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Central Effects of Neuromedin U in the Regulation of Energy Homeostasis

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Neuromedin U (NMU) is a brain-gut peptide whose peripheral activities are well-understood but whose central actions have yet to be clarified. The recent identification of two NMU receptors in rat brain has provided a springboard for further investigation into its role in the central nervous system. Intracerebroventricular administration of NMU to free-feeding rats decreased food intake and body weight. Conversely, NMU increased gross locomotor activity, body temperature, and heat production. NMU, a potent endogenous anorectic peptide, serves as a catabolic signaling molecule in the brain. Further investigation of the biochemical and physiological functions of NMU will help our better understanding of the mechanisms of energy homeostasis.

Key Words: **neuromedin U (NMU); energy expenditure; locomotor activity; body temperature; heat production.**

Orphan G-protein-coupled receptors (GPCRs) have been used as targets for identification of novel neurotransmitters, which facilitates the discovery of unknown neuronal functions and development of new drugs. Cells expressing an orphan GPCR have been used in assay systems to identify unknown endogenous ligands, such as orexin, orphanin FQ, and prolactin-releasing peptide (1–3). Using an intracellular calcium influx assay in a stable cell line expressing a previously described orphan GPCR, FM-3 (4) (also called GPR66), we and three other groups have recently identified NMU as an endogenous ligand for this receptor (5–8). Another NMU receptor, FM-4, has also been identified in rats and humans (5). FM-3 and FM-4 are cognate receptors for NMU, and have been designated NMU1R and NMU2R, respectively. NMU, a 23-amino-acid peptide, is a smooth-muscle-contracting peptide first isolated from porcine spinal cord and later from the brain, spinal cord, and intestine of other species (9–11). NMU also is involved in the alteration of ion transport in the gut, control of mesenteric blood flow, and regulation of blood pressure and adrenocortical function (12–15). The C-terminal, biologically active region of the NMU peptide is widely conserved across various species.

Rat NMU1R is expressed at relatively high levels in the small intestine and lung, and at a very low level in the brain (5–7). In contrast, the expression of rat NMU2R is mostly restricted to specific regions of the brain, such as the hypothalamic paraventricular nucleus, along the wall of the third ventricle in the hypothalamus and CA1 region of the hippocampus (5). Intracerebroventricular (ICV) administration of NMU to rats suppressed dark-(fasted) phase food intake and fasting-induced feeding (5, 8). Anti-NMU IgG, in turn, increased dark-phase feeding compared with preimmune serum IgG (8). The NMU mRNA level was downregulated upon fasting (5). These results indicate that NMU is a potent endogenous anorectic peptide. NMU neurons were found in the ventromedial hypothalamic regions and some nuclei of the caudal brainstem as well as in the pituitary (5, 16). This widespread distribution of NMU and its receptors suggests the additional, as yet undefined, central roles of NMU. In this paper, we have investigated the physiological role of centrally-administered NMU in rats. In addition to the previously established role in appetite control, NMU

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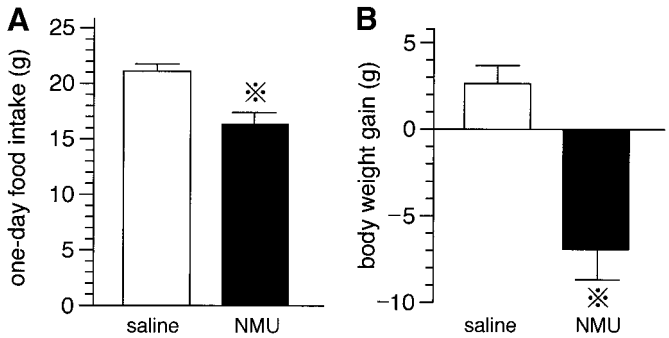

FIG. 1. Effects of rat NMU (1 nmol) on (A) food intake and (B) body weight gain. NMU was administered ICV at 0700 and 1800 h and one-day food consumption and body weight gain were measured at 0700 h the following morning. ※$P < 0.01$.

was found to function in the regulation of energy homeostasis.

