The Effects of Neuropeptide S on General Anesthesia in Rats

Tetsuya Kushikata, MD, PhD,* Hitoshi Yoshida, MD,* Mihoko Kudo, PhD,* Severo Salvadori, MD, PhD,† Girolamo Calo, MD, PhD,‡ and Kazuyoshi Hirota, MD, FRCA*

BACKGROUND: Neuropeptide S (NPS) and its receptor (NPSR) is a novel neuropeptide system that regulates arousal and anxiety. A link between natural sleep and general anesthesia has been suggested. Therefore, we hypothesized that the NPS neuronal system may also modulate general anesthesia.
METHODS: The effects of intracerebroventricular NPS and [D-Cys(tBu)^5]NPS, a peptide NPSR antagonist, on ketamine and thiopental anesthesia time were measured in rats. Anesthesia time was defined as the interval between the loss of righting reflex and its recovery.
RESULTS: Intracerebroventricular NPS 1 to 30 nmol significantly reduced ketamine anesthesia time, showing a bell-shaped dose-response curve. [D-Cys(tBu)^5]NPS 20 nmol antagonized NPS 1 nmol effects and was per se able to increase ketamine anesthesia time. Similar results were obtained investigating thiopental anesthesia time that was significantly reduced by NPS and prolonged by [D-Cys(tBu)^5]NPS.
CONCLUSION: NPS via selective NPSR activation stimulates the wakefulness-promoting pathway, thus reducing anesthesia duration. The endogenous NPS/NPSR system seems to tonically control these pathways. (Anesth Analg 2011;112:845–49)

Recent articles[1,2] suggest a link between natural sleep and general anesthesia. Several nuclei have been recognized as important loci for initiation of sleep and anesthesia emergence.[3,4] The hypothalamic ventrolateral preoptic nucleus promotes sleep by activation of GABAergic neurons, which inhibit wakefulness-promoting circuits, such as the hypothalamic tuberomammillary nucleus. Another critical arousal-promoting or arousal-stabilizing locus is the lateral hypothalamus where orexin-producing neurons contribute to promoting and maintaining arousal.[5] In addition, accumulating evidence indicates that sleep, especially non–rapid eye movement (REM) sleep, and general anesthesia involve common neuronal and genetic substrates. Both seem to reduce or abolish spontaneous movement and sensory responsiveness. Sleep deprivation increases the drive to sleep, reflecting homeostatic regulation. Similarly, sleep deprivation also enhances the action of the volatile anesthetics.[6] General anesthetics seem to act through sleep neural circuits. General anesthetics induce a similar electroencephalographic state as that recorded during non-REM sleep[7,8] and act on the same sleep-wake neural circuits, as suggested by brain imaging studies.[9] Importantly, the sedative effects of propofol require γ-aminobutyric acid $(GABA)_A$ receptor function in the wake-promoting tuberomammillary nucleus.[10]

General anesthetics are classified into 2 types.[11] Most general anesthetics such as barbiturates, propofol, benzodiazepines, and volatile anesthetic drugs produce anesthesia by enhancing the activity of inhibitory $GABA_A$ receptors ($GABA_A$-type anesthetics). However, the effects of ketamine, xenon, and nitrous oxide on these receptors are negligible. Instead, these anesthetics potently inhibit the excitatory *N*-methyl-D-aspartate (NMDA) receptors (NMDA-type anesthetics). We previously found significant interactions among the wakefulness-promoting neuropeptide, orexins, and both $GABA_A$ and NMDA types of anesthetics.[12,13] Intracerebroventricular (icv) orexin A reduces barbiturates ($GABA_A$-type) and ketamine (NMDA-type) anesthesia duration. In addition, clinically relevant concentrations of both anesthetics significantly antagonized orexin A–evoked norepinephrine release from rat cerebrocortical slices.[12,13]

Neuropeptide S (NPS) and its receptor (NPSR) is a novel neuropeptide system that regulates arousal and anxiety.[14] The NPS-producing neurons are mainly located between the noradrenergic locus coeruleus and the Barrington's nucleus.[14] Reports show that central administration of NPS significantly increases wakefulness and decreases REM sleep and non-REM sleep in rats.[14] Therefore, we hypothesized that the NPS neuronal system may also modulate general anesthesia similar to orexinergic systems.

