Adult male Japanese quails (*Coturnix coturnix japonica*), weighing 110–120 g, were reared in individual net cages (*W*: 14 × *L*: 26 × *H*: 17 cm) in a room with a 12-h light (300 lx)/12-h dark (dim light, 25 lx) period (lights on at 07:00 h), at a temperature of 28 ± 1 °C. The birds were given free access to food and water. Rat NMS or rat NMU (Peptide Institute, Osaka, Japan) was dissolved in 0.9% saline and several doses were administered i.c.v. to each of six free-feeding male birds in each experimental group. Each experiment was set for measurement only one parameter to avoid the effect of one parameter on the other. All the experiments were performed twice in order to confirm the results obtained in each experiment. We performed 1-week interval between the first and second time experiment to avoid the residual effects of repeated injection. All procedures were performed in accordance with the Japanese Physiological Society's guidelines for animal care.

For implantation of the i.c.v. cannula, each bird was anesthetized with 5% sodium pentobarbital (1.4 μl/g body weight) and placed in a stereotaxic frame. A stainless steel guide cannula (outer diameter: 550 μm; length: 14 mm) was stereotaxically implanted into the third cerebral ventricle using a modification of a previously reported method [1]. The coordinates were 5 mm anterior to the interaural axis and 6.5 mm below the dura at the midline. One stainless steel anchoring screw was fixed to the skull, and the guide cannula was secured in place with acrylic dental cement. The birds were returned to their individual cages and allowed to recover for at least 4 days. They were acclimatized to handling every day before the start of the experiments. The i.c.v. injections were administered through the implanted guide cannulae without anesthesia or restraining of the birds. At the end of the experiments, proper placement of the cannulae was verified by administering Evans Blue dye (10 μl), followed by sacrifice and brain sectioning (20 μm intervals). Data for birds lacking dye in the third ventricle were excluded from the analysis.

Before the feeding experiment, the birds were weighed and assigned to an experimental group based on their body weight. The average body weight (110–120 g) in each group was kept as uniform as possible. To examine the orexigenic or anorexic effect of NMS, rat NMS (0.1, 0.5 or 1.0 nmol/10 μl saline) or saline (control) was administered i.c.v. at 07:00 h. Food consumption was determined in the free-fed birds at 2, 4 and 12 h after administration by measuring the disappearance of food from a pre-weighed feeder placed in each individual cage. Care was taken to collect and weigh any spillage, thus making the determination of food intake as accurate as possible.

The quails' body temperature was measured at 0 min (before injection), then at 5, 10, 20, 40, 60 and 120 min after i.c.v. injection of rat NMS, rat NMU (each at doses of 0.1, 0.5 or 1.0 nmol/10 μl saline) or saline vehicle ($n = 6$ in each group) at 10:00 h using a previously reported method [1]. Briefly, temperature was measured electronically with a small sensor (measurable range: 25–50 °C; measurement error: 0.05 °C) connected to a line (outer diameter: 0.7 mm; length: 45 cm). The sensor tip was inserted into the cloaca, and part of the line was fixed to the body of the bird.

Locomotor activity was measured in each bird under light/dark conditions for 1 week, and thereafter under constant dim light at an intensity of about 30 lx. Locomotion was measured using a rat locomotor activity recording system (Muromachi Co. Ltd., Tokyo, Japan) comprising infrared sensors, an interface and a computer [8]. The infrared sensors were placed above the cages and measured all locomotor activity (e.g. eating, perch-hopping and flying). Each cage with its infrared sensor was placed in an isolated chamber with a controlled light/dark cycle. Data were collected at 15-min intervals and analyzed using CompactACT AMS software (Muromachi Co.). Rat NMS, rat NMU (each at doses of 0.1, 0.5 or 1.0 nmol/10 μl saline) or saline vehicle was administered i.c.v. at 10:00 h ($n = 8$ per group). After the injections, the birds were immediately returned to their individual cages. Locomotor activity counts were made every 15 min and summed for the 2-h period following administration.

All results are expressed as mean ± S.E.M. The data were analyzed using analysis of variance and the post hoc Fisher's test.

I.c.v. administration of NMS 0.5 and 1 nmol significantly ($P < 0.05$) decreased food intake in a time-dependent manner compared with saline alone (Fig. 1A). This anorexigenic action of NMS was apparent by 2 h and continued for 12 h after i.c.v. administration. The effect was no longer observable on the following day (data not shown). Concomitantly, a significant ($P < 0.05$) decrease in body weight was observed at 2, 4 and 12 h after i.c.v. injection of NMS (Fig. 1B). The decrease in body weight was more pronounced than the decrease in food intake, and became quite considerable by 12 h after the injection. The effect of a smaller dose of NMS (0.01 nmol i.c.v.) was examined, but this dose effected no significant change in food intake ($n = 6$; data not shown).

