Central Neuropeptide S inhibits food intake in mice through activation of Neuropeptide S receptor

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1. Introduction

Neuropeptide S (NPS), a 20-amino-acid peptide, was recently identified via reversal pharmacology techniques [29]. The peptide is called Neuropeptide S, because the N-terminal residue is serine (S) in all animal species, and the primary sequence of the peptide is highly conserved in different species [34]. NPS selectively binds and activates an orphan G-protein coupled receptor named NPS receptor (NPSR), which also known as GPR154 or GPRA [34]. In cells expressing the recombinant NPSR, NPS induces mobilization of intracellular calcium ions and stimulation of cAMP [34]. NPS/NPSR system has been shown to modulate a variety of physiological and pathological functions [13], including wakefulness [3,28,34], stress and anxiety [17,24,28,32,34], locomotion [6,12,24,28,30,34], memory [15,16,20,25,36], drug abuse [1,5,19,24], gastrointestinal functions [4,9,14,31], and nociception [18,26].

In rat, NPS and NPSR mRNA are expressed in both central nervous system and peripheral tissues [33,34]. In brain, high level of NPSR mRNA was found in hypothalamus which is the predominant brain center regulating energy homeostasis [33]. The distribution of NPSR mRNA indicated that NPS/NPSR system might play a role in regulating food intake. In fact, previous studies have shown that central NPS could inhibit food intake in rats and chicks, and the hypothalamus was involved [2,7,8,10,22,30]. However, the modulatory role and the underlying mechanisms of NPS on food intake in mice remain to be elucidated. Therefore, we investigated the role of central NPS on feeding behavior in fasted mice, and detected the underlying mechanism(s) by using NPSR antagonist [D-Val5]NPS and Corticotropin-Releasing Factor 1 (CRF1) Receptor antagonist NBI-27914. The present results indicated that intracerebroventricular injection of NPS (0.001–0.1 nmol) dose-dependently inhibited food intake in fasted mice. The anorectic effect of NPS reached the maximum at the dose of 0.1 nmol, which could be antagonized by co-injection of 10 nmol NPSR antagonist [D-Val5]NPS. Furthermore, CRF1 receptor antagonist NBI-27914 at the dose of 2 μg antagonized the hyperlocomotor action of NPS, but did not affect the role of NPS on food intake. In conclusion, our results demonstrated central NPS inhibited food intake in fasted mice, mediated by its cognate NPSR, but not by CRF1 receptor.
vivo [24]. Thus, we also examined whether the effect of NPS on food intake involved CRF1 receptor.

2. Materials and methods

2.1. Animals

Male Kunming strain mice (20–24 g) were obtained from the Experimental Animal Center of Lanzhou University. All animals were cared for and experiments were carried out in accordance with the European Community guidelines for the use of experimental animals (86/609/EEC). Animals were housed in an animal room that was maintained at 22 ± 2 °C with a 12-h light:12-h dark cycle. Food and water were available ad libitum before experiment. All the protocols in this study were approved by the Ethics Committee of Lanzhou University, China.

2.2. Implantation of cannula into lateral ventricle

Surgical implantation of cannula into lateral ventricle was conducted according to our previous report [19]. Mice (20–24 g) were anesthetized intraperitoneally (i.p.) with pentobarbital sodium (80 mg/kg), and placed in a stereotaxic apparatus. A vertical incision was made in the skin to expose the skull. A stainless steel guide cannula was inserted into the guide cannula. The dummy cannula protruded 0.5 mm from the guide cannula. After surgery, the animals were allowed to recover for at least 5 days, and during this period, mice were gently handled daily to minimize the stress associated with manipulation of the animals throughout the experiments. For intracerebroventricular (i.c.v.) injection, awake mice were gently restrained by hand and injected manually 2 μl drug(s) or vehicle at a rate of 2 μl/min through a 10 μl syringe. Each mouse was used only once.

After completion of testing, mice were injected i.c.v. with methylene blue dye (2 μl) which was allowed to diffuse for 10 min. Then mice were decapitated, and their brains were removed and frozen. Gross dissection of the brain was used to verify the placement of the cannula. Only the data from those animals with dispersion of the dye throughout the ventricles were used.

2.3. Food intake in fasted mice

Food was removed from each animal’s cage at 6:00 PM on the day before i.c.v. injection, and the animals of each group (n = 7–14) were food-deprived for 18 h. After i.c.v. injection of 2 μl vehicle or drug(s), preweighed standard chow pellets were reintroduced. Food intake was measured at 0.5, 1, 2, 4 and 24 h thereafter.