MATERIALS AND METHODS

Animals. Male Wistar rats were maintained in individual cages under controlled conditions of temperature (21–23°C) and light-dark cycle (light on 0700–1900 h). Food and water were provided *ad libitum*. ICV cannulae were implanted into the lateral cerebral ventricle. Proper placement of the cannulae was verified at the end of the experiments by dye administration (17). Rats were sham injected before the study, and weighed and handled daily. Only animals that showed progressive weight gain after the surgery were used in subsequent experiments. Rat NMU (M_r = 2641.3) was synthesized by the solid phase technique in our laboratory. Purity of the peptide was ascertained by reverse-phase high performance liquid chromatography, amino acid sequencing, and mass spectrometry (MALDI-MS). It was dissolved in 0.9% saline and 10 μl of this solution was administered ICV to free-feeding male Wistar rats. All experiments were performed twice.

Comparisons between groups of data (mean ± SEM) were made using ANOVA with a *post-hoc* Fisher's test. All procedures were done in accordance with the Japanese Physiological Society's guidelines for animal care.

Feeding and body weight change. NMU (1 nmol/10 μl saline) or vehicle was administered ICV at 0700 and 1800 h to rats (n = 8 per group) weighing 270–280 g (Japan SLC, Inc., Shizuoka, Japan). One-day food consumption and body weight gain were measured at 0700 h the following morning.

Locomotor activity. Rats (n = 10 per group) were housed individually in sound- and light-proof cages equipped with infrared light-beam detectors. Movement of rats that had received an ICV administration of NMU (1 or 5 nmol) or vehicle was measured using a Rat Locomotor Activity Recording Systems device (Muromachi Co. Ltd., Tokyo, Japan) as described previously (18). Locomotor activity counts were made every 15 min and summed up for 180 min after administration.

Body temperature. The body temperature was measured −45 to 120 min after an ICV administration of 1 nmol NMU or saline (n = 8 per group). A sensor tip (measurable range: 25 to 50°C and measuring error: ± 0.02°C) was inserted into the rectum and the digital signal was transferred to Thermometer MT-1 (Senko Co. Ltd., Tokyo, Japan).

Oxygen consumption and energy output. Oxygen consumption and energy output were measured with an Oxymax apparatus (Columbus Instruments, Columbus, OH). Rats (n = 5 per group) were given ICV administrations of 1 nmol NMU or saline, and then were individually returned to a sealed chamber with an air flow of 1 liter per minute for 4 h. The O_2 levels in air going into and out of the chamber were measured every 30 s. The O_2 level in air going out of the chamber became stable 15 min after the rats were returned to the chamber. Oxygen consumption and heat production were measured 15 to 30 min after the ICV administration.

RESULTS AND DISCUSSION

An ICV administration of NMU (1 nmol) to rats decreased food intake to 76% the saline-injected group (16.6 g vs 21.8 g, Fig. 1A). NMU also reduced body weight by 6.9 g one day after its administration, whereas the weight of the rats in the saline-injected group increased (Fig. 1B). A single ICV administration of NMU increased gross locomotor activity in a dose-dependent manner (Fig. 2). NMU-induced locomotor activity lasted more than 3 h. An ICV administration of

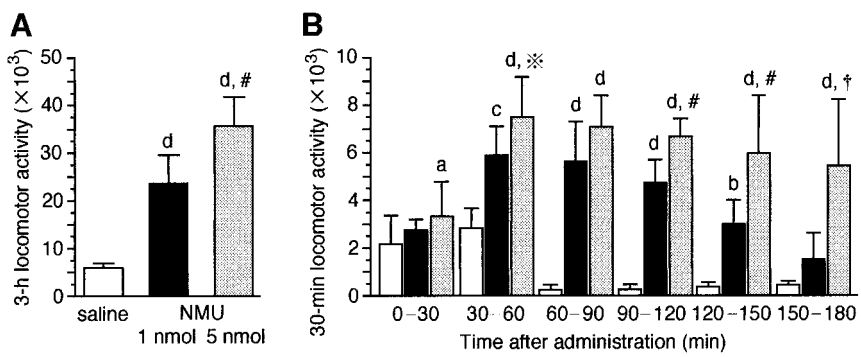

FIG. 2. Movement of rats given an ICV administration of saline (white bars), 1 nmol (black bars) or 5 nmol (dotted bars) NMU. (A) Total locomotor activity counts for 180 min after administration and (B) locomotor activity counts at 30-min intervals after administration. Gross locomotor activity was stimulated non-specifically during the first h even when the control vehicle was administered, but NMU-induced locomotor activity lasted more than 3 h. $^{a}P < 0.05$, $^{b}P < 0.005$, $^{c}P < 0.0005$, $^{d}P < 0.0001$ versus saline-injected group and ※$P < 0.05$, $^{\#}P < 0.001$, $^{\dagger}P < 0.0005$ versus 1 nmol NMU-injected group.