I.c.v. injection of NMS also significantly ($P < 0.05$) increased body temperature and locomotor activity (Fig. 1C and D). An increment of about 2 °C was observed in body temperature 40–60 min after i.c.v. injection of 1.0 nmol NMS. Although 0.1 nmol NMS also caused an increase in body temperature, the change was not significantly different from that seen with saline alone. Locomotor activity was increased 1.5-fold during the 2-h period following i.c.v. injection of 1 nmol NMS.

When the effects of i.c.v. injection of the same doses of rat NMU and rat NMS on food intake, body temperature and locomotor activity were compared in Japanese quails, opposite effects were observed. Fig. 2 shows that rat NMU produced an increase in food intake but decreases in body temperature and locomotor activity.

The present study demonstrates that rat NMS suppresses food intake but promotes locomotor activity and increases body temperature in avian species. The suppression of feeding is unlikely to be due to any side effect of NMS, since the quails in the treated group did not show any abnormal behavior. The noticeable decrease in body weight after i.c.v. injection of NMS may be due to both a decrease in food intake and an increase in energy expenditure. These results therefore suggest that central NMS may play important roles in the regulation of feeding and the sympathetic nervous system in avian species.

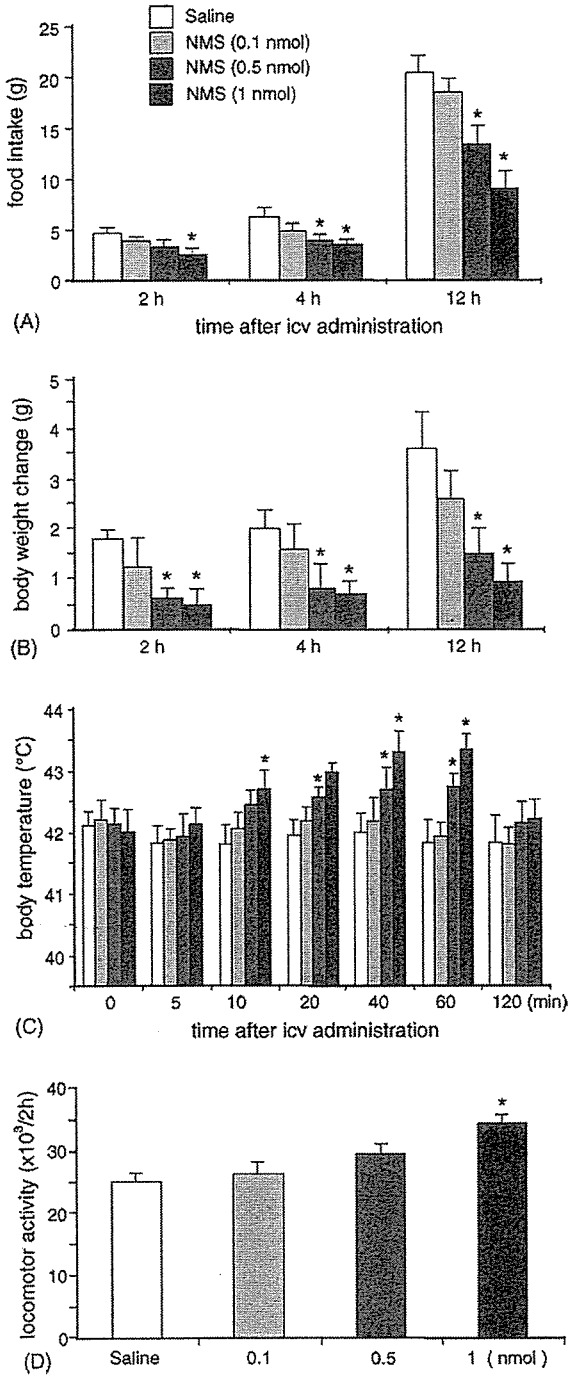

Fig. 1. Effect of intracerebroventricular (i.c.v.) administration of rat NMS on food intake (A), body weight change (B), body temperature (C), and gross locomotor activity (D) in the Japanese quail. Saline (vehicle control) or NMS (0.1, 0.5 or 1.0 nmol) was injected i.c.v. at 07:00 h for food intake assessments or 10:00 h for body temperature and gross locomotor activity assessments. Each bar and vertical line represents the mean ± S.E.M. ($n = 12$ for food intake assessments; $n = 6$ for body temperature assessments and $n = 8$ for gross locomotor activity assessments). * Significantly different from the saline-treated group; $P < 0.05$.

