Peptides 31 (2010) 2259–2263

Contents lists available at ScienceDirect

Peptides

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

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

Ya-Li Peng[a,1], Ren-Wen Han[a,b,1], Min Chang[a], Lei Zhang[a], Rui-San Zhang[a], Wei Li[a], Yi-Fan Han[b,*], Rui Wang[a,b,**]

[a] Key Laboratory of Preclinical Study for New Drugs of Gansu Province, School of Basic Medical Sciences, and Institute of Biochemistry and Molecular Biology, School of Life Sciences, and State Key Laboratory of Applied Organic Chemistry, Lanzhou University, 222 Tian Shui South Road, Lanzhou 730000, PR China
[b] State Key Laboratory of Chinese Medicine and Molecular Pharmacology, Department of Applied Biology and Chemical Technology, Hong Kong Polytechnic University, Hung Hom, Hong Kong, China

ARTICLE INFO

Article history:
Received 23 July 2010
Received in revised form 17 August 2010
Accepted 17 August 2010
Available online 26 August 2010

Keywords:
Neuropeptide S (NPS)
Neuropeptide S receptor (NPSR)
CRF
Food intake
Mice

ABSTRACT

Neuropeptide S (NPS), the endogenous ligand of NPS receptor (NPSR), can regulate a variety of biological functions, including arousal, anxiety, locomotion, memory and drug abuse. Previous studies have shown that central NPS inhibited food intake in rats and chicks. In the present study, we investigated the role of central NPS on food intake in fasted mice, and detected the underlying mechanism(s) by using NPSR antagonist [D-Val^5]NPS and Corticotropin-Releasing Factor 1 (CRF_1) 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-Val^5]NPS. Furthermore, CRF_1 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 CRF_1 receptor.

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 synthesis, which imply that NPSR couples to both G_q and G_s proteins [27,34]. 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. Recently, the pure and potent antagonists for NPSR were identified [3,11,12,21,23,35], in which [D-Val^5]NPS was reported to fully antagonized the role of NPS on locomotion, distal colonic transit and nociception in mice [12,14,26]. Thus, [D-Val^5]NPS was used to confirm whether the effect of NPS on food intake was mediated though NPSR. Additionally, it is noted that Corticotropin-Releasing Factor (CRF) release was up-regulated by NPS stimulation in hypothalamus in vitro [22], and CRF_1 receptor mediated the hyperlocomotion action of NPS in

* Corresponding author at: Department of Applied Biology and Chemical Technology, the Hong Kong Polytechnic University, Hung Hom, Hong Kong, PR China. Tel.: +852 3400 8695; fax: +852 2364 9932.
** Corresponding author at: School of Basic Medical Sciences, Lanzhou University, 222 Tian Shui South Road, Lanzhou 730000, PR China. Tel.: +86 931 8912567/852 34003755; fax: +86 931 8911255/852 23649932.
E-mail addresses: bcyfhan@polyu.edu.hk (Y.-F. Han), wangrui@lzu.edu.cn, bcrwang@polyu.edu.hk (R. Wang).
[1] Both authors contributed equally to this work.

0196-9781/$ – see front matter. Crown Copyright © 2010 Published by Elsevier Inc. All rights reserved.
doi:10.1016/j.peptides.2010.08.015

vivo [24]. Thus, we also examined whether the effect of NPS on food intake involved CRF_1 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 implanted into lateral ventricle and was fixed with dental cement. Coordinates toward the bregma were *L* +1 mm, *A* −0.5 mm, *V* +2 mm. To prevent occlusion, a dummy 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 Evaluated 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-Val^5]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-Val^5]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 ate 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-Val^5]NPS on the action of NPS

Our results indicated that 10 nmol [D-Val^5]NPS (i.c.v.) did not affect food intake compared with vehicle-treated mice (Fig. 2). Co-injection of 10 nmol [D-Val^5]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-Val^5]NPS ($p < 0.05$; Fig. 2B).