METHODS

After approval of our protocol by our University Animal Ethics Committee, an 8-mm length of stainless-steel guide cannula (outside diameter = 0.5 mm, AG-8; Eicom, Kyoto, Japan) was implanted unilaterally into the lateral cerebroventricle of 30 Sprague-Dawley rats under pentobarbital anesthesia (50 mg/kg intraperitoneal [IP] administration). In pilot experiments, we found that both ketamine 100 mg IP and thiopental 45 mg IP produced 30 to 40 minutes of

From the *Department of Anesthesiology, Hirosaki University Graduate School of Medicine, Hirosaki, Japan; †Department of Pharmaceutical Sciences and Biotechnology Centre, University of Ferrara; and ‡Department of Experimental and Clinical Medicine, Section of Pharmacology, and National Institute of Neuroscience, University of Ferrara, Ferrara, Italy.

Accepted for publication November 30, 2010.

Supported by grants-in-aid for scientific research (21390430 and 20581848) from the Ministry of Education, Science and Culture in Japan.

The authors declare no conflicts of interest.

Reprints will not be available from the authors.

Address correspondence to Kazuyoshi Hirota, MD, FRCA, Department of Anesthesiology, Hirosaki University Graduate School of Medicine, Hirosaki 036-8562, Japan. Address e-mail to hirotak@cc.hirosaki-u.ac.jp.

DOI: 10.1213/ANE.0b013e31820b990d

anesthesia time in Sprague-Dawley rats. Therefore, these doses were selected and used in the present study.

Effects of icv NPS and [D-Cys(tBu)^5]NPS on Ketamine Anesthesia Time

Rats received 4 μL pyrogen-free saline icv via the cannula simultaneously with IP ketamine (100 mg/kg) on a separate control day. Five days later (treatment day), the same animals received icv NPS (0 [vehicle], 0.3, 1, 3, 10, and 30 nmol/4 μL; $n = 7$ each) and IP ketamine at the same dose as on the control day. To test the effects of the NPSR antagonist [D-Cys(tBu)^5]NPS, rats received IP ketamine (100 mg/kg) and icv [D-Cys(tBu)^5]NPS 20 nmol with or without icv NPS 1.0 nmol ($n = 7$ each).

Effects of icv NPS and [D-Cys(tBu)^5]NPS on Thiopental Anesthesia Time

Rats received 4 μL pyrogen-free saline icv via the cannula simultaneously with IP thiopental (45 mg/kg) on a separate control day. Five days later (treatment day), the same animals received icv NPS (0 [vehicle], 0.1, 0.3, 1, and 10 nmol/4 μL; $n = 8$ each) and IP thiopental at the same dose as on the control day. To test the reversal effects of [D-Cys(tBu)^5]NPS, rats received IP thiopental (45 mg/kg) and icv [D-Cys(tBu)^5]NPS 20 nmol with or without icv NPS 1.0 nmol ($n = 8$ each).

Definition of Anesthesia Time

Induction time of anesthesia was defined as the duration from anesthetic administration to loss of 3 successive righting reflexes. Anesthesia maintenance time was defined as the duration from the loss of righting reflex to its recovery, i.e., the ability to perform 3 successive righting reflexes. The effects of NPS or NPSR antagonist were estimated as the anesthesia time differences between control and treatment day.

Data Analysis

All data are presented as mean ± SEM. Where appropriate, statistical analysis was performed using the Student t test or 1-way analysis of variance followed by the Student-Newman-Keuls test. A P value <0.05 was considered significant.

