Biochemical and Biophysical Research Communications **289,** 225–228 (2001)
doi:10.1006/bbrc.2001.5945, available online at http://www.idealibrary.com on IDEAL®

A Role for Neuromedin U in Stress Response

Reiko Hanada,* Masamitsu Nakazato,†,[1] Noboru Murakami,‡ Satoru Sakihara,§ Hironobu Yoshimatsu,* Koji Toshinai,† Takeshi Hanada,¶ Toshihiro Suda,§ Kenji Kangawa,‖ Shigeru Matsukura,† and Toshiie Sakata*

**First Department of Internal Medicine, Oita Medical University, Oita 879-5593, Japan; †Department of Internal Medicine, Miyazaki Medical College, Miyazaki 889-1692, Japan; ‡Department of Veterinary Physiology, Faculty of Agriculture, Miyazaki University, Miyazaki 889-2192, Japan; §Third Department of Internal Medicine, Hirosaki University School of Medicine, Hirosaki 036-8562, Japan; ¶Suntory Institute for Medicinal Research & Development, Gunma 370-0503, Japan; and ‖Department of Biochemistry, National Cardiovascular Center Research Institute, Osaka 565-8565, Japan*

Received October 2, 2001

Neuromedin U (NMU) is a hypothalamic peptide that has been recently found to reduce food intake, but few is known about its other functions in the central nervous system. We here studied behavioral activities induced by an intracerebroventricular (ICV) administration of NMU in rats and mice. NMU increased gross locomotor activity, face washing behavior, and grooming. NMU-induced stress response was significantly abolished by pretreatment with an antagonist of corticotropin-releasing hormone (CRH), α-helical CRH (9–41) (α-hCRH), or anti-CRH IgG. NMU did not induce locomotor activity in CRH knockout mice. NMU that interacts anatomically and/or functionally with the CRH system is a novel physiological regulator of stress response.

Key Words: **neuromedin U; hypothalamus; CRH; stress; CRH KO mice.**

NMU, a 23-amino-acid peptide, was first isolated as a smooth-muscle-contracting peptide from the porcine spinal cord and later from the brains of other species (1). Two receptors for NMU, NMU1R, and NMU2R, have recently been identified by using an intracellular calcium influx assay in a cell line expressing NMU1R (2–7). Rat NMU1R is expressed at a low level in the brain, but NMU2R is abundantly expressed in the hypothalamic paraventricular nucleus (PVN), along the wall of the third ventricle in the hypothalamus, and CA1 region of the hippocampus (2). We and another group have shown that an ICV administration of NMU suppressed feeding in rats (2, 3, 8). The PVN is a major part that produces CRH, a 41-amino-acid peptide, which functions in stress response, anorectic behavior, autonomic regulation, and the hypothalamo-pituitary-adrenal axis (9, 10). An ICV injection of CRH to rodents increased gross locomotor activity, especially grooming, and face washing behavior (11–13). We here studied behavioral activities of NMU given to rats centrally. We also investigated the functional relationship between NMU and CRH in the stress response. Furthermore, we examined the effect of NMU on behavioral responses to stress by the use of CRH-deficient (knockout) mice.

[1] To whom correspondence should be addressed. E-mail: nakazato@post.miyazaki-med.ac.jp.

MATERIALS AND METHODS

Rat experiment. Ten-week-old male Wistar rats weighing 300–325 g (Charles River Japan, Inc., Shiga, Japan) were maintained in individual cages under controlled temperature (21–23°C) and light (light on 0700–1900 h) with *ad libitum* access to chow and water. ICV cannulae were implanted into the lateral cerebral ventricle. Proper placement of the cannulae was verified at the end of the experiment by the administration of dye. Rat NMU (M_r = 2641.3) was synthesized by the solid phase technique in our laboratory. All experiments were performed twice. All procedures were done in accordance with the Japanese Physiological Society's guidelines for animal care.

First, movement of rats (n = 8 per group) that had received an ICV administration of NMU (1 nmol), CRH (1 nmol), or vehicle was measured using a Rat Locomotor Activity Recording Systems Device (Muromachi Co. Ltd., Tokyo, Japan) as described previously (14). After materials were injected to free-moving rats at 0900 h, they were immediately returned to the individual sound- and light-proof cages equipped with infrared light-beam detectors. Locomotor activity counts were made every 15 min and summed up for 60 min after administration.

