Effects of ∆9-Tetrahydrocannabinol and Diazepam on Feeding Behavior in Mice

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ABSTRACT. The present study examined effects of diazepam (DZP) alone or in combination with ∆9-tetrahydrocannabinol (THC) on feeding behavior as well as body weight in male ddY strain mice at 5 weeks of age. Because we saw no hyperphagic effect of DZP with or without THC in mice, we explored the hyperphagia elicitable by DZP. THC [2 (THC2) or 4 (THC4) mg/kg/day s.c.] was given daily for 7 days. For the last day the mice were starved and injected i.p. with DZP (2 mg/kg) 10 min prior to a food or maze test. Controls received vehicle injections. Feeding behavior was measured after giving food for 2 hr. THC4 significantly reduced body weight gain. DZP, with or without THC, induced hyperphagia. THC4 alone also induced hyperphagia that was not significantly affected by DZP. Time taken to find food was extended by DZP and further with THC. Both DZP and THC can therefore interact on food ingestion but synergize on food seeking in mice through different mechanisms.—KEY WORDS: diazepam, feeding behavior, hyperphagia, mouse, tetrahydrocannabinol.


Tetrahydrocannabinol (THC) is thought to interact with the GABAergic system [10, 11]. Because diazepam (DZP) induces hyperphagia via a GABAergic action [16], THC and diazepam might interact with each other to elicit feeding behavior. The effects of THC on feeding behavior are controversial and depend on the doses used, time of injection, and whether or not the animal is food-deprived, as well as on the method of data collection. Because THC can modify levels of receptor mRNA and binding to the receptor in a time-dependent manner [18, 19], acute and chronic exposure to THC might have different effects on feeding behavior. THC has been reported to facilitate feeding, achieving a maximum stimulatory effect on the rate of food consumption within the first 3-hr after administration [5].

The present experiments examined the possibility that THC-treatment modifies the feeding behavior elicited by the GABA agonist, DZP, which is known to disrupt working memory [2, 3, 8, 13, 15] and to facilitate feeding behavior [4].

MATERIALS AND METHODS

Male ddY strain mice (5 weeks of age) were used. The mice were maintained individually in a plastic cage in a room with a 12 hr light/dark cycle with lights on from 06:00 to 18:00 hr. In Experiment 1, mice were injected i.p. with DZP (2 mg/kg/day, n=20) or 0.9% saline solution (n=10) for 5 weeks at 17:00 hr. In the 3rd week of treatment, half of the DZP-treated group were injected daily with THC (2 mg/kg s.c.) for 1 week. Food consumed was monitored daily and body weight gain was measured weekly.

In Experiment 2, mice were injected daily with THC s.c. (2 mg/kg: THC2, n=40, or 4 mg/kg: THC4, n=40) or 0.9% saline (n=40) for 7 days at 06:00 hr. On the 6th day of treatment, the mice were food-deprived from 09:00 to 09:00 on the 7th day. At 08:50 on the last day, DZP (2 mg/kg) or 0.9% saline solution was injected i.p. into half of the mice in both groups. Half of the mice administered DZP and half of the mice administered saline were given food at 09:00. Their food and water consumption were measured every 30 min for 2 hr. In the other half of each of the two groups the time to seek for food in a maze (time to banquet room) was measured.

The statistical analysis was by analysis of variance (ANOVA). Differences were considered statistically significant at p values of less than 0.05. The data were then analyzed further by two-way Student’s t-tests.

RESULTS

Chronic DZP treatment, alone or in combination with THC, had no significant effect on body weight gain or daily food intake (Figs. 1 and 2). DZP alone had no effect on the time to banquet room, but increased it in combination with THC (Fig. 3). THC4, but not THC2, reduced body weight gain (Fig. 4). The effect of THC2 on percentile gain of body weight was significant after 24-hr food deprivation (Fig. 5). Controls gained approximately 10% of their body weight during the 6 days prior to food deprivation. This gain was reduced by THC in a dose-dependent manner. After 24-hr food deprivation, the control group lost the 10% gained over the 6 days, but the THC group lost more weight in a dose-dependent manner.

THC2, but not THC4, significantly reduced water ingestion (Fig. 6). The reduced water consumption caused by THC2 was significantly increased with simultaneous DZP. This facilitatory effect of DZP disappeared in combination with THC4. DZP had a significant hyperphagic effect on total food intake for 2 hr after 24 hr starvation, but
 Assessing the total food intake after 24 hr starvation, the hyperphagia caused by DZP was not reduced in combination with THC. In assessing food intake for the 30-min intervals (Fig. 8), DZP attained the hyperphagic effect throughout the entire 2 hr period. This effect was not reduced in combination with THC2. THC4 alone had a hyperphagic effect on the food consumption rate for the 2nd 30-min interval. In this period the facilitative effect of DZP was attenuated.

Effects of both agents on the time to banquet room are summarized in Fig. 9. DZP alone significantly increased the time. This effect was more pronounced in combination with THC, thus implying that both agents are synergistic on seeking food.

**DISCUSSION**

The suppressive effect of THC on body weight gain is consistent with the observations of Drewnowski and Grinker...
Our results did not, however, support the inhibitory effect of THC on daily water intake observed by them. This contradiction might be due to differences in the time of injection and in the period of measurement of water intake. They administered injections at night and measured water intake for a whole day, but in the present study injections were done in the morning, and water intake was only measured for 2 hr.

DZP-induced hyperphagia was sustained for 2 hr, and measured at 30-min intervals. THC alone at a larger dose also had hyperphagic effect in a restricted interval in which the hyperphagic effect of DZP was attenuated. This probably means that the effect of DZP did not continue long after accomplishing the effect of THC. DZP might, therefore, modify food intake via a similar mechanism but not synergistically with THC.

It has been suggested that DZP acts at the benzodiazepine receptors in the ventromedial hypothalamus (VMH) to modify GABA levels, leading to a reduction in the satiety response, and thereby eliciting hyperphagia [1]. If THC...
also acts at the benzodiazepine receptor, it might induce a reciprocal hunger-satiety regulation to activate the satiety function, thus inhibiting the effect of DZP [1]. It has been reported that THC receptors coexist with dopamine receptors [6, 7, 9, 12]. An increase in the dopamine concentration in the VMH might increase appetite or motivation for feeding [1, 21], and a decrease in the dopamine concentration in the VMH might lead to an increase in the dopamine concentration in the lateral hypothalamus (LHA) and terminate feeding [14]. THC might activate this system in the LHA, thus reducing the hyperphagic effects of DZP. Another possible system underlying the THC-DZP interaction is the dopamine pathway arising in substantia nigra that can modify LHA function to elicit feeding [20]. GABA might interact with the dopamine system to reduce LHA function and suppress feeding [16]. In this regard, THC in the present study may facilitate food intake by regulating the reciprocal hunger-satiety balance and the THC-DZP interaction might be revealed in the GABA-dopamine system. On the other hand, both agents were synergistic in seeking food.

In view of the above mentioned information, it is likely that both DZP and THC can affect feeding behavior through different mechanisms in mice.

REFERENCES


