Reevaluation of anti-obesity action of mazindol and elucidation of its effect on the reward system

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**HIGHLIGHTS**
- Mazindol reduces body weight gain and hyperphagia induced by high-fat diet.
- Mazindol decreases preference for lipid emulsion.
- Mazindol decreases conditioned place preference for high-fat diet.
- Mazindol at the dose required for these effects does not elicit abuse potential.

**ABSTRACT**
In this study, we evaluated the preventive effect of mazindol on the development of obesity and sought to elucidate the drug's effects on the reward system. In mice, body weight gain and hyperphagia induced by high-fat diet (HFD) were decreased by 38.6% and 13.9%, respectively, by subcutaneous infusion of mazindol (1.5 mg/kg/day) for 28 days. A single intraperitoneal administration of mazindol (1.5 mg/kg) significantly reduced lipid preference, as assessed using the two-bottle preference paradigm (vehicle, 89.98 ± 1.66%; mazindol, 75.65 ± 5.47%; p < 0.05). In addition, the conditioned place preference (CPP) test demonstrated that mazindol (1.5 mg/kg) significantly decreased CPP score for HFD as compared with vehicle (vehicle, 330.44 ± 58.61 s; mazindol, 144.72 ± 43.02 s; p < 0.05). Moreover, at the dose required for these effects, mazindol did not elicit abuse potential or induce psychostimulant-like behavior. These results confirm that mazindol prevents diet-induced obesity without addictive behavior and demonstrate that its action is mediated at least in part via the reward system, advancing our understanding of mazindol in clinical practice.

**1. Introduction**
The World Health Organization has recognized that the number of obese people has more than doubled since 1980 worldwide, although frequency and severity differ among countries and regions [1,8]. Obesity is associated with elevated risk not only for cardiovascular diseases and type 2 diabetes, but also for non-alcoholic steatohepatitis, sleep apnea syndrome, and certain cancers [4,10,17]. Given that some of these diseases are the main causes of mortality in developed countries, primary prevention of obesity is crucial [5,24].

Mazindol causes weight loss and anorexia, and is currently marketed as an anorectic agent for the treatment of obesity in certain countries, including Japan. Despite the fact that the drug was brought to market more than 40 years ago, very little recent evidence is available regarding its effects [7,13,28,30].

This study was designed to evaluate the effect of mazindol on the development of obesity in a mouse model and to determine whether it has an effect on the reward system.
2. Materials and methods

2.1. Animals

Male C57BL6/J mice were purchased from Japan SLC (Shizuoka, Japan). All experiments were performed with 14–17-week-old mice. Mice were housed under a 12/12 h light/dark cycle at a constant room temperature of 24 ± 1 °C with free access to water and a normal chow diet (NCD; Oriental BioService, Kyoto, Japan) unless otherwise indicated. All experimental procedures were conducted in accordance with the guidelines for animal experiments of Kyoto University and were approved by the Animal Research Committee of Kyoto University.

2.2. Materials

Mazindol was supplied by FUJIFILM Pharma Co., Ltd. (Tokyo, Japan). Mazindol was dissolved in hydrochloric acid, pH-adjusted to 6.0, and injected intraperitoneally in a volume of 10 ml/kg or infused subcutaneously. Cocaine hydrochloride was purchased from Takeda Pharmaceutical Co., Ltd. (Osaka, Japan). Cocaine was dissolved in saline and injected intraperitoneally in a volume of 10 ml/kg. 20% lipid emulsion was purchased from Otsuka Pharmaceutical Co., Ltd. (Tokyo, Japan). Lipid emulsion was diluted in water and given orally.

2.3. Mazindol infusion experiments

Mice were housed individually with continuous access to NCD and water. A mini-osmotic pump (Alzet model 2004; Alza, Palo Alto, CA) was implanted subcutaneously in the mid-scarpal region of each mouse at 14–16 weeks of age. After implantation, mice were administered a high-fat diet (HFD) (protein 20 kcal%; carbohydrate 20 kcal%; fat 60 kcal%; Research Diets, Inc., New Brunswick, NJ; No. D12492). The pump delivered vehicle or mazindol subcutaneously at a dose of 1.5 mg/kg/day for 28 days. Body weight and food intake were measured at 1500 ± 0100 h. Mice were sacrificed to weigh epididymal fat tissue 28 days after implantation.

2.4. Two-bottle preference test

For the two-bottle preference test, mice were housed individually and acclimated to cages with two bottles of water for 4 days. At 1730 h on the test day, mice received an intraperitoneal injection of vehicle or mazindol at a dose of 1.5 mg/kg. Starting at 1800 h, mice were then given access to bottles with water and lipid emulsion with free access to NCD. Water and lipid emulsion intake were measured for 15 h. To exclude position effects, the same procedure was conducted the next day after exchanging the positions of the bottles. Lipid preference was calculated as lipid emulsion intake divided by total fluid intake for 2 consecutive days and expressed as percentage values, with 50% representing indifference. To avoid saturation of taste receptors by high doses of lipid, lipid emulsion concentrations were at the low end of the dynamic range. The concentration of lipid emulsion increased every 2 days (0.078%, 0.156%, and 0.312%).