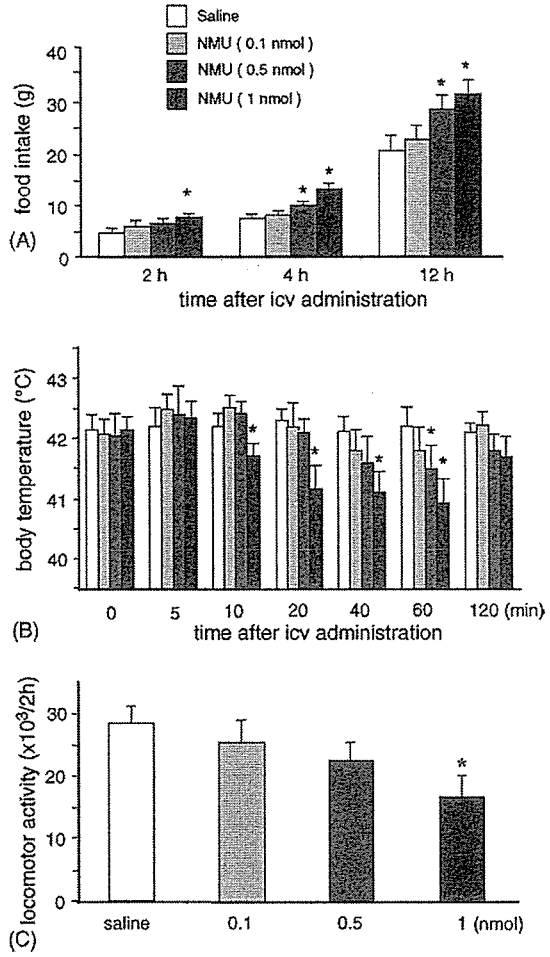

Fig. 2. Effect of i.c.v. administration of rat NMU on food intake (A), body temperature (B) and gross locomotor activity (C) in the Japanese quail. Saline (vehicle control) or NMU (0.1, 0.5 or 1.0 nmol) was injected i.c.v. at 07:00 h for food intake assessments or 10:00 h for body temperature and gross locomotor activity assessments. Each bar and vertical line represents the mean ± S.E.M. ($n = 12$ for food intake assessments; $n = 6$ for body temperature assessments and $n = 8$ for gross locomotor activity assessments). * Significantly different from the saline treated group; $P < 0.05$.

In a previous study, the distribution of NMS mRNA in various rat tissues was investigated using a quantitative reverse-transcriptase polymerase chain reaction technique [9]. NMS mRNA was expressed mainly in the hypothalamus, spleen and testis. Within the hypothalamus, NMS mRNA was expressed predominantly in the SCN; there was only very slight expression in other brain regions, such as the paraventricular nucleus (PVN) and arcuate nucleus (Arc) [9]. In situ hybridization histochemistry also showed that NMS mRNA expression was restricted to the SCN. No hybridization signal was observed in any other brain region [9]. In the case of NMS, therefore, its action on the PVN and Arc through the NMS projection from the SCN may be important.

Recently, we observed that i.c.v. NMS also suppressed food intake in rats [4]. In that case, cFos expression was detected in preopiomelanocortin (POMC)-neuron in the arcuate nucleus and corticotropin-releasing hormone (CRH)-secreting cells in

the paraventricular nucleus. This suggests that neuron containing POMC (a precursor of α-melanocyte-stimulating hormone; α-MSH) and CRH may be the targets for suppression of food intake by NMS, because CRH and α-MSH are known to be anorexigenic hormones in chicken [6,13]. However, further study is required to elucidate the mechanism of action of NMS in avian species.

Because NMS contains the active core C-terminus of NMU and binds to the same receptors (NMU1R and NMU2R), rat NMS and rat NMU would be expected to have very similar actions on food intake, locomotor activity and body temperature in Japanese quails. However, opposite effects were observed. Previously, we reported that Japanese quail NMU, but not rat NMU, suppressed food intake in Japanese quails, and that pretreatment with rat NMU inhibited the Japanese quail NMU-induced suppression of food intake [11]. Rat NMU therefore appears to have an antagonistic action on Japanese quail NMU, possibly through competition for NMU receptors. If this is so, why did rat NMS not show similar antagonism? The reason for the discrepancy is unclear from the present study; however, the following considerations may provide possible explanations. First, the structure of avian NMS may be close to that of rat NMS. If this is so, rat NMS may not act antagonistically at NMU receptors, and may be able to have same physiological function as avian NMS. Although we tried cloning Japanese quail NMS using essentially the same method as that used for cloning Japanese quail NMU [11], we were unsuccessful and could not therefore perform direct experiments with Japanese quail NMS. Second, there may be a specific receptor for NMS other than the NMU1R and NMU2R, and NMS may act on feeding and locomotion through it.