3.3. The effects of CRF_1 receptor antagonist NBI-27914 on the actions of NPS

It has been reported that the effect of NPS on locomotion was blocked by CRF_1 antagonist antalarmin and no longer evident in CRF_1 receptor knock-out mice [24]. Here, we used another CRF_1 receptor antagonist NBI-27914 to study whether the role of NPS on food intake was also mediated by CRF_1 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].

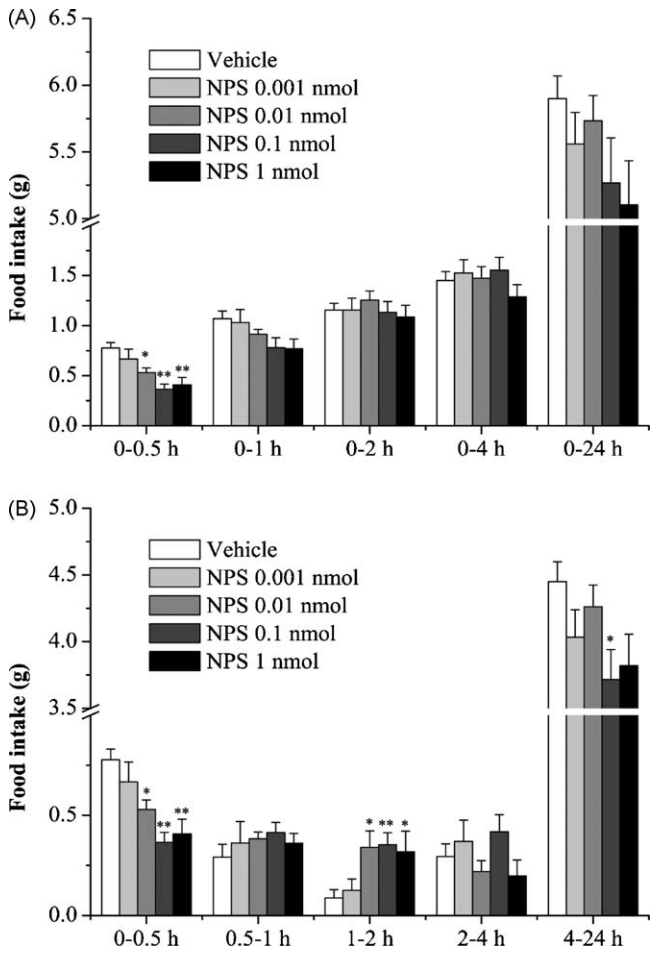

Fig. 1. The effect of NPS on cumulated food intake (A) and intake by period of time (B) in fasted mice. NPS was injected i.c.v. from 0.001 to 1 nmol. Results were presented as mean ± S.E.M. (n = 8–12). *p < 0.05 and **p < 0.01 versus vehicle control according to ANOVA followed by Dunnett's test.