RESULTS

Ketamine Anesthesia

Ketamine induction and maintenance time on the control day after icv saline was 2.2 ± 0.1 and 35.9 ± 1.0 minutes, respectively ($n = 56$). These data were not different from those on the treatment day (2.3 ± 0.3 and 35.0 ± 3.5 minutes, respectively; $n = 7$). Anesthesia induction time of IP ketamine was not affected by icv NPS. In contrast, icv NPS in the range 1 to 10 nmol dose dependently reduced the anesthesia maintenance time of ketamine. At the highest dose, i.e., 30 nmol, NPS was found inactive; thus, the dose response curve to the peptide was bell shaped (Fig. 1A). [D-Cys(tBu)^5]NPS at 20 nmol fully prevented the effect of NPS 1 nmol. Interestingly, when given alone, the NPSR antagonist significantly increased the anesthesia maintenance time of ketamine (Fig. 1B) without modifying the anesthesia induction time. All rats were sensitive to NPS in the range 1 to 10 nmol and 6 of 7 rats were sensitive to [D-Cys(tBu)^5]NPS 20 nmol.

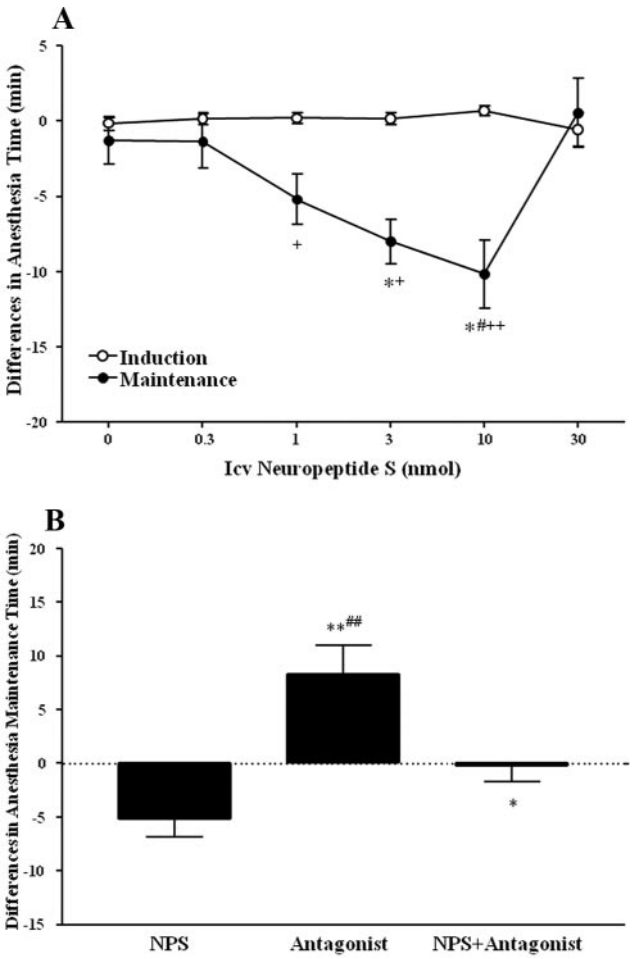

Figure 1. Effects of intracerebroventricular (icv) neuropeptide S (NPS) (A) and [D-Cys(tBu)^5]NPS (B) on ketamine anesthesia time. Anesthesia time differences = Anesthesia maintenance time on control day − Anesthesia maintenance duration on the NPS treatment day. All data are mean ± SEM. A, *$P < 0.05$ versus NPS 0, #$P < 0.05$ versus NPS 0.3, +$P < 0.05$, ++$P < 0.01$ versus NPS 30. B, *$P < 0.05$, **$P < 0.01$ versus NPS, ##$P < 0.01$ versus NPS + antagonist.