2.4. Locomotor activity

Locomotor activity of mice was measured using the Evalu- ated Plus Maze Tracking System (TME, Chengdu, China). Mice (n = 8 in each group) were placed individually in a plexiglas box (40 cm × 40 cm × 40 cm) for 10 min, and horizontal activity (distance traveled) was recorded. Then animals were injected i.c.v. with 2 μl vehicle or drug(s). Immediately after injection, horizontal activity was recorded for 30 min.

The locomotion index = distance traveled after i.c.v. injection/distance traveled before i.c.v. injection.

2.5. Drugs

Neuropeptide S (mouse) and [D-Val5]NPS were synthesized by manual solid-phase synthesis using standard Fmoc chemistry as described in our previous report [19]. Crude peptides were purified by reversed-phase HPLC using a Water Delta 600 system with an Xbridge BEH130 C18 column (19 mm × 250 mm). NBI-27914 was purchased from Sigma. NPS and [D-Val5]NPS were dissolved in saline (pH 7.4) and were stored frozen in aliquots. NBI-27914 was dissolved in cremaphor/DMSO at 1:1 to a concentration of 100 μg/μl, this solution was kept at 4 °C and was diluted in saline immediately before i.c.v. injection. NBI-27914 (1 μg/μl containing 0.5% cremaphor and 0.5% DMSO), the identical cremaphor/DMSO/saline combination (vehicle), NPS (containing identical cremaphor/DMSO), and the mixture of NPS and NBI-27914 (containing identical cremaphor/DMSO) were administered i.c.v. in the experiments with the use of NBI-27914, respectively.

2.6. Statistical analysis

Data were expressed as mean ± S.E.M. Significant differences between groups were determined by independent Student’s t-test or Dunnett’s test for multiple comparisons after analysis of variance (ANOVA). In all statistical comparisons, p < 0.05 was used as the criterion for statistical significance.

3. Results

3.1. Effect of i.c.v. NPS on food intake in fasted mice

During the first half hour, NPS (0.001–1 nmol, i.c.v.) dose-dependently inhibited food intake in fasted mice versus i.c.v. vehicle-treated mice (F(4,47) = 7.922, p < 0.001; Fig. 1A). The inhibitory effect of NPS on food intake reached statistically significant level at the relatively low dose of 0.01 nmol (p < 0.05 versus vehicle; Fig. 1A) and almost obtained maximum at the dose of 0.1 nmol (p < 0.01 versus vehicle; Fig. 1A). There was still a tendency for diminished food intake until 24 h (F(4,47) = 1.832, p = 0.138; Fig. 1A). However, during the 1–2 h period, the mice injected with NPS are significantly more than the control mice (F(4,47) = 4.446, p < 0.01; Fig. 1B). During the 4–24 h period, NPS at the dose of 0.1 nmol could significantly inhibit food intake (p < 0.05 versus vehicle; Fig. 1B).

3.2. The antagonist effect of [D-Val5]NPS on the action of NPS

Our results indicated that 10 nmol [D-Val5]NPS (i.c.v.) did not affect food intake compared with vehicle-treated mice (Fig. 2). Co-injection of 10 nmol [D-Val5]NPS (i.c.v.) could significantly antagonize the anorectic effect of 0.1 nmol NPS 0.5 and 1 h after injection (Fig. 2A). During the period of 1–2 h, the rebound of food intake induced by NPS was also antagonized by [D-Val5]NPS (p < 0.05; Fig. 2B).

3.3. The effects of CRF1 receptor antagonist NBI-27914 on the actions of NPS

It has been reported that the effect of NPS on locomotion was blocked by CRF1 receptor antalarmin and no longer evident in CRF1 receptor knock-out mice [24]. Here, we used another CRF1 receptor antagonist NBI-27914 to study whether the role of NPS on food intake was also mediated by CRF1 receptor.