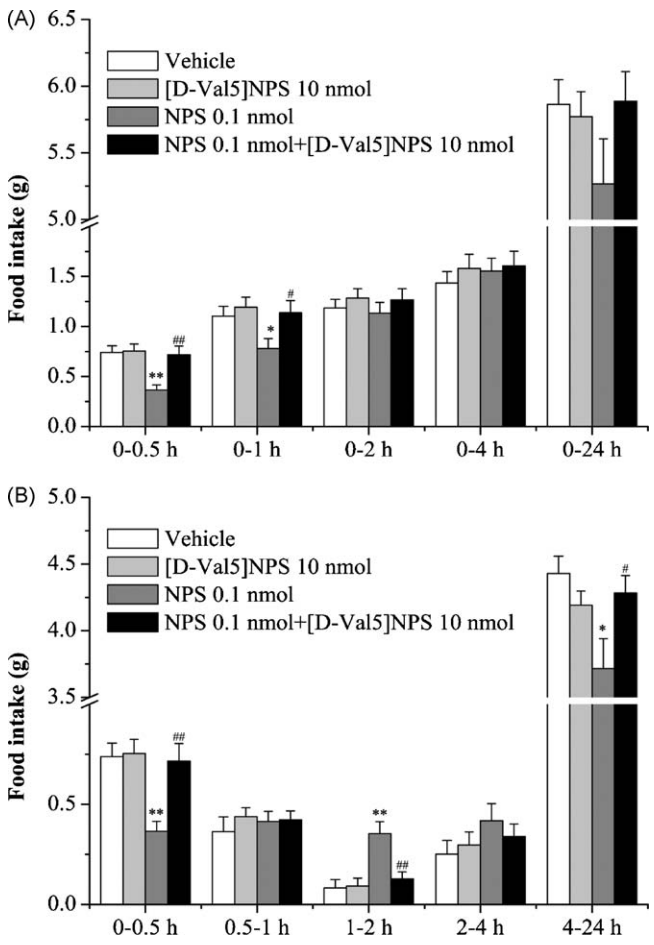

Fig. 2. Antagonism by [D-Val^5]NPS on NPS-induced cumulated food intake (A) and intake by period of time (B) in fasted mice. Results were presented as mean ± S.E.M. (n = 10–14). *p < 0.05 and **p < 0.01 versus vehicle control, $^{\#}p$ < 0.05 and $^{\#\#}p$ < 0.01 versus NPS-treated mice according to independent Student's t-test.

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 CRF_1 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-Val^5]NPS could almost fully

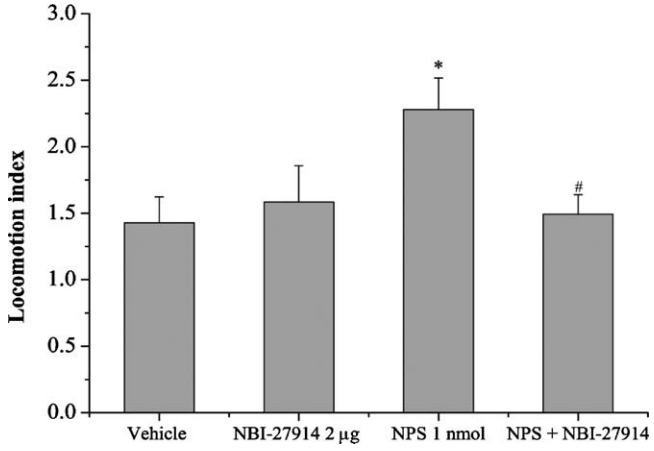

Fig. 3. Antagonism by NBI-27914 on hyperlocomotor effect of central NPS in mice. Results were presented as mean ± S.E.M. (n = 8). *p < 0.05 versus vehicle control, and $^{\#}p$ < 0.05 versus NPS-treated mice according to independent Student's t-test.