Second, rats (n = 10 per group) were administered an ICV injection at 0900 h with the following reagents: NMU 1 nmol, NMU 1 nmol + α-hCRH (50 μg, Sigma Chemical Co., St. Louis, MO), NMU 1 nmol + anti-CRH IgG (1 μg, Peptide Institute, Inc., Osaka, Japan), NMU 1 nmol + control serum IgG (1 μg), CRH 1 nmol (Peptide Institute, Inc.), CRH 1 nmol + anti-CRH IgG, CRH 1 nmol + control serum IgG. IgG was injected 2 h before peptide administration. Locomotor activity counts were made as above.

Third, rats (n = 6 per group) were administered an ICV injection at 0900 h with following reagents: 5 nmol NMU, 5 nmol NMU + 100

0006-291X/01 $35.00

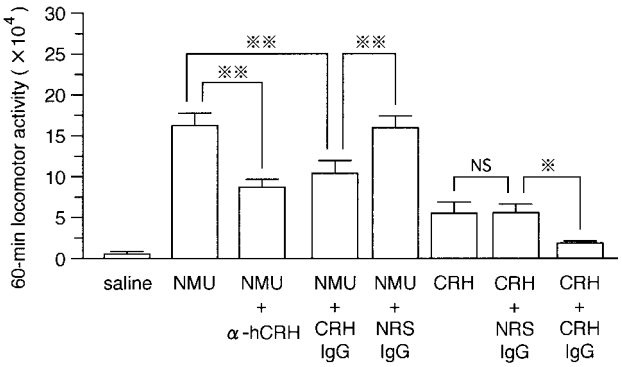

FIG. 1. NMU-induced locomotor activity and effects of α-hCRH and anti-CRH IgG on NMU-induced locomotor activity. Total locomotor activity count was measured for 1 h after injection. *$P < 0.001$; **$P < 0.0001$. NS, not significant.

μg α-hCRH, or saline. The behavior of the rats was monitored for 2 h with a video camera and recorded on videotape. Face washing and grooming were defined as follows (15): face washing, rubbing and stroking the face and head (with) both forepaws; grooming, face grooming with the hindpaws, cleaning the hindlegs, body, tail, and genitals. The behavioral analysis was performed according to two criteria. First, the total time taken carrying out each type of behavioral activity during 2 h period was measured. Second, the time taken carrying out each type of behavioral activity was measured during consecutive 10-min intervals.

Mouse experiment. CRH knockout (KO) mice generated by targeted mutation in embryonic stem cells (16) and wild-type mice (n = 4 per group, 6-month-old male) were housed individually in sound- and light-proof cages equipped with infrared light-beam detectors. ICV cannulation was done as described above. Movement of mice given an ICV administration of NMU (2.5 nmol/2 μl saline) or the vehicle was measured in the same Locomotor Activity Recording Systems Device used above. Locomotor activity counts were made every 15 min and summed up from 30 min to 150 min after administration.

Statistics. Comparisons between groups of data (mean ± SEM) were made using ANOVA with a *post-hoc* Fisher's test. Time-course curves were compared by analysis of variance followed by Duncan's multiple range test. $P < 0.05$ was accepted as statistically significant.

RESULTS

A single ICV administration of NMU markedly increased total locomotor activity in rats (Fig. 1). CRH also increased locomotor activity but the NMU-induced locomotor activity count was three times that induced by the same molar dose of CRH. Although administration of either α-hCRH or anti-CRH IgG also had no effect on locomotor activity (data not shown), they significantly reduced NMU-induced locomotor activity (Fig. 1). Anti-CRH IgG also canceled CRH-induced locomotor activity.

An ICV administration of NMU robustly increased face washing behavior and grooming for 2 h (Fig. 2A). α-hCRH significantly reduced NMU-induced behavior. Face washing and grooming began within 10 min after NMU injection and lasted for 2 h after the injection (Fig. 2B).

An ICV administration of NMU increased total locomotor activity in wild-type mice (Fig. 3A). Neuromedin U-induced locomotor activity lasted for 2 h after the injection (Fig. 3, upper panel). In contrast, NMU did not affect locomotor activity in CRH KO mice.

