Appetite 118 (2017) 120–128

Contents lists available at ScienceDirect

Appetite

journal homepage: www.elsevier.com/locate/appet

Intraperitoneal injection of D-serine inhibits high-fat diet intake and preference in male mice

Tsutomu Sasaki [a, *], Yasunobu Yasoshima [b], Sho Matsui [a], Hiromi Yokota-Hashimoto [a], Masaki Kobayashi [a], Tadahiro Kitamura [a]

[a] Laboratory for Metabolic Signaling, Institute for Molecular and Cellular Regulation, Gunma University, 3-39-15 Showa-machi, Maebashi, Gunma, 371-8512, Japan
[b] Division of Behavioral Physiology, Department of Behavioral Sciences, Graduate School of Human Sciences, Osaka University, 1-2 Yamadaoka, Suita, Osaka, 565-0871, Japan

ARTICLE INFO

Article history:
Received 19 June 2017
Received in revised form
8 August 2017
Accepted 9 August 2017
Available online 10 August 2017

Keywords:
Diet selection
Feeding behavior
Food preference
Intra-strain variability
NMDA receptor

ABSTRACT

D-serine is a co-agonist of the N-methyl D-aspartate (NMDA) receptor, an important modulator of glutamatergic excitatory synaptic transmission. We previously reported that oral D-serine ingestion inhibited the intake of highly preferred food and promoted the intake of less preferred food in mice. Here, we analyzed the effects of intraperitoneal (IP) D-serine injections on feeding behavior in mice. We assessed the effects of D-serine during both the acquisition and maintenance of a preference for high-fat diets (HFDs). Aversiveness of IP D-serine was analyzed in the conditioned taste aversion paradigm. The effects on food intake were assessed by providing liquid meals with different fat contents. Finally, we measured brain D-serine and L-serine levels after D-serine administration. We found that IP-injected D-serine effectively inhibited the acquisition of a HFD preference, but failed to prevent expression of a previously learned HFD preference. IP-injected D-serine was not sufficient to condition taste aversion. The effect on HFD preference acquisition was associated with increases in D-serine levels in the cerebral cortex, hypothalamus, and cerebellum. IP-injected D-serine most effectively inhibited the intake of liquid meals with high fat content. This effect was dose-dependent, but the responses varied significantly among male C57BL/6J mice. The differential responses to D-serine were consistent among multiple trials in each mouse. In summary, IP-injected D-serine inhibited HFD intake and the acquisition of an HFD preference. Individual mice with the same genetic background showed different sensitivities to D-serine; thus, D-serine sensitivity may be associated with unidentified traits.

1. Introduction

Organisms are made of what they ingest. Therefore, it would make sense from homeostatic point of view for organisms to preferentially ingest nutritionally well-balanced diet. However, in reality, both humans and experimental animals prefer diet rich in fat over diet with proper macronutrient balance. Diet rich in fat promotes weight gain by increasing caloric intake (Thaler et al., 2012) and altering feeding patterns (Kohsaka et al., 2007), and excessive consumption of high-fat diet is detrimental to health. To overcome the issue, better understandings on the molecular and neural mechanisms that controls diet selection are necessary.

D-serine is a co-agonist for the N-methyl-D-aspartate (NMDA) receptor; thus, it facilitates excitatory glutamatergic neurotransmission at synapses (Johnson & Ascher, 1987; Kleckner & Dingledine, 1988; Papouin et al., 2012). Co-agonism of NMDA receptors is the only known *in vivo* function of D-serine (Hashimoto & Oka, 1997), and its activity is necessary for the NMDA receptor to function as an ion channel (Cheriyan, Mezes, Zhou, Balsara, & Castellino, 2015). D-serine production and degradation are regulated *in vivo* by serine racemase (SR) (Kartvelishvily, Shleper, Balan, Dumin, & Wolosker, 2006; Wolosker et al., 1999; Yoshikawa et al., 2007) and D-amino acid oxidase (Weimer & Neims, 1977), respectively. D-serine is also supplied from dietary sources, such as fermented foods, microorganisms, plants, and marine invertebrates (Friedman, 1999). The cerebral cortex of SR-knockout mice had only 10% of the D-serine levels present in wild-type mice (Basu et al., 2009; Horio et al., 2011). This finding indicated that 90% of brain

* Corresponding author.
E-mail address: tsutomus@gunma-u.ac.jp (T. Sasaki).

http://dx.doi.org/10.1016/j.appet.2017.08.011

D-serine was maintained by endogenous production and 10% was supplied from the gastrointestinal tract.

We previously reported that, in mice, oral ingestion of D-serine inhibited the intake of highly preferred food (Sasaki et al., 2015). That effect depended on co-agonism of the NMDA receptor, but it was independent of any particular macronutrient, sensory input, or intact leptin receptor signaling. Oral D-serine ingestion resulted in a reverse preference (chow preferred over high-fat food), when given at the time that mice acquired a food preference, and it prevented expression of a previously learned food preference, when given at later times. In the absence of food choices (access provided to only one food type), oral D-serine ingestion inhibited food intake. However, it took approximately 2 days for oral D-serine ingestion to affect feeding behaviors; thus, we could not rule out any potential effects mediated by incretions and gut microbiota. In that study, the central nervous system was the implied main target of orally administered D-serine, because D-serine maintained effectiveness in mice treated with capsaicin to remove sensory afferent transmission. However, we lacked evidence to show that D-serine levels changed within the central nervous system with D-serine administration.