Thiopental Anesthesia

Similar to IP ketamine, IP thiopental after icv saline produced anesthesia with 2.3 ± 0.1 and 35.6 ± 1.5 minutes of anesthesia induction and maintenance time, respectively. These data were not different from those on the treatment day (2.0 ± 0.3 and 37.1 ± 2.2 minutes, respectively; $n = 8$). Icv NPS did not affect the anesthesia induction time of IP thiopental. In contrast, the anesthesia maintenance time of thiopental was significantly decreased by icv NPS in the range 0.1 to 1 nmol. At 10 nmol, the peptide was inactive (Fig. 2A). [D-Cys(tBu)^5]NPS 20 nmol antagonized the stimulatory effect of 1 nmol NPS. When the same dose of antagonist was given alone, it elicited a robust enhancement of thiopental's anesthesia maintenance time and fully reversed NPS-induced reduction in anesthesia maintenance time (Fig. 2B). Fifteen of 16 rats were sensitive to NPS at 0.3

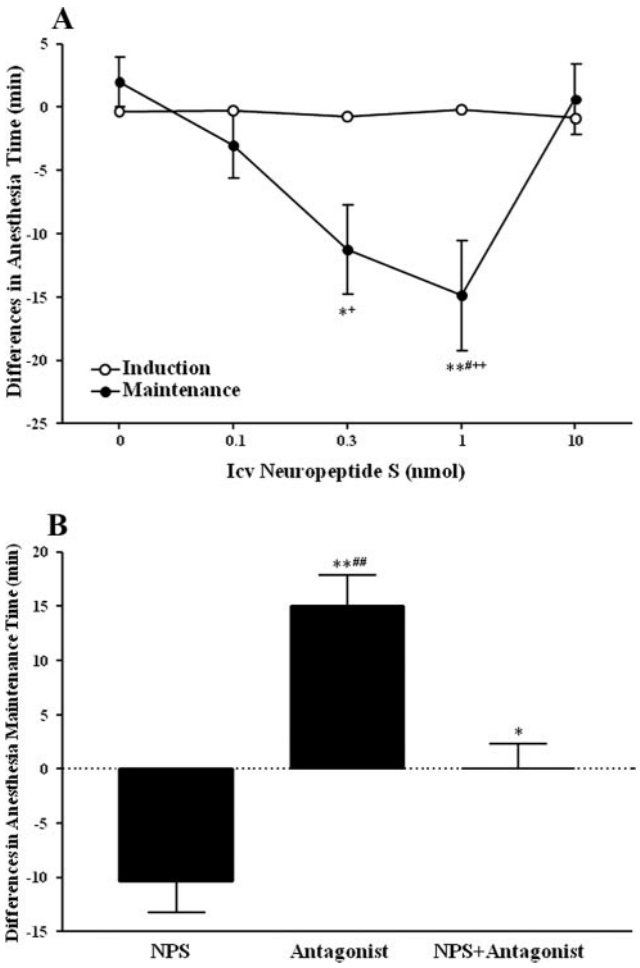

Figure 2. Effects of intracerebroventricular (icv) neuropeptide S (NPS) (A) and [D-Cys(tBu)^5]NPS (B) on thiopental anesthesia time. Anesthesia time differences = Anesthesia maintenance time on control day − Anesthesia maintenance duration on the NPS treatment day. All data are mean ± SEM. A, $*P < 0.05$, $**P < 0.01$ versus NPS 0, $\#P < 0.05$ versus NPS 0.1, $+P < 0.05$, $++P < 0.01$ versus NPS 10. B, $*P < 0.05$, $**P < 0.01$ versus NPS, $\#\#P < 0.01$ versus NPS + antagonist.

and 1 nmol and all rats were sensitive to [D-Cys(tBu)^5]NPS 20 nmol.

DISCUSSION

There may be a significant link between general anesthesia and sleep/wakefulness circuits.[1,2] Electroencephalographic studies showed that icv NPS increased the amount of wakefulness and reduced both non-REM and REM sleep.[15] We previously reported that anesthetics such as barbiturates,[12] benzodiazepines,[16] and ketamine[13] could interact with a wakefulness-promoting orexinergic system. Thus, it might be likely that another wakefulness-promoting peptide, namely, NPS, could reduce anesthesia duration. Indeed, the present study demonstrated that icv NPS dose dependently reduced both thiopental and ketamine anesthesia duration in rats. In line with the present findings, Rizzi et al.[17] reported that icv NPS dose dependently reduced anesthesia duration (duration of loss of righting reflex) after IP diazepam 15 mg/kg in mice. Therefore, NPS may promote wakefulness not only from natural sleep but also general anesthesia, and its effects are robust among animal species.