DISCUSSION

We studied here behavioral activities of NMU administered centrally to rats and mice. NMU increased gross locomotor activity and induced certain stress reaction, such as face washing and grooming. NMU2R mRNA is expressed in the PVN, ependymal layer in the wall of the third ventricle, and CA1 layer of the hippocampus (2). NMU-immunoreactive fibers are abundantly projected to the PVN (17), which produces CRH that induces anxiety-related behaviors such as face washing, grooming, and gross locomotor activity when administered ICV to rats (10–13). We thus studied the functional relationship between NMU and CRH. The anti-CRH IgG and antagonist of CRH partially abolished NMU-induced stress reaction. NMU induced gross locomotor activity in wild-type mice but not in CRH KO mice. Taken together, NMU is thought to

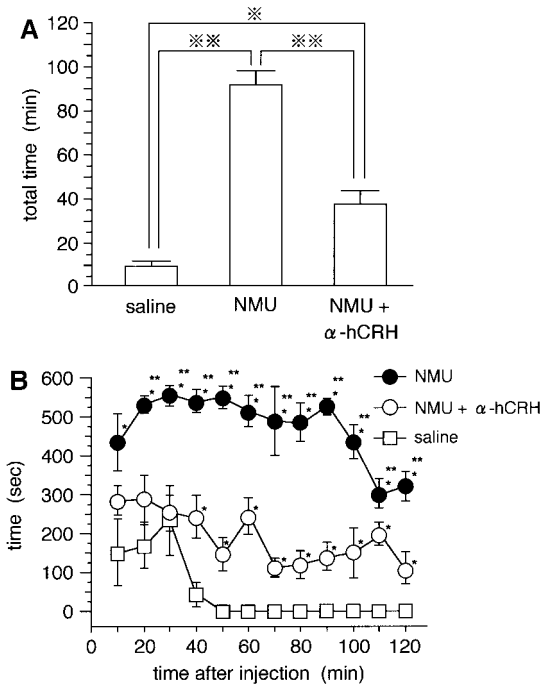

FIG. 2. Effects of ICV injections of 5 nmol NMU and 5 nmol NMU + 100 μg α-hCRH on face washing and grooming behavior. (A) Total time spent carrying out face washing and grooming during a 2 h period after administration of peptides or saline. *$P < 0.005$; **$P < 0.001$. (B) Time course of face washing and grooming after administration. *$P < 0.001$ vs saline; **$P < 0.01$ vs NMU + α-hCRH.

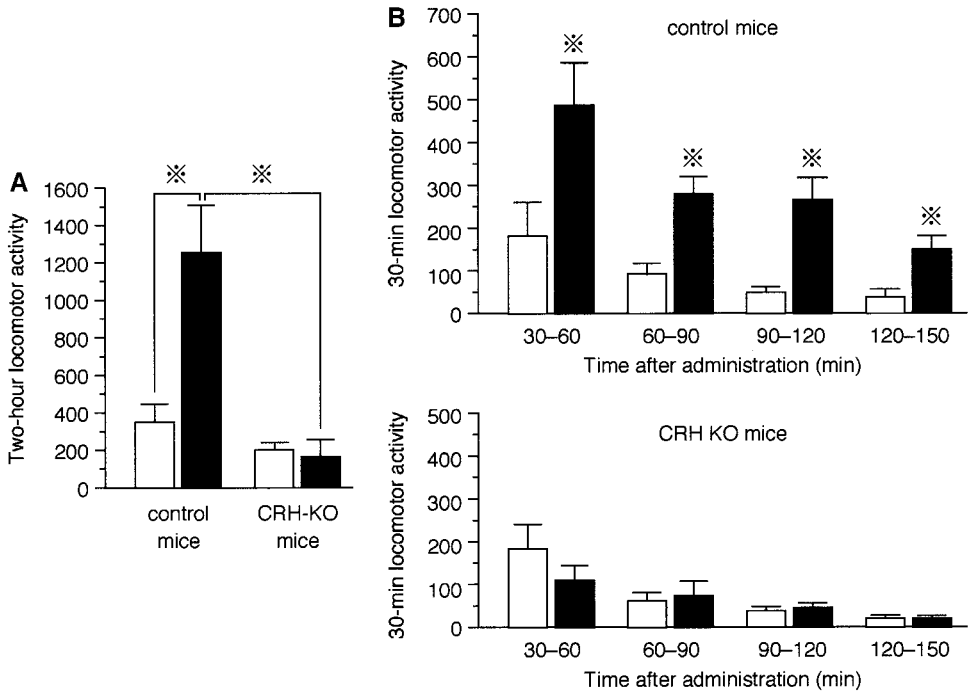

FIG. 3. Movement of CRH KO mice and wild-type mice given ICV administration of saline (white bars) or 2.5 nmol NMU (black bars). (A) Total locomotor activity counts for 2 h after administration and (B) every 30-min locomotor activity counts after administration. The first 30-min locomotor activity was not used in analysis, because locomotor activity was stimulated nonspecifically during the first 30 min even when control vehicle was administered to mice. $*P < 0.05$ in (A). $*P < 0.05$ vs saline-injected group in (B).