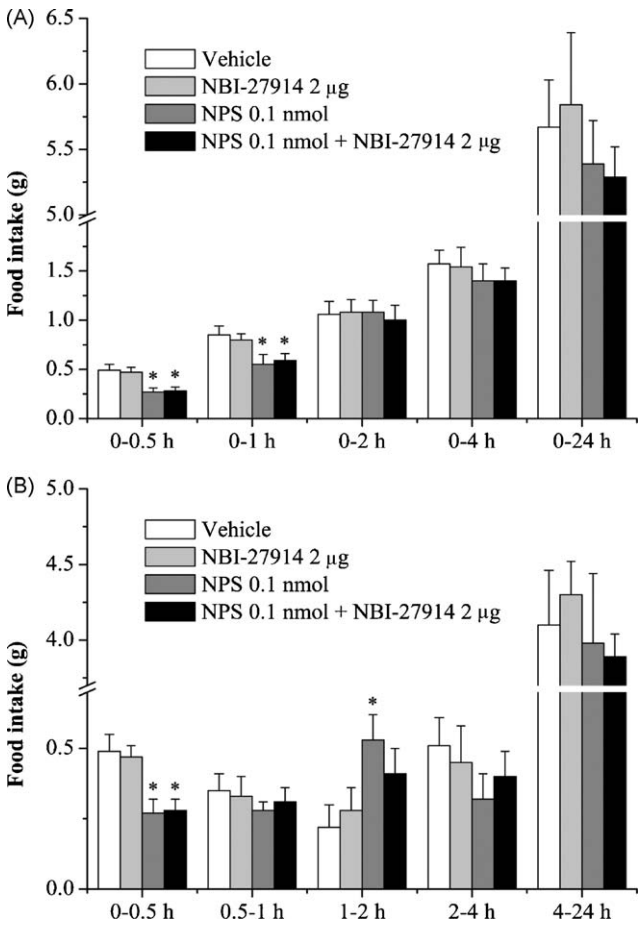

Fig. 4. The effect of NBI-27914 on NPS-induced cumulated food intake (A) and intake by period of time (B) in fasted mice. Results were presented as mean ± S.E.M. (n = 7–8). *p < 0.05 versus vehicle control according to independent Student's t-test.

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. Therefore, we utilized 10 nmol [D-Val5]NPS to antagonize the anorectic action induced by 0.1 nmol NPS. In the present study, central [D-Val^5]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-Val^5]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-Cys^5]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 CRF_1 receptor antagonist antalarmin and no longer evident in CRF_1 receptor knock-out mice [24]. Thus, we used another CRF_1 receptor antagonist NBI-27914 to investigate whether CRF_1 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 CRF_1 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 CRF_1 receptor. Moreover, our results also indicated that the anorectic activity of NPS was not secondary to its enhanced locomotor activity.

Acknowledgements

This study was supported by the grants from the National Natural Science Foundation of China (Nos. 20932003, 90813012 and 30900445), Gansu province Natural Science Foundation of China (0708NKCA111), and Shenzhen Shuanbai Fund 2008.

