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Research article

Midazolam impairs the retrieval of conditioned taste aversion via opioidergic transmission in mice

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HIGHLIGHTS

- Retrieval mechanisms of conditioned taste aversion (CTA) in mice were explored.
- Effects of systemic midazolam (MDZ) on CTA retrieval were tested.
- Systemic MDZ increased conditioned stimulus (CS) intake in conditioned mice.
- Systemic MDZ disrupted aversive orofacial reactions to the CS in conditioned mice.
- An opioid antagonist, naloxone, precluded midazolam's effect on CTA retrieval.

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ABSTRACT

Midazolam is a benzodiazepine agonist that affects the acquisition, retention, and retrieval of malaise-induced conditioned taste aversion (CTA) in rats. Our previous study suggested that the palatability-enhancing rather than amnesic effects of midazolam were responsible for impaired retrieval of conditioned aversion to palatable conditioned stimuli (CSs). However, it remains unclear whether this effect is opioid-dependent. In the present study, we examined the involvement of opioid signaling with the ability of peripheral midazolam administration to transiently impair CTA retrieval in mice. CTA was established by pairing 5 mM saccharin ingestion (conditioned stimulus, CS) with an intraperitoneal (i.p.) injection of 0.15 M lithium chloride (LiCl, 2% body weight) (unconditioned stimulus) for two consecutive days. Conditioned mice that received midazolam (1.5 mg/kg, i.p.) before the first retention test consumed significantly more saccharin (CS) than conditioned mice that received vehicle (phosphate-buffered physiological saline, PBS; i.p.). On the next day, both conditioned groups showed strong aversions to the CS. Next, naloxone, an opioid receptor antagonist, was peripherally administered prior to the midazolam injection before the retention test. Pre-administration of naloxone but not PBS attenuated midazolam-induced increases in CS intake. Finally, we examined aversive orofacial taste reactions (TRs) to an oral infusion of the CS with pre-administration of naloxone or PBS prior to midazolam using a taste reactivity test. Conditioned mice that received midazolam showed significantly longer latencies to express aversive orofacial TRs than those that received PBS. Pre-administration of naloxone eliminated the effect of midazolam on latency to express aversive TRs. Taken together, these data suggest that midazolam activates opioidergic transmission and opioid-dependent palatability enhancement of the CS to eliminate conditioned aversion to a sweet taste.

1. Introduction

Conditioned taste aversion (CTA) is a robust aversive learning paradigm in which animals are trained to avoid consumption of a novel taste substance (conditioned stimulus, CS) after pairing the taste CS with visceral malaise (unconditioned stimulus, US) [1,2]. When conditioned animals are exposed to the CS after the CS-US pairing, they show conditioned avoidance behaviors (i.e.,

Abbreviations: ANOVA, analysis of variance; CS, conditioned stimulus; CTA, conditioned taste aversion; MDZ, midazolam; NAL, naloxone; PBN, parabrachial nucleus; PBS, phosphate buffered saline; TR, taste reaction; US, unconditioned stimulus.

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they reject the CS) despite thirst or hunger. In addition, when conditioned animals receive an intraoral infusion of the CS after CTA acquisition, they show conditioned disgust behaviors such as aversive orofacial taste reactions (TRs) [3–5]. Malaise-induced CTA retrieval is associated with taste avoidance and disgust reactions due to conditioned fear and a shift in taste palatability, respectively [4–6].

Midazolam (MDZ) is a benzodiazepine agonist that has anxiolytic and sedative effects [7–10]. In addition, MDZ is known to have effects on memory, inducing anterograde and retrograde amnesia in both humans [10] and animals [11]. Although the amnesic effects of MDZ on CTA acquisition and retention have been previously reported [12], few studies have examined the effects of MDZ on CTA retrieval except for a previous study by our group [13]. MDZ is known to increase the intake of palatable but not unpalatable tastants [14–16]. Previously, we showed that peripheral administration of MDZ impaired the retrieval of CTA to a sweet CS, but not to a salty or sour CS [13]. This finding suggested that the ability of MDZ to enhance the palatability of a sweet tastant impaired the process of CTA retrieval. However, the exact neural mechanisms underlying the ability of MDZ to impair CTA retrieval or shift the hedonic evaluation of a CS are unknown.

Opioid receptor agonists also increase the intake of palatable foods [17–19]. In contrast, naloxone, an opioid receptor antagonist, reduces sweet taste-driven food intake [20]. Some reports suggest that benzodiazepine-induced enhancements in the intake and palatability of palatable foods are opioid-dependent [21–23]. On this premise, we hypothesized that MDZ activates the opioidergic system to enhance the taste palatability of a sweet CS, resulting in the transient elimination of a conditioned aversion to the CS after CTA acquisition. Our preliminary study published in the Japan literature [24] showed that pre-administration of naloxone prevented MDZ-induced impairment of CTA retrieval for sucrose as a CS. However, to clarify whether MDZ-induced impairment of CTA retrieval is mediated by opioidergic activation and subsequent enhanced palatability of a CS, the following questions were evaluated. First, we examined whether MDZ impaired CTA retrieval to a non-caloric sweet tastant (saccharin) CS in mice. Saccharin was used to eliminate possible effects of MDZ on postingestive consequences. Second, we examined effects of naloxone pre-administration on MDZ-induced CTA retrieval impairment in the one-bottle test using different doses of naloxone based on our previous study [24]. Third, we examined effects of MDZ administration on affective hedonic evaluation of a CS in conditioned mice using an orofacial taste reactivity test. Finally, we examined whether naloxone pre-administration would prevent MDZ-induced shifts in hedonic reactions to a CS in conditioned mice.