NPS and NPSR antagonist did not modulate the anesthesia induction time of ketamine and thiopental. Similarly, Kelz et al.[18] reported that inhibition of orexinergic signaling does not alter the anesthesia induction time of sevoflurane and isoflurane. Thus, activation or inhibition of the wakefulness-promoting pathway by neuropeptides such as NPS and orexins or their antagonists may impact emergence from, but not entry into, the anesthesia state.

In the present study, the dose-response curve to NPS was clearly bell shaped. In fact, the peptide completely lost its stimulatory effects at the highest dose tested, i.e., 30 nmol versus ketamine and 10 nmol versus thiopental. We do not have an obvious explanation for this phenomenon. However, it is worthy of mention that several sleep/wakefulness-related substances have been reported to display similar dose-response curves. Clonidine, an α_2 agonist, from 0.3 to 5 mg/kg dose dependently increased the sleep duration in chicks, but 10 mg/kg clonidine completely lost the effects.[19] Oxidized glutathione, an active sleep-promoting substance, significantly enhanced both non-REM and REM sleep at the dosage range from 20 to 50 nmol, but maximal enhancement occurred at 25 nmol.[20] Polc et al.[21] found that IV administration of δ-sleep-inducing peptide 30 nmol/kg decreased waking time and enhanced both non-REM and REM sleep whereas δ-sleep-inducing peptide 300 nmol/kg did not affect sleep and waking time in cats. Uridine[22] and muramyl dipeptide,[23] which are sleep-promoting substances, have also been reported to show a bell-shaped dose-response curve. Moreover, regarding arousal-promoting agents, we previously reported that yohimbine 1 mg/kg IP reduced propofol anesthesia time by 28% whereas yohimbine 10 mg/kg IP increased propofol anesthesia time by 55%.[24]

[D-Cys(tBu)^5]NPS completely prevented NPS effects on ketamine and thiopental anesthesia duration, demonstrating the exclusive involvement of the NPSR in the wakefulness-promoting action of NPS in rats. The NPSR antagonist properties of [D-Cys(tBu)^5]NPS were first demonstrated in vitro in cells expressing the murine NPSR,[25,26] and then confirmed in vivo in mice versus the NPS arousal-promoting[25] and antinociceptive actions,[27] and in rats versus the anxiolytic-like effects[28] and inhibitory action on palatable food intake[29] elicited by NPS. In all these studies, as well as in the present experiments, [D-Cys(tBu)^5]NPS was active when tested versus NPS in a 20 to 100/1 antagonist/agonist dose ratio. Therefore, the present results and previous literature findings suggest that [D-Cys(tBu)^5]NPS is a pharmacologically reliable tool for neurobiological investigations in the field of the NPS/NPSR system.

Moreover, this study also demonstrated that [D-Cys(tBu)^5]NPS per se prolonged both ketamine and thiopental anesthesia duration. This finding may be interpreted assuming that under the present experimental conditions the endogenous NPS/NPSR system tonically controls the anesthesia state, i.e., the increase in anesthesia duration elicited by the NPSR antagonist might be attributable to inhibition of the stimulatory effects of endogenously released NPS. Similarly, we previously reported

that another wakefulness-promoting neuropeptide orexin A reduced barbiturate anesthesia duration and orexin receptor-1 antagonist produced per se opposite effects.[12] Therefore, endogenous sleep/wakefulness-related neuropeptide signaling may affect anesthesia duration. However, Camarda et al.[25] showed that [D-Cys(tBu)^5]NPS did not affect diazepam-induced sleeping time in mice at doses capable of counteracting the stimulatory effects of NPS. Similar results were recently obtained with the nonpeptide NPSR antagonist SHA68.[30] In addition, NPSR gene knockout mice were found to be as sensitive as their wild-type littermates to the hypnotic effects of diazepam.[25] The discrepancy between the present results and those reported by Camarda et al.[25] may be attributable to differences in experimental settings including anesthesia duration (35 minutes versus 100 minutes), animal species (rat versus mouse), or anesthetic drugs used (ketamine and thiopental versus diazepam). Clearly, further studies performed with different NPSR antagonists, anesthetic drugs, and animal species are needed before drawing firm conclusions on the role of the endogenous NPS/NPSR system in controlling sleep and the anesthesia state.

