaka) and tap water except for training and testing periods as described below. All rats were placed on a 20 h water-deprivation schedule and were allowed a 4 h access to distilled water (DW) for 5 days and amounts of DW intake for the first 20 min were measured. Means of the amount for last 3 were employed as control values (SDW) of preconditioning DW intake. The percent of saccharine consumption was calculated from the ratio of Sac intake on each of test day 1, day 2 and day 3 (D1, D2 and D3).

On day 6, the animals were offered 0.1% sodium saccharin (Sac) as a CS for 20 min, and after 30 min interval, rats received an intraperitoneal (i.p.) injection of 0.15 M LiCl (2% of body weight) as an US. The animals were placed on the 20 h water-deprivation schedule again, and the amounts of Sac intake for 20 min were measured on the successive 3 days.

Nine groups of rats were trained according to the procedure described above. Each group of rats received i.p. injection of saline (0.9% NaCl solution, Otsuka, Japan), several doses of anesthetics, midazolam (Dormicum, Roche, Basel) or yohimbin hydrochloride (Sigma, St. Louis, MO), after CS-exposure. The ten groups are as follows: Group 1: CS + US (Li(+)), Group 2: CS + saline + no US (Li(−)), Group 3: CS + saline + US (Li(+)), Group 4: CS + 5 mg/kg midazolam + Li(−), Group 5–9: CS + (0.5, 1, 5, 20 or 30 mg/kg midazolam) + US (Li(+)) and Group 10: CS + 1 mg/kg yohimbin + US (Li(+)).

We calculated the following two categories of indices as the parameters for acquisition and retention of the long-term CTAM from volumes of pre- and postconditioning intake of DW and Sac [33]: acquisition index (AI) = 1 − D1/DW; retention index (RI) = 1 − (D2 + D3)/2DW, where D1, D2 and D3 are total Sac intakes on the 1st, 2nd and 3rd test days, and DW is preconditioning mean DW intake. Results are expressed as mean ± S.E. The amount of Sac consumed on D1 and D2 was analyzed by a one-way analysis of variance (ANOVA). Sac consumption across the days of preconditioning (DW) and extinction (D1–D3) was analyzed with a two-way (group × day) ANOVA with repeated measures on days in each experiment. Post hoc analysis was performed using Bonferroni test. Comparison of two independent groups was analyzed using the two-tailed unpaired *t*-test. The significance level of all statistical analysis was set at $p < 0.05$. All data analyses were conducted using StatView software (SAS Instruments Inc.).

When Sac as a CS was paired with LiCl as a US, animals examined in this study readily acquired CTA as judged by comparing the amount of Sac intake on the 1st and 2nd test days with that of the preconditioning DW intake as shown in our previous paper [15]. However, the amount of Sac intakes gradually increased toward the 3rd test day (D3: 5.9 ± 1.5 ml) as shown in previously reports [3,5,6,10,15,33]. When CS was paired with saline instead of LiCl, CTA was never induced, and rather significant increase of Sac intake was observed on the 2nd to 3rd test day rather than decreases shown in our previous paper [15].

When rats received i.p. injection of a hypnotic dose of midazolam (30 mg/kg) or the same amount of saline, the properties of acquisition and extinction in CTAM unchanged (DW: 14.8 ± 0.7 ml, D1: 0.8 ± 0.4 ml ($p < 0.0001$), D2: 2.4 ± 0.5 ml ($p < 0.001$) and D3: 6.0 ± 1.4 ml ($p < 0.05$), $n = 5$, for 30 mg/kg midazolam injection (Fig. 1A); DW: 13.2 ± 1.1 ml, D1: 1.8 ± 0.2 ml ($p < 0.001$), D2: 4.2 ± 1.0 ml ($p < 0.01$), D3: 8.4 ± 0.9 ml ($p < 0.05$), $n = 5$, for saline injection. These results are consistent with those of previous reports [6,32].

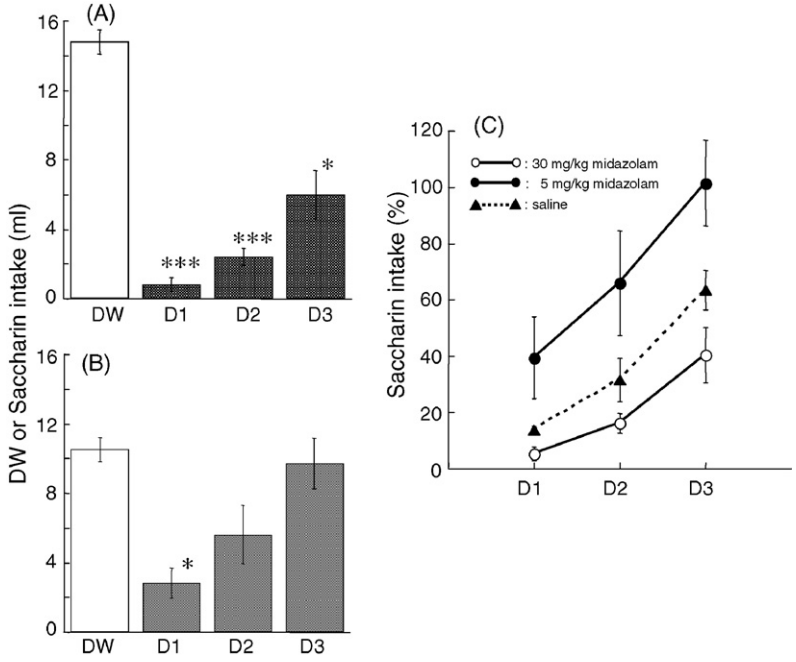

Fig. 1. Dose-dependent effect of midazolam on acquisition and retention of CTAM. (A) Hypnotic dose of midazolam (30 mg/kg). (B) Subhypnotic dose of midazolam (5 mg/kg). Midazolam was administrated between CS- and US-exposures. Open column shows preconditioning deionized water (DW) intake and hatched columns show saccharin intake on the 1st, 2nd and 3rd test days (D1, D2 and D3, respectively) in this and other figures. (C) Ratio of saccharin intake after CTA acquisition with administrations of hypnotic and subhypnotic doses of midazolam. Ratio is expressed as percentages of preconditioning DW intake on D1, D2 and D3. The data are represented as mean ± S.E.M. in this and other figures. $^{*}p < 0.05$; $^{***}p < 0.001$.