References

[1] Badia-Elder NE, Henderson AN, Bertholomey ML, Dodge NC, Stewart RB. The effects of Neuropeptide S on ethanol drinking and other related behaviors in alcohol-preferring and -nonpreferring rats. Alcohol Clin Exp Res 2008;32:1380–7.
[2] Beck B, Fernette B, Stricker-Krongrad A. Peptide S is a novel potent inhibitor of voluntary and fast-induced food intake in rats. Biochem Biophys Res Commun 2005;332:859–65.
[3] Camarda V, Rizzi A, Ruzza C, Zucchini S, Marzola G, Marzola E, et al. In vitro and in vivo pharmacological characterization of the Neuropeptide S receptor antagonist [D-Cys(tBu)^5]Neuropeptide S. J Pharmacol Exp Ther 2009;328:549–55.
[4] Camilleri M, Carlson P, Zinsmeister AR, McKinzie S, Busciglio I, Burton D, et al. Neuropeptide S receptor induces neuropeptide expression and associates with intermediate phenotypes of functional gastrointestinal disorders. Gastroenterology, 138 98–107, e4.
[5] Cannella N, Economidou D, Kallupi M, Stopponi S, Heilig M, Massi M, et al. Persistent increase of alcohol-seeking evoked by Neuropeptide S: an effect mediated by the hypothalamic hypocretin system. Neuropsychopharmacology 2009;34:2125–34.
[6] Castro AA, Moretti M, Casagrande TS, Martinello C, Petronilho F, Steckert AV, et al. Neuropeptide S produces hyperlocomotion and prevents oxidative stress damage in the mouse brain: a comparative study with amphetamine and diazepam. Pharmacol Biochem Behav 2009;91:636–42.
[7] Cline MA, Godlove DC, Nandar W, Bowden CN, Prall BC. Anorexigenic effects of central Neuropeptide S involve the hypothalamus in chicks (Gallus gallus). Comp Biochem Physiol A: Mol Integr Physiol 2007;148:657–63.
[8] Cline MA, Prall BC, Smith ML, Calchary WA, Siegel PB. Differential appetite-related responses to central Neuropeptide S in lines of chickens divergently selected for low or high body weight. J Neuroendocrinol 2008;20:904–8.
[9] D'Amato M, Bruce S, Bresso F, Zucchelli M, Ezer S, Pulkkinen V, et al. Neuropeptide S receptor 1 gene polymorphism is associated with susceptibility to inflammatory bowel disease. Gastroenterology 2007;133:808–17.
[10] Fedeli A, Braconi S, Economidou D, Cannella N, Kallupi M, Guerrini R, et al. The paraventricular nucleus of the hypothalamus is a neuroanatomical substrate for the inhibition of palatable food intake by Neuropeptide S. Eur J Neurosci 2009;30:1594–602.
[11] Guerrini R, Camarda V, Trapella C, Calo G, Rizzi A, Ruzza C, et al. Further studies at Neuropeptide S position 5: discovery of novel Neuropeptide S receptor antagonists. J Med Chem 2009;52:4068–71.
[12] Guerrini R, Camarda V, Trapella C, Calo G, Rizzi A, Ruzza C, et al. Synthesis and biological activity of human Neuropeptide S analogues modified in position 5: identification of potent and pure Neuropeptide S receptor antagonists. J Med Chem 2009;52:524–9.
[13] Guerrini R, Salvadori S, Rizzi A, Regoli D, Calo G. Neurobiology, pharmacology, and medicinal chemistry of Neuropeptide S and its receptor. Med Res Rev 2009, doi:10.1002/med.20180.
[14] Han RW, Chang M, Peng YL, Qiao LY, Yin XQ, Li W, et al. Central Neuropeptide S inhibits distal colonic transit through activation of central Neuropeptide S receptor in mice. Peptides 2009;30:1313–7.
[15] Han RW, Yin XQ, Chang M, Peng YL, Li W, Wang R. Neuropeptide S facilitates spatial memory and mitigates spatial memory impairment induced by N-methyl-d-aspartate receptor antagonist in mice. Neurosci Lett 2009;455:74–7.
[16] Jungling K, Seidenbecher T, Sosulina L, Lesting J, Sangha S, Clark SD, et al. Neuropeptide S-mediated control of fear expression and extinction: role of intercalated GABAergic neurons in the amygdala. Neuron 2008;59:298–310.
[17] Leonard SK, Dwyer JM, Sukoff Rizzo SJ, Platt B, Logue SF, Neal SJ, et al. Pharmacology of Neuropeptide S in mice: therapeutic relevance to anxiety disorders. Psychopharmacology (Berl) 2008;197:601–11.