In conclusion, NPS significantly reduced anesthesia duration via selective stimulation of the NPSR and subsequent activation of wakefulness-promoting pathways. These same pathways seem to be tonically active, at least under the present experimental conditions, because blocking the endogenous NPS/NPSR signaling with a selective NPSR antagonist produces the opposite result of longer-lasting anesthesia.

DISCLOSURES

Name: Tetsuya Kushikata, MD, PhD.
Contribution: Study design, conduct of study, data analysis, manuscript preparation.
Name: Hitoshi Yoshida, MD.
Contribution: Data analysis, manuscript preparation.
Name: Mihoko Kudo, PhD.
Contribution: Conduct of study, data analysis.
Name: Severo Salvadori, MD, PhD.
Contribution: Study design.
Name: Girolamo Calo, MD, PhD.
Contribution: Study design, data analysis, manuscript preparation.
Name: Kazuyoshi Hirota, MD, FRCA.
Contribution: Study design, data analysis, manuscript preparation.

REFERENCES

1. Zecharia AY, Nelson LE, Gent TC, Schumacher M, Jurd R, Rudolph U, Brickley SG, Maze M, Franks NP. The involvement of hypothalamic sleep pathways in general anesthesia: testing the hypothesis using the GABAA receptor beta3N265M knock-in mouse. J Neurosci 2009;29:2177–87
2. Franks NP. General anaesthesia: from molecular targets to neuronal pathways of sleep and arousal. Nat Rev Neurosci 2008;9:370–86
3. Saper CB, Scammell TE, Lu J. Hypothalamic regulation of sleep and circadian rhythms. Nature 2005;437:1257–63
4. Lydic R, Baghdoyan HA. Sleep, anesthesiology, and the neurobiology of arousal state control. Anesthesiology 2005; 103:1268–95
5. Sakurai T. The neural circuit of orexin (hypocretin): maintaining sleep and wakefulness. Nat Rev Neurosci 2007;8:171–81
6. Tung A, Szafran MJ, Bluhm B, Mendelson WB. Sleep deprivation potentiates the onset and duration of loss of righting reflex induced by propofol and isoflurane. Anesthesiology 2002; 97:906–11
7. Sleigh JW, Andrzejowski J, Steyn-Ross A, Steyn-Ross M. The bispectral index: a measure of depth of sleep? Anesth Analg 1999;88:659–61
8. Tung A, Lynch JP, Roizen MF. Use of the BIS monitor to detect onset of naturally occurring sleep. J Clin Monit Comput 2002;17:37–42
9. Alkire MT, Haier RJ, Fallon JH. Toward a unified theory of narcosis: brain imaging evidence for a thalamocortical switch as the neurophysiologic basis of anesthetic-induced unconsciousness. Conscious Cogn 2000;9:370–86
10. Nelson LE, Guo TZ, Lu J, Saper CB, Franks NP, Maze M. The sedative component of anesthesia is mediated by GABA(A) receptors in an endogenous sleep pathway. Nat Neurosci 2002;5:979–84
11. Hirota K. Special cases: ketamine, nitrous oxide and xenon. Best Pract Res Clin Anaesthesiol 2006;20:69–79
12. Kushikata T, Hirota K, Yoshida H, Kudo M, Lambert DG, Smart D, Jerman JC, Matsuki A. Orexinergic neurons and barbiturate anesthesia. Neuroscience 2003;121:855–63
13. Tose R, Kushikata T, Yoshida H, Kudo M, Furukawa K, Ueno S, Hirota K. Orexin A decreases ketamine-induced anesthesia time in the rat: the relevance to brain noradrenergic neuronal activity. Anesth Analg 2009;108:491–5
14. Reinscheid RK, Xu YL. Neuropeptide S as a novel arousal promoting peptide transmitter. FEBS J 2005;272: 5689–93
15. Xu YL, Reinscheid RK, Huitron-Resendiz S, Clark SD, Wang Z, Lin SH, Brucher FA, Zeng J, Ly NK, Henriksen SJ, de Lecea L, Civelli O. Neuropeptide S: a neuropeptide promoting arousal and anxiolytic-like effects. Neuron 2004;43:487–97
16. He Y, Kudo M, Kudo T, Kushikata T, Li E, Hirota K. The effects of benzodiazepines on orexinergic systems in rat cerebrocortical slices. Anesth Analg 2007;104:338–40
17. Rizzi A, Vergura R, Marzola G, Ruzza C, Guerrini R, Salvadori S, Regoli D, Calo G. Neuropeptide S is a stimulatory anxiolytic agent: a behavioural study in mice. Br J Pharmacol 2008; 154:471–9
18. Kelz MB, Sun Y, Chen J, Cheng Meng Q, Moore JT, Veasey SC, Dixon S, Thornton M, Funato H, Yanagisawa M. An essential role for orexins in emergence from general anesthesia. Proc Natl Acad Sci USA 2008;105:1309–14
19. Roach AG, Doxey JC, Strachan DA, Cavero I. Sleeping times evoked by alpha adrenoceptor agonists in two-day-old chicks: an experimental model to evaluate full and partial agonist at central alpha-2 adrenoceptors. J Pharmacol Exp Ther 1983;227:421–8
20. Honda K, Komoda Y, Inoue S. Oxidized glutathione regulates physiological sleep in unstrained rats. Brain Res 1994; 636:253–8
21. Polc P, Schneeberger J, Haefely W. Effect of the delta sleep-inducing peptide (DSIP) on the sleep-wakefulness cycle of cats. Neurosci Lett 1978;9:33–6
22. Honda K, Komoda Y, Nishida S, Nagasaki H, Higashi A, Inoue S. Uridine as an active component of sleep-promoting substances: its effects on nocturnal sleep in rats. Neurosci Res 1984;1:243–52
23. Krueger JM, Pappenheimer JR, Karnovsky ML. Muramyl peptides: variation of somnogenic activity with structure. J Exp Med 1984;159:68–76
24. Hirota K, Kushikata T, Kudo M. Role of noradrenergic and orexinergic neurons in general anaesthesia. Curr Neuropharmacol 2003;1:333–40
25. Camarda V, Rizzi A, Zucchini S, Marzola G, Marzola E, Guerrini R, Salvadori S, Reinscheid RK, Regoli D, Calò G. In vitro and in vivo pharmacological characterization of the neuropeptide S receptor antagonist [D-Cyst(tBu)^5]neuropeptide S. J Pharmacol Exp Ther 2009;328:549–55