2. Materials and methods

2.1. Animals

Eight-week-old C57/BL6J (B6) male mice (93 animals in total) were purchased from CREA Japan and housed individually in a controlled-temperature environment (22 ± 3 °C) on a 12 h light/dark cycle (light on at 07:00). Mice were had *ad libitum* access to normal chow (normal pellet, MF; Oriental Yeast, Japan) and water except for in the pre-training, conditioning, and testing phases as noted below. All experimental procedures were approved by the animal experiment committee of the Graduate School of Human Sciences at Osaka University. All animals were handled in accordance with the guidelines of the *Guide for the Care and Use of Laboratory Animals* (National Institute of Health, 1996) and the *Guiding Principles for the Care and Use of Animals in the Fields of Physiological Sciences* (The Physiological Society of Japan, 2003).

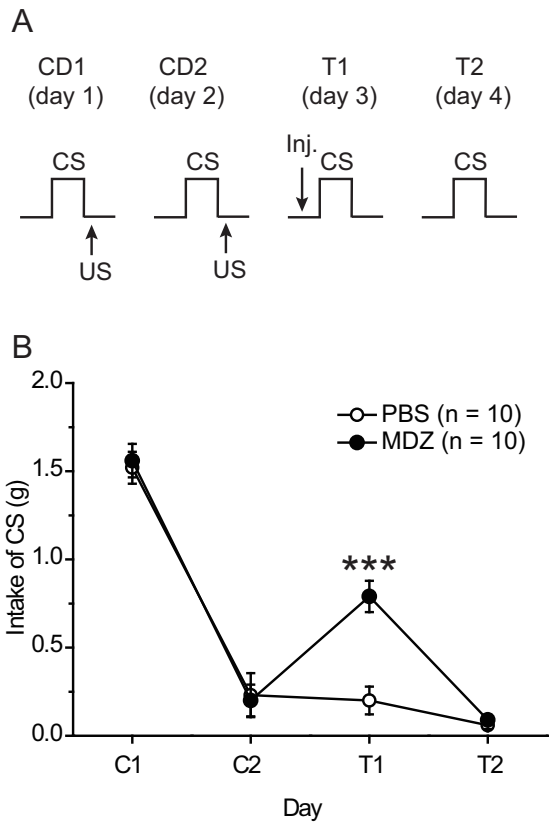

Fig. 1. Transient impairment of retrieval of conditioned taste aversion (CTA) by systemic midazolam administration in the one-bottle test. (A) Schematic illustration of the CTA conditioning and testing procedure among groups receiving midazolam (MDZ; 1.5 mg/kg, i.p.; n = 10) or vehicle (phosphate-buffered physiological saline, PBS; n = 10). (B) Intake of 5 mM saccharin (conditioned stimulus, CS) during two conditioning (C1, C2) and testing (T1, T2) days. Inj., injection; US, unconditioned stimulus. ***: $p < 0.001$, CS intake between the MDZ-injected and PBS-injected groups on T1 (Tukey's test).

2.2. Experimental design

2.2.1. CTA conditioning and testing with the bottle method

All mice were water-deprived for 20 h from 17:00 to 13:00 the next day with *ad libitum* access to food except during water or saccharin access as noted below. During the pre-training phase, mice were trained to ingest distilled water without access to food for 10 min starting at 13:00 each day. All mice were allowed to access to water for one additional hour from 16:00 to 17:00 to avoid dehydration through the pre-training, conditioning, and testing phases. After the water intake during the 10-min period became stable under the water-deprivation schedule (5–7 days), mice received 5 mM saccharin as a CS instead of distilled water for 10 min followed by an intraperitoneal (i.p.) injection of 0.15 M lithium chloride (LiCl, 2% of body weight (BW)) for conditioning on day 1 (C1). The same conditioning procedure was conducted on the next day (day 2) (C2). On day 3 (retention test 1, T1), mice in a bottle-testing subgroup (n = 20) received access to the same saccharin CS to test CTA retrieval; 20 min beforehand, half (n = 10) of the conditioned mice received physiological phosphate-buffered saline (PBS) as a vehicle control and the other half (n = 10) received peripheral administration of MDZ (1.5 mg/kg [13]; Astellas Pharma Inc. Tokyo, Japan). Both groups were subjected to the same CTA test (T2) the next day without any injection. CS intake was measured on the conditioning and testing days. Fig. 1A illustrates the behavioral procedure of conditioning and testing with MDZ or PBS administration.