2.5. Conditioned place preference (CPP) test

CPP for HFD was carried out in a three-chamber apparatus with a small middle chamber that connected two large side chambers (MED Associates, St. Albans, VT). The two large chambers were made distinguishable by varying the wall color (white vs black) and floor texture (mesh vs bar). On day 0, mice were allowed to move freely among the three chambers for 20 min. On days 1–3, mice were intraperitoneally injected with vehicle or mazindol at a dose of 1.5 mg/kg. Thirty minutes after the injection, the mice were confined to one side chamber containing a control diet (protein 20 kcal%; carbohydrate 70 kcal%; fat 10 kcal%; Research Diets, Inc.; No. D12450K) for 30 min. Five hours later, they were confined to the other side chamber containing HFD for 30 min. The conditioning order was reversed for half of the mice. Vehicle- and mazindol-treated mice consumed comparable amounts of HFD during the 30-min conditioning period (vehicle-treated: 0.038 ± 0.004 g; mazindol-treated: 0.036 ± 0.009 g; not significant). On day 4, mice were placed in the middle chamber and allowed to move freely among the three chambers for 20 min in the absence of either diet. CPP score was calculated based on the difference in time spent in the paired chambers, as follows: (the time spent in HFD-paired chamber – the time spent in control-paired chamber).

To evaluate CPP for drug, the procedures on day 0 and day 4 were as described for CPP for HFD. On days 1–3, mice were confined to one side chamber for 30 min just after receiving intraperitoneal injection of vehicle, mazindol (1.5 mg/kg), or cocaine (10 mg/kg). Five hours later, they were confined to the other side chamber for 30 min just after receiving intraperitoneal injection of vehicle. The conditioning order was reversed for half of the mice. CPP score was evaluated as follows: (the time spent in drug-paired chamber – the time spent in vehicle-paired chamber).

2.6. Drug-induced locomotor response

Horizontal locomotor activity was measured using a comprehensive lab animal metabolic monitoring system (CLAMS; Columbus Instruments, Columbus, OH), which monitors animal activity via a grid of infrared light beams that traverse the animal cage. All mice were habituated to the monitoring apparatus for 3 consecutive days before the experiment. At 1300 h on the day of the experiment, mice received a single intraperitoneal injection of saline (10 mL/kg), mazindol (1.5 mg/kg), or cocaine (20 mg/kg). Locomotor activity was recorded for 180 min after each injection. The locomotor response to each drug was determined by calculating area under the curve (AUC) during the observation period.

2.7. Statistical analysis

Data are expressed as means ± SEM. Statistical analyses of body weight, weight gain, lipid preference, and CPP for HFD were performed using two-way repeated measures analysis of variance (ANOVA). Greenhouse–Geisser-corrected p values were used whenever Mauchly’s test of sphericity was significant. Data for food intake was analyzed by one-way ANOVA after the homogeneity of variance assumption was checked by Bartlett’s test. Subsequent post hoc comparisons of groups were conducted with Tukey’s test. Data for fat weight and AUC for locomotor activity were analyzed with the Kruskal–Wallis test. For CPP for drug, data were analyzed with paired t-test for each drug. p < 0.05 was considered statistically significant.

3. Results

3.1. Mazindol infusion experiments

In response to HFD feeding, vehicle-treated mice exhibited a significant increase in body weight relative to vehicle-treated mice on NCD. By contrast, mazindol-treated mice gained less body weight than vehicle-treated mice (Fig. 1A). On day 28, body weight gain decreased by 38.6% in the mazindol-treated group (Fig. 1A, HFD-vehicle, 8.79 ± 0.89 g; HFD-mazindol, 5.40 ± 0.76 g; p < 0.01). Epididymal fat pad weight
Fig. 1. Effects of subcutaneous infusion of mazindol for 28 days at 1.5 mg/kg/day by mini osmotic pump on body weight, adiposity, and food intake in mice fed a HFD. 
(A) Body weight and weight gain during treatment (n = 10 in each group).
(B) Epididymal fat weight on day 28 of the treatment (n = 5-6 in each group).
(C) Energy intake during 24 h on day 21 of the treatment (n = 10 in each group).
*p < 0.05 vs HFD-vehicle, **p < 0.01, #p < 0.05.

Fig. 2. Effect of single injection of mazindol at 1.5 mg/kg on preference for lipid emulsion at the indicated concentrations (0.078%, 0.156%, 0.312%), assessed by two-bottle assays (n = 11 for vehicle and n = 13 for mazindol). *p < 0.05.

was significantly lower in mazindol-treated mice than in vehicle-treated mice (Fig. 1B, HFD-vehicle, 1.19 ± 0.07 g; HFD-mazindol, 0.73 ± 0.09 g; p < 0.01), and energy intake was significantly reduced (Fig. 1C, HFD-vehicle, 16.06 ± 0.73 kcal/day; HFD-mazindol, 13.83 ± 0.47 kcal/day; p < 0.05).