[18] Li W, Chang M, Peng YL, Gao YH, Zhang JN, Han RW, et al. Neuropeptide S produces antinociceptive effect at the supraspinal level in mice. Regul Pept 2009;156:90–5.
[19] Li W, Gao YH, Chang M, Peng YL, Yao J, Han RW, et al. Neuropeptide S inhibits the acquisition and the expression of conditioned place preference to morphine in mice. Peptides 2009;30:234–40.
[20] Meis S, Bergado-Acosta JR, Yanagawa Y, Obata K, Stork O, Munsch T. Identification of a Neuropeptide S responsive circuitry shaping amygdala activity via the endopiriform nucleus. PLoS ONE 2008;3:e2695.
[21] Melamed JY, Zartman AE, Kett NR, Gotter AL, Uebele VN, Reiss DR, et al. Synthesis and evaluation of a new series of Neuropeptide S receptor antagonists. Bioorg Med Chem Lett 2010;20:4700–3.
[22] Niimi M. Centrally administered Neuropeptide S activates orexin-containing neurons in the hypothalamus and stimulates feeding in rats. Endocrine 2006;30:75–9.
[23] Okamura N, Habay SA, Zeng J, Chamberlin AR, Reinscheid RK. Synthesis and pharmacological in vitro and in vivo profile of 3-oxo-1,1-diphenyl-tetrahydro-oxazolo[3,4-a]pyrazine-7-carboxylic acid 4-fluoro-benzylamide (SHA 68), a selective antagonist of the Neuropeptide S receptor. J Pharmacol Exp Ther 2008;325:893–901.
[24] Paneda C, Huitron-Resendiz S, Frago LM, Chowen JA, Picetti R, de Lecea L, et al. Neuropeptide S reinstates cocaine-seeking behavior and increases locomotor activity through corticotropin-releasing factor receptor 1 in mice. J Neurosci 2009;29:4155–61.
[25] Pape HC, Jungling K, Seidenbecher T, Lesting J, Reinscheid RK. Neuropeptide S: a transmitter system in the brain regulating fear and anxiety. Neuropharmacology, 58:29–34.
[26] Peng YL, Zhang JN, Chang M, Li W, Han RW, Wang R. Effects of central Neuropeptide S in the mouse formalin test. Peptides 2010, doi:10.1016/j.peptides.2010.06.027.
[27] Reinscheid RK, Xu YL, Okamura N, Zeng J, Chung S, Pai R, et al. Pharmacological characterization of human and murine Neuropeptide S receptor variants. J Pharmacol Exp Ther 2005;315:1338–45.
[28] Rizzi A, Vergura R, Marzola G, Ruzza C, Guerrini R, Salvadori S, et al. Neuropeptide S is a stimulatory anxiolytic agent: a behavioural study in mice. Br J Pharmacol 2008;154:471–9.
[29] Sato S, Shintani Y, Miyajima N, Yoshimura K. Novel G Protein Coupled Receptor Protein and Dna Thereof. World Patent Application WO 02/31145 A1 (2002).
[30] Smith KL, Patterson M, Dhillo WS, Patel SR, Semjonous NM, Gardiner JV, et al. Neuropeptide S stimulates the hypothalamo-pituitary-adrenal axis and inhibits food intake. Endocrinology 2006;147:3510–8.
[31] Sundman L, Saarialho-Kere U, Vendelin J, Lindfors K, Assadi G, Kaukinen K, et al. Neuropeptide S receptor 1 expression in the intestine and skin—putative role in peptide hormone secretion. Neurogastroenterol Motil, 22, 79–87, e30.
[32] Vitale G, Filaferro M, Ruggieri V, Pennella S, Frigeri C, Rizzi A, et al. Anxiolytic-like effect of Neuropeptide S in the rat defensive burying. Peptides 2008;29:2286–91.
[33] Xu YL, Gall CM, Jackson VR, Civelli O, Reinscheid RK. Distribution of Neuropeptide S receptor mRNA and neurochemical characteristics of Neuropeptide S-expressing neurons in the rat brain. J Comp Neurol 2007;500:84–102.
[34] Xu YL, Reinscheid RK, Huitron-Resendiz S, Clark SD, Wang Z, Lin SH, et al. Neuropeptide S: a neuropeptide promoting arousal and anxiolytic-like effects. Neuron 2004;43:487–97.
[35] Zhang Y, Gilmour BP, Navarro HA, Runyon SP. Identifying structural features on 1,1-diphenyl-hexahydro-oxazolo[3,4-a]pyrazin-3-ones critical for Neuropeptide S antagonist activity. Bioorg Med Chem Lett 2008;18:4064–7.
[36] Zhao Z, Huang L, Wu H, Li Y, Zhang L, Yin Y, et al. Neuropeptide S mitigates spatial memory impairment induced by rapid eye movement sleep deprivation in rats. Neuroreport, 21:623–8.