interact anatomically and/or functionally with the pathway of CRH. A central administration of NMU suppressed food intake in rats (2, 3, 8). CRH is also capable of acting centrally to inhibit feeding (12). Further investigation is needed to define the interaction between NMU and CRF in the regulation of feeding.

In addition to NMU and CRH, many neuropeptides such as adrenocorticotropic hormone (ACTH), alpha-melanocyte-stimulating hormone, bombesin, cholecystokinin, and oxytocin induce stress reaction when administered centrally (18, 19). In the first stage of stress reaction, the anterior pituitary secretes ACTH to stimulate the adrenal cortex, thereby releasing glucocorticoids. If exposure to a stressor is prolonged, a second stage of stress response develops during which synthesis and secretion of glucocorticoids and catecholamines increase, facilitating metabolic processes crucial for survival, whereas inhibiting inessential ones, including feeding. NMU stimulates the secretion of ACTH (20). NMU as well as all the above peptides suppress feeding, which appears to adapt to stressful environment.

Neuromedin U-producing cells are most abundant in the pituitary, and next in the arcuate nucleus, median eminence, solitary tract, area postrema, dorsal motor nucleus of vagus, inferior olive, and spinal cord (2, 17), suggesting that the NMU system is in a position to modulate the autonomic functions. To examine this possibility, we investigated the systemic effects of centrally administered NMU on the autonomic nervous system. NMU increased body temperature and heat production in conscious unrestrained rats (8). An ICV administration of NMU also dose-dependently increased heart rate and blood pressure (H. Kannan and M.N., unpublished observations). The NMU system is probably involved in the central regulation of autonomic functions. In conclusion, NMU may be a novel physiological regulator of stress response and autonomic functions.

ACKNOWLEDGMENTS

This study was supported in part by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science, and Technology, Japan, the Ministry of Health, Labour, and Welfare, Japan, the Novartis Foundation (Japan) for the Promotion of Science, and Mitsui Life Foundation to M.N.

REFERENCES

1. Minamino, N., Kangawa, K., and Matsuo, H. (1985) Neuromedin U-8 and U-25: Novel uterus stimulating and hypertensive peptides identified in porcine spinal cord. *Biochem. Biophys. Res. Commun.* **130,** 1078–1085.
2. Howard, A. D., Wang, R., Pong, S. S., Mellin, T. N., Strack, A., Guan, X. M., Zeng, Z., Williams, D.L., Jr., Feighner, S. D., Nunes, C. N., Murphy, B., Stair, J. N., Yu, H., Jiang, Q., Clements, M. K., Tan, C. P., Mckee, K. K., Hreniuk, D. L., McDonald, T. P., Lynch, K. R., Evans, J. F., Austin, C. P., Caskey, C. T.,

Ploeg, L. H. T. V., and Liu, Q. (2000) Identification of receptors for neuromedin U and its role in feeding. *Nature* **406,** 70–74.