First, we investigated whether NBI-27914 could antagonize the hyperlocomotion role of NPS in mice. NBI-27914 (2 μg, i.c.v.) did not affect locomotor activity per se (Fig. 3). Co-injection of NBI-27914 (2 μg, i.c.v.) could significantly antagonize the hyperlocomotion action of 1 nmol NPS (Fig. 3). The dose of NPS used here was based on our previous reported results that the action of NPS on locomotor activity obtained maximum at the dose of 1 nmol [19].
In fasted mice, NBI-27914 (2 μg, i.c.v.) did not affect food intake compared with vehicle-treated mice (Fig. 4). Unexpectedly, co-injection of NBI-27914 (2 μg, i.c.v.) could not antagonize the role of NPS on food intake (Fig. 4). These results indicated that the central role of NPS on food intake was not mediated by CRF₁ receptor.

4. Discussion

The present results first indicated the role of NPS on food intake in fasted mice. Our results are consistent with the anorectic effects of NPS reported in rats and chicks [2,7,8,10,22,30], suggesting the role of NPS on feeding behavior is a robust phenomenon among animal species.

In fasted mice, NPS could significantly reduce food intake during the first hour, and induce a significant rebound during the second hour. Our results were consistent with that obtained in fasted rats that central NPS induced a diminish of food intake followed by a rebound of food intake during the 1–3 h post-injection [2]. Interestingly, the role of NPS on food intake was well correlated with the effect of NPS on arousal and sleep [34]. That is, the increase in wakefulness during the first hour post-NPS injection was also followed by a rebound in the amount of non-REM sleep at the second hour [34]. In fasted mice, the effective dose of central NPS on food intake was 0.01 nmol, which was lower than that obtained in rats [2,10,30]. This might due to the difference between species, as the effective dose of NPS was also low in the modulation of arousal, anxiety, locomotion, nociception and distal colonic transit in mice [13].

Recently, several studies reported the pure and potent NPSR antagonists [3,11,12,21,23,35], which were used to investigate whether the actions of NPS were mediated through its cognate receptor. Among these antagonists, [D-Val⁵]NPS could almost fully...
induced by 0.1 nmol NPS. In the present study, central [D-Val5]NPS of NPS reached maximum at the dose of 0.1 nmol. Therefore, we rats that the CRF receptor antagonist CRF 9-41 did not prevent the previous report[24]. However, NBI-27914 did not affect the anorectic role of central NPS on locomotion, which was consistent with the Our results indicated that NBI-27914 almost fully antagonized the antagonist role of NPS on locomotion and distal colonic transit intake by period of time (B) in fasted mice. Results were presented as mean ± S.E.M. *p < 0.05 versus vehicle control according to independent Student’s t-test.

antagonize the role of NPS on locomotion and distal colonic transit in a molar ratio of 100/1[12,14]. In addition, the anorectic effect of NPS reached maximum at the dose of 0.1 nmol NPS. In the present study, central [D-Val5]NPS (10 nmol) did not affect food intake in fasted mice, indicating that endogenous central NPS could not tonically affect food intake. As expected, our results indicated that 10 nmol [D-Val5]NPS well antagonized the role of central NPS (0.1 nmol) on food intake, suggesting the anorectic effect of central NPS was mediated through NPSR. These results supported a recently reported result that another NPSR antagonist [D-Cys5]NPS could block the inhibition of palatable food intake by NPS in rats[10].

In rats, the inhibitory role of NPS on feeding appears to be independent of NPY, ghrelin and leptin pathways[2]. But it seems to be correlated with CRF system, since NPS could increase the release of CRF in hypothalamus in vitro[30]. In addition, the hyperlocomotion action of NPS was blocked by CRF1 receptor antagonist antalarmin and no longer evident in CRF1 receptor knock-out mice[24]. Thus, we used another CRF1 receptor antagonist NBI-27914 to investigate whether CRF1 receptor was involved in the anorectic role of NPS. Our results indicated that NBI-27914 almost fully antagonized the role of central NPS on locomotion, which was consistent with the previous report[24]. However, NBI-27914 did not affect the anorectic activity of central NPS. Recently, a similar result was obtained in rats that the CRF receptor antagonist CRF-9-41 did not prevent the inhibitory role of NPS on palatable food intake[10]. Our results indicated that the role of NPS on locomotion, but not food intake, was mediated by the downstream activation of CRF1 receptor, which indicated that the action of NPS on food intake and locomotion might act via different neuronal network, and the anorectic activity was not secondary to the increased exploratory activity induced by NPS.

In conclusion, the present study demonstrated the inhibitory role of central NPS on food intake in fasted mice, which was mediated by the activation of NPSR, but not by the activation of CRF1 receptor. Moreover, our results also indicated that the anorectic activity of NPS was not secondary to its enhanced locomotor activity.

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