In conclusion, NMS, a novel peptide, appears to play important roles in the regulation of feeding, locomotor activity and body temperature in avian species. As this is the first paper to describe the actions of NMS in avian species, further research will be required to elucidate the exact mechanisms of action of NMS and any further physiological functions that it may have.

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References

[1] J.D. Bayle, F. Ramade, J. Oliver, Stereotaxic topography of the brain of the quail (*Coturnix coturnix japonica*), J. Physiol. (Paris) 68 (1974) 219–241.

[2] R. Hanada, H. Teranishi, J.T. Pearson, M. Kurokawa, H. Hosoda, N. Fukushima, Y. Fukue, R. Serino, H. Fujihara, Y. Ueta, M. Ikawa, M. Okabe, N. Murakami, M. Shirai, H. Yoshimatsu, K. Kangawa, M. Kojima, Neuromedin U has a novel anorexigenic effect independent of the leptin signaling pathway, Nat. Med. 10 (2004) 1067–1073.

[3] A.D. Howard, R. Wang, S.S. Pong, T.N. Mellin, A. Strack, X.M. Guan, Z. Zeng, D.L. Williams Jr., S.D. Feighner, C.N. Nunes, B. Murphy, J.N. Stair, H. Yu, Q. Jiang, M.K. Clements, C.P. Tan, K.K. McKee, D.L. Hreniuk, T.P. McDonald, K.R. Lynch, J.F. Evans, C.P. Austin, C.T. Caskey, L.H.T. Van der Ploeg, Q. Liu, Identification of receptors for neuromedin U and its role in feeding, Nature 406 (2000) 70–74.

[4] T. Ida, K. Mori, M. Miyazato, Y. Egi, S. Abe, K. Nakahara, M. Nishihara, K. Kangawa, N. Murakami, Neuromedin S is a novel anorexigenic hormone. Endocrinology, available online: 23 June 2005.

[5] T.R. Ivanov, C.B. Lawrence, P.J. Stanley, S.M. Luckman, Evaluation of neuromedin U actions in energy homeostasis and pituitary function, Endocrinology 143 (2002) 3813–3821.

[6] S.-I. Kawakami, T. Bungo, R. Ando, A. Ohgushi, M. Shimojo, Y. Masuda, M. Furuse, Central administration of alpha-melanocyte stimulating hormone inhibits fasting- and neuropeptide Y-induced feeding in neonatal chicks, Eur. J. Pharmacol. 398 (2000) 361–364.

[7] M. Kojima, R. Haruno, M. Nakazato, Y. Date, N. Murakami, R. Hanada, H. Matsuo, K. Kangawa, Purification and identification of neuromedin U as an endogenous ligand for an orphan receptor GPR66 (FM3), Biochem. Biophys. Res. Commun. 276 (2000) 435–438.

[8] N. Marumato, N. Murakami, T. Katayama, H. Kuroda, T. Murakami, Effect of daily injections of melatonin on locomotor activity rhythms in rats maintained under constant bright or dim light, Physiol. Behav. 60 (1996) 767–773.

[9] K. Mori, M. Miyazato, T. Ida, N. Murakami, R. Serino, Y. Ueta, M. Kojima, K. Kangawa, Identification of neuromedin S and its possible role in the mammalian circadian oscillator system, EMBO J. 24 (2005) 325–335.

[10] M. Nakazato, R. Hanada, N. Murakami, Y. Date, M.S. Mondal, M. Kojima, H. Yoshimatsu, K. Kangawa, S. Matsukura, Central effects of neuromedin U in the regulation of energy homeostasis, Biochem. Biophys. Res. Commun. 277 (2000) 191–194.

[11] S. Shousha, K. Nakahara, M. Miyazato, K. Kangawa, N. Murakami, Endogenous neuromedin U has anorectic effects in the Japanese quail, Gen. Comp. Endocrinol. 140 (2005) 156–163.

[12] A.M. Wren, C.J. Small, C.R. Abbott, P.H. Jethwa, A.R. Kennedy, K.G. Murphy, S.A. Stanley, A.N. Zollner, M.A. Ghatei, S.R. Bloom, Hypothalamic actions of neuromedin U, Endocrinology 143 (2002) 4227–4234.

[13] R. Zhang, T. Nakanishi, A. Ohgushi, R. Ando, T. Yoshimatsu, D.M. Denbow, M. Furuse, Suppression of food intake induced by corticotropin-releasing factor family in neonatal chicks, Eur. J. Pharmacol. 427 (2001) 37–41.