Here, we aimed to resolve these unaddressed issues by analyzing the effects of intraperitoneal injections of D-serine on feeding behavior in mice. In addition, we measured D-serine and L-serine concentrations in the brain. We analyzed the effects of D-serine on a high-fat diet (HFD) preference, during both the acquisition and the expressed phases. Effects on food intake were assessed by providing liquid meals with different fat contents. We also assessed the aversiveness of IP D-serine to condition taste aversion. Better time resolution of IP D-serine results over PO D-serine results unexpectedly revealed variability in responses even in age-matched isogenic B6 male mice. Therefore, we also assessed the consistency of the differential responses over multiple trials.

2. Materials and methods

2.1. Animals

All mice (male C57BL/6J) used in experiments were purchased from CLEA Japan (Tokyo, Japan). All experiments, except for the conditioned taste aversion (CTA) experiment, were performed with mice between eight to ten weeks of age and different cohorts of mice were used in each experiment. To reduce number of animals, male C57/BL6J mice between twelve to fourteen-weeks of age, which had been mice used in other studies (open-field and sucrose chow intake tests), were subjected to CTA experiment with multiple conditioning paradigm. For the duration of this study, all mice were housed in individual cages in a temperature-controlled facility on a 12-h light/dark cycle (6:00 a.m. - 6:00 p.m.) and had *ad libitum* access to food and water. Body weight (BW) was measured daily. Cages and water bottles were changed on a weekly basis. All animal care and experimental protocols were approved by the Institutional Animal Care and Use Committees at Gunma University and Osaka University.

2.2. Food

Normal chow (CE-2, here referred to as NC) and high-fat diet chow (HFD32, here referred to as HFD) were purchased from CLEA Japan. We decided to use these food because it was used in our previous study assessing the effect of oral D-serine ingestion (Sasaki et al., 2015), and the use of these food allowed us to compare the results between oral versus intraperitoneal administration of D-serine. We also used the following liquid meal to manipulate the fat content of the diet. SanET2.0 liquid meal was purchased from Sanwa Kagaku Kenkyusho (Nagoya, Japan). IntraLipos lipid emulsion was purchased from Otsuka Pharmaceutical Factory (Naruto, Japan). Based on the preliminary experiments testing various mixture ratio of SI liquid meals (data not shown), we decided to perform the experiments with the following liquid meals: one was prepared by mixing SanET2.0 and distilled water at a 1:1 ratio (SW), the other liquid meal was prepared by mixing SanET2.0 and IntraLipos at a 1:1 ratio (SI). Liquid meal intakes were measured with drink bottles (SN-950 H) purchased from Shinano (Tokyo, Japan). A separate water bottle was provided in each cage during the liquid meal studies. The calorie-based macronutrient composition of the food used in this study is shown in Table 1.

2.3. D-serine effect on the acquisition phase of HFD preference

To test the dose dependence of intraperitoneal D-serine injection on feeding behavior, we chose to test it on the acquisition phase of a HFD preference, during which orally ingested D-serine showed the strongest effect in our previous study. We used the Feeding, Drinking, and Activity Monitoring System for mice (FDAMS, Shinfactory, Fukuoka, Japan) to monitor food intake, food access, water intake, and locomotor activity every minute (Sasaki et al., 2015), and these data were analyzed in 1-h bins. C57Bl/6J male mice were acclimated to FDAMS, and received two food trays that both contained NC (NC vs. NC). After three-day acclimation, on the day of the experiment, mice were given an intraperitoneal injection of vehicle (water) or D-serine (1, 2, or 4 g/kg BW) at 5:45 p.m., just before the start of the dark cycle at 6:00 p.m., and subsequently, mice were given two foods to choose from: NC and HFD (two-food choice). Observation was continued for the next 24 h. We analyzed 6, 7, 8, and 7 mice for 0, 1, 2, and 4 g/kg BW D-serine doses, respectively.

2.4. Conditioned taste aversion experiment

To assess if D-serine injection conditioned avoidance of novel food (HFD), we performed the conditioned taste aversion experiment using saccharine solution as conditioned stimulus (CS) and lithium chloride injection as unconditioned stimulus (US). Male C57/BL6J (twelve to fourteen-week old at beginning of experiment) mice were placed on a 20-h water deprivation schedule (from 17:00 to 13:00 the next day) in which they received one-bottle access to distilled water for 10 min starting at 13:00 each day and additional 1-h water access from 16:00 to 17:00 to avoid dehydration throughout behavioral procedure. The animals received the one-bottle training for 5 days to stabilize the 10-min water intake. The subjects were then divided into two groups to match water intake. For conditioned aversion to saccharin, all mice were given 10-min access to 5 mM sodium saccharin (Sigma, UK) as a conditioned stimulus (CS) instead of distilled water for 10 min followed by an intraperitoneal (i.p.) injection of either 0.15 M lithium chloride (LiCl, 2% of BW, $n = 8$) or D-serine (2 g/kg BW, 0.1 ml/10 g BW, $n = 9$) for conditioning on day 1 (C1). Each group received the same conditioning procedure on additional two days (days 2 and 3) (C2 and C3, respectively). On day 4 (retention test 1, T1), all mice received access to the same saccharin solution to test conditioned aversion to the CS. Both groups received the same CTA test (T2) on

Table 1
Calorie-based macronutrient composition of the food used in this study.

	Protein	Fat	Carbohydrate	Calories
Normal chow (CE2)	28.9%	12.0%	59.1%	3.50 (kcal/g)
High-fat diet (HFD32)	20.1%	56.7%	23.2%	5.08 (kcal/g)
SW (SanET2.0/water)	16.0%	34.0%	50.0%	1.00 (kcal/ml)
SI (SanET2.0/IntraLipos)	8.0%	67.0%	25.0%	2.00 (kcal/ml)