NMU significantly increased body temperature by 1°C for 1 h (Fig. 3). The effects of NMU on oxygen consumption and heat production were measured by indirect calorimetry. Both measures of energy consumption increased upon NMU administration (Fig. 4).

In situ hybridization histochemistry and semiquantitative RT-PCR analysis of NMU mRNA in the rat brain showed that its expression was most abundant in the pituitary, and slightly less in the ventromedial hypothalamic regions (lateral arcuate nucleus and median eminence), caudal brainstem (nucleus of solitary tract, area postrema, dorsal motor nucleus of the vagus and inferior olive) and spinal cord (5, 6). In the arcuate nucleus and median eminence, NMU-producing neurons are coextensive, but not co-localized with pro-opiomelanocortin neurons which produce α-melanocyte-stimulating hormone (α-MSH), a potent anorectic peptide (19). NMU-containing fibers are found throughout the rat brain, with a particularly dense projection to the hypothalamus, thalamus, and the brainstem, suggesting that NMU has multiple unknown activities in the central nervous system.

We here showed that ICV administration of NMU reduced feeding. Although rats given NMU consumed 16.6 g of food on the day of administration, their body weights decreased. This suggested that NMU enhances energy expenditure. To investigate this potent action of NMU, we studied locomotor activity, heat production, and body temperature changes induced by NMU, because exercise and thermogenesis are primary mechanisms of energy expenditure.

ICV administration of NMU indeed increased gross locomotor activity as measured by light-beam detectors. Furthermore, ICV administration of NMU increased body temperature. Heat production by the metabolic systems is increased by shivering and sympathetic excitation in the brown adipose tissue and skeletal muscle. The latter mechanism, also called chemical thermogenesis, is thought to be the primary mechanism of NMU-induced thermogenesis, because shivering was not seen in rats given NMU. A number of studies have now implicated brown adipose tissue as

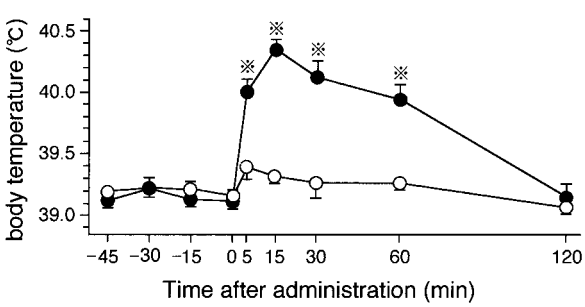

FIG. 3. Change in rectal temperature in response to an ICV administration of NMU (1 nmol). ※$P < 0.0001$ versus saline-injected group (○).

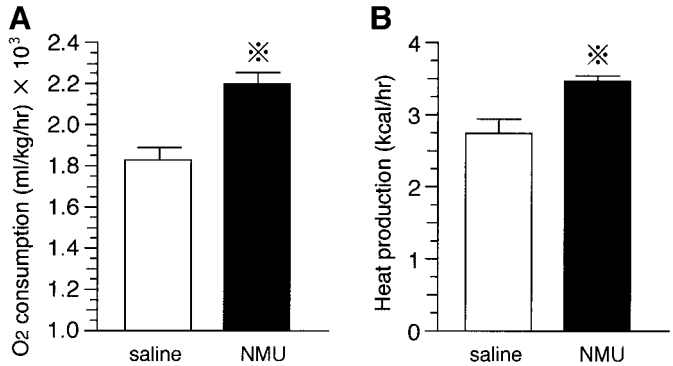

FIG. 4. (A) Oxygen consumption and (B) heat production induced by ICV administration of NMU (1 nmol). These parameters were measured by indirect calorimetry. ※$P < 0.01$ versus saline-injected group.

an important regulator of energy homeostasis in rodents (20–22).

ICV administration of NMU suppresses food intake, and conversely anti-NMU-IgG stimulates feeding (5, 8). Furthermore, NMU mRNA expression in the ventromedial hypothalamus is reduced upon fasting (5). We here showed that NMU increases energy expenditure and reduces body weight. These findings indicate that NMU meets the criteria for a catabolic signaling molecule. Further investigations of the functions of NMU will promote our understanding of physiological feeding mechanisms and should facilitate the study of eating disorders.

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