3.2. Two-bottle preference test

At concentrations of 0.078% and 0.156%, lipid emulsion and water were consumed at almost equal volumes by both vehicle- and mazindol-treated mice, whereas lipid emulsion preference increased dramatically at a concentration of 0.312% in both groups (Fig. 2). However, mazindol-treated mice had a significantly lower preference for lipid emulsion than vehicle-treated mice (vehicle, 89.98 ± 1.66%; mazindol, 75.65 ± 5.47%; p < 0.05).

3.3. CPP test for HFD

CPP score for HFD was higher after the 3-day conditioning period than before conditioning in both vehicle- and mazindol-treated mice. Notably, the increase in CPP score for HFD was significantly suppressed in mazindol-treated mice (Fig. 3, vehicle: post-conditioning, 330.44 ± 58.61 s; mazindol: post-conditioning, 144.72 ± 43.02 s; p < 0.05). Administration of mazindol throughout the conditioning period reduced CPP for HFD.

3.4. Evaluation of addictive aspects of mazindol

CPP for mazindol was evaluated to examine the drug’s abuse potential or rewarding properties. CPP score did not significantly change after conditioning in mice conditioned with mazindol, as in mice conditioned with vehicle (Fig. 4A, vehicle: pre-
4. Discussion

This study confirms that mazindol prevents diet-induced obesity, and demonstrates that its anorectic effect is mediated at least in part via hedonic regulation of feeding in the reward system.

The purpose of this study was to evaluate the effects of mazindol on body weight and food intake. Our results show that infusion of mazindol for 28 days suppresses HFD-induced body weight gain and hyperphagia by 38.6% and 13.9%, respectively, relative to vehicle.

Our results also reveal that mazindol has an effect on hedonic regulation of feeding. Because feeding behavior is primarily regulated in the hypothalamus by homeostatic information about the current metabolic state, mazindol has been proposed to exert its effects on body weight and food intake via the hypothalamus [14,19]. Apart from hormonal input into this feeding circuitry, hedonic regulation that controls the desire to consume highly palatable foods can also drive feeding behavior, thereby promoting weight gain and obesity [16,21]. In this study, we used behavioral analysis to test the hypothesis that mazindol has an effect on hedonic regulation of feeding. The results clearly show that mazindol inhibits preference for lipid emulsion, indicating that it has effects on the reward system involved in feeding behavior. The test of CPP for HFD confirmed this effect on the reward system.

In this study, the effects of mazindol on hedonic regulation were observed only 2 or 3 days after a single injection of mazindol, implying that these effects are likely to be mediated by instantaneous action, such as modulation of neurotransmitters in the synaptic cleft or electric activities of involved neurons. These findings are consistent with previous studies demonstrating that mazindol is a centrally acting reuptake inhibitor for the monoamines, such as noradrenaline, dopamine, and to a lesser extent serotonin, and increases the amount of each neurotransmitter in the synaptic cleft [6,11,26]. Both noradrenaline and dopamine play roles in the acquisition of natural rewards, such as food, water, and sex [3,20,25,29]. Mazindol has been reported to bind not only to the hypothalamus, but also to noradrenaline uptake sites in the cerebral cortex and lateral neuronal dopamine uptake sites in the corpus striatum [2,15]. Therefore, it has been speculated that mazindol suppresses hedonic regulation of feeding via the interaction between the hypothalamus and the brain reward regions by modulating the synaptic level of noradrenaline and/or dopamine in these brain areas. Because this study could not determine more specific brain areas where this effect might occur, further studies should be performed in which mazindol is directly administered into candidate brain regions.

The CPP test is widely used in drug abuse studies and can be applied to food preference [12,23,27]. In this study, we conducted CPP in mice that were not food-deprived in their home cage. Because homeostatic and hedonic systems interact and the CPP is enhanced by fasting or energy restriction [18], this approach enabled us to exactly examine the effect of mazindol on the reward system involved in feeding behavior. The test of CPP for HFD confirmed this effect on the reward system.

Although many appetite suppressants are variants of the amphetamine molecule, these drugs should not be associated with drug dependence, as was the case for amphetamine. Mazindol 1.5 mg/kg, the dose used in the present study, was found not to be addictive, as demonstrated by CPP test and drug-induced locomotor response. It is possible that mazindol does not elicit an addiction because it is quite different from amphetamine in its structure, which is chemically unrelated to the phenethylamines [7,30]. Thus mazindol can block monoamine reuptake, but has...
no effect on direct release of either noradrenaline or dopamine in the synaptic cleft [6]. Although the self-administration rate of mazindol increases in rhesus monkey when the drug is injected intravenously [31], there have been no reports of abuse potential of mazindol.

In summary, administration of mazindol has a substantial preventive effect on the development of obesity and significantly suppresses lipid preference in mice, indicating that the drug exerts its anorectic effect partly through regulation by controlling the preference for energy-dense food in the reward system. These findings provide new insight into the function of mazindol in clinical practice.

Author contribution

DA and CS designed the study; DA, YS, and HN performed the experiments; TH contributed to CPP analysis; CS, TK, TM, KH, and KN provided useful discussion regarding the interpretation of obtained results. All authors contributed to and approved the final version of the paper.

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Conflict of interest

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