26. Guerrini R, Camarda V, Trapella C, Calo G, Rizzi A, Ruzza C, Fiorini S, Marzola E, Reinscheid RK, Regoli D, Salvadori S. 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
27. Li W, Chang M, Peng YL, Gao YH, Zhang JN, Han RW, Wang R. Neuropeptide S produces antinociceptive effect at the supraspinal level in mice. Regul Pept 2009;156:90–5
28. Ruggieri V, Filaferro M, Romano M, Guerrini R, Trapella C, Salvadori S, Rizzi A, Calò G, Vitale G. Neuropeptide S evokes anxiolytic-like effects in rats via NPSR selective activation. 34° Congresso Nazionale della Società Italiana di Farmacologia, Rimini, October 14–17, 2009
29. Fedeli A, Barconi S, Economidou D, Cannella N, Kallupi M, Guerrini R, Calò G, Cifani C, Massi M, Ciccocioppo R. 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
30. Ruzza C, Rizzi A, Trapella C, Pelà M, Camarda V, Ruggieri V, Filaferro M, Cifani C, Reinscheid RK, Vitale G, Ciccocioppo R, Salvadori S, Guerrini R, Calò G. Further studies on the pharmacological profile of the neuropeptide S receptor antagonist SHA 68. Peptides 2010;31:915–25