3. Kojima, M., Haruno, R., Nakazato, M., Date, Y., Murakami, N., Hanada, R., Matsuo, H., and Kangawa, K. (2000) Purification and identification of neuromedin U as an endogenous ligand for an orphan receptor GPR66 (FM3). *Biochem. Biophys. Res. Commun.* **276,** 435—438.
4. Szekeres, P. G., Muir, A. I., Spinage, L. D., Miller, J. E., Butler, S. I., Smith, A., Rennie, G. I., Murdock, P. R., Fitzgerald, L. R., Wu, H-l., McMillan, L. J., Guerrera, S., Vawter, L., Elshourbagy, N. A., Mooney, J. L., Bergsma, D. J., Wilson, S., and Chambers, J. K. (2000) Neuromedin U is a potent agonist at the orphan G protein-coupled receptor FM3. *J. Biol. Chem.* **275,** 20247–20250.
5. Fujii, R., Hosoya, M., Fukusumi, S., Kawamata, Y., Habata, Y., Hinuma, S., Onda, H., Nishimura, O., and Fujino, M. (2000) Identification of neuromedin U as the cognate ligand of the orphan G protein-coupled receptor FM-3. *J. Biol. Chem.* **275,** 21068–21074.
6. Raddatz, R., Wilson, A. E., Artymyshyn, R., Bonini, J. A., Borowsky, B., Boteju, L. W., Zhou, S., Kouranova, E. V., Nagorny, R., Guevarra, M. S., Dai, M., Lerman, G. S., Vaysse, P. J., Branchek, T. A., Gerald, C., Forray, C., and Adham, N. (2000) Identification and characterization of two neuromedin U receptors differentially expressed in peripheral tissues and the central nervous system. *J. Biol. Chem.* **275,** 32452–32459.
7. Shan, L., Qiao, X., Crona, J. H., Behan, J., Wang, S., Laz, T., Bayne, M., Gustafson, E. L., Monsma, F. J., Jr., and Hedrick, J. A. (2000) Identification of a novel neuromedin U receptor subtype expressed in the central nervous system. *J. Biol. Chem.* **275,** 39482–39486.
8. Nakazato, M., Hanada, R., Murakami, N., Date, Y., Muhtashan, S. M., Kojima, M., Yoshimatsu, H., Kangawa, K., and Matsukura, S. (2000) Central effects of neuromedin U in the regulation of energy homeostasis. *Biochem. Biophys. Res. Commun.* **277,** 191–194.
9. Vale, W., Spiess, J., Rivier, C., and Rivier, J. (1981) Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. *Science* **213,** 1394–1397.
10. Sutton, R. E., Koob, G. F., Le Moal, M., Rivier, J., and Vale, W. (1982) Corticotropin releasing factor produces behavioural activation in rats. *Nature* **297,** 331–333.
11. Britton, D. R., Koob, G. F., Rivier, J., and Vale, W. (1982) Intraventricular corticotropin-releasing factor enhances behavioral effects of novelty. *Life Sci.* **31,** 363–367.
12. Morley, J. E., and Levine, A. S. (1982) Corticotrophin releasing factor, grooming, and ingestive behavior. *Life Sci.* **31,** 1459–1464.
13. Dunn, A. J., Berridge, C. W., Lai, Y. I., and Yachabach, T. L. (1987) CRF-induced excessive grooming behavior in rats and mice. *Peptide* **8,** 841–844.
14. Murakami, N., Marumoto, N., Nakahara, K., and Murakami, T. (1997) Daily injections of melatonin entrain the circadian activity rhythms of nocturnal rats but not diurnal chipmunks. *Brain Res.* **775,** 240–243.
15. Ida, T., Nakahara, K., Murakami, T., Hanada, R., Nakazato, M., and Murakami, N. (2000) Possible involvement of orexin in the stress reaction in rats. *Biochem. Biophys. Res. Commun.* **270,** 318–323.
16. Muglia, L., Jacobson, L., Dikkes, P., and Majzoub, J. A. (1995) Corticotropin-releasing hormone defeciency reveals major fetal but not adult glucocorticod need. *Nature* **373,** 427–432.
17. Domin, J., Yiangou, Y., Spokes, R. A., Aitken, A., Parmar, K. B., Chrysanthou, B. J., and Bloom, S. R. (1989) The distribution, purification, and pharmacological action of an amphibian neuromedin U. *J. Biol. Chem.* **264,** 20881–20885.
18. Kulkosky, P. J., Gibbs, J., and Smith, G. P. (1982) Feeding suppression and grooming repeatedly elicited by intraventricular bombesin. *Brain Res.* **242,** 194–196.
19. Kaltwasser, M. T., and Crawley, J. N. (1987) Oxytocin and cholecystokinin induce grooming behavior in the ventral tegmentum of the rat. *Brain Res.* **426,** 1–7.
20. Malendowicz, L. K., Nussdorfer, G. G., Markowska, A., Tortorella, C., Nowak, M., and Warchol, J. B. (1994) Effects of neuromedin U (NMU)-8 on the rat hypothalamo-pituitary-adrenal axis. *Neuropeptides* **26,** 47–53.