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Neuromedin S exerts an antidiuretic action in rats

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Abstract

We recently identified neuromedin S (NMS) as an endogenous ligand for the FM-4/TGR-1 receptor. Here, we examined the possible involvement of central NMS in regulation of urinary output and vasopressin (AVP) release in rats. Intracerebroventricular (icv) injection of NMS induced a dose-dependent increase in the plasma level of AVP, followed by a decrease of nocturnal urinary output. Expression of cFos after icv injection of NMS was observed in the supachiasmatic nucleus (SCN), arcuate nucleus, paraventricular nucleus (PVN), and supraoptic nucleus (SON). The cFos expressing cells in PVN and SON, but not SCN, were then double-stained using antibodies against the vasopressin. On the other hand, icv injection of neuromedin U, which also binds to the FM-4/TGR-1 receptor, required a concentration ten times higher than that of NMS in order to exert the same antidiuretic potency. These results suggest that central NMS may exert a physiological antidiuretic action via vasopressin release.

Keywords: Neuromedin S; Neuromedin U; Vasopressin; Antidiuretic action

In addition to neuromedin U, we have recently discovered the novel peptide neuromedin S (NMS) as an endogenous ligand for two orphan G-protein-coupled receptors, FM-3/GPR66 and FM-4/TGR-1, using a reverse-pharmacological technique [1]. FM-3/GPR66 and FM-4/TGR-1 had already been identified as neuromedin U receptor type-1 (NMUR1) and type-2 (NMUR2), respectively [2–4]. Rat NMS is a 36-amino acid neuropeptide that is specifically expressed in the suprachiasmatic nucleus (SCN) [1]. Although NMS shares a C-terminal core structure (7 amino acid residues) with NMU and activates both recombinant NMU1R and NMU2R expressed in Chinese hamster ovary cells, NMS is not a splice variant of neuromedin U, because both the NMS and neuromedin U genes have been mapped to discrete chromosomes. In addition, although neuromedin U mRNA has been detected in peripheral and central organs, the distribution of NMS is limited to the testis, spleen, and hypothalamus, especially the SCN [1–3]. NMS has been shown to have several physiological roles in rats, including involvement in circadian oscillation systems, since intracerebroventricular (icv) administration of NMS induces phase-dependent phase shifts in the circadian rhythm of locomotor activity in rats kept under constant darkness [1]. In addition, NMS may be involved in feeding regulation, because icv injection of NMS decreases food intake in a dose-dependent manner [5]. Recently, it has been shown that NMS regulate luteinizing hormone secretion [6].

NMUR1 is located in a wide range of peripheral tissues such as intestine, testis, pancreas, uterus, lung, and kidney. On the other hand, expression of NMUR2 is limited to areas of the central nervous system [2,3]. Therefore, NMS may have an unknown role in the central nervous system. We examined the expression of cFos after icv injection of

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NMS to search for the site of action of NMS in the central nervous system. When NMS was injected icv, cFos expression was observed specifically in the paraventricular nucleus (PVN), arcuate nucleus (Arc), supraoptic nucleus (SON), and SCN [5]. Although the expression of cFos in the PVN, Arc, and SCN may be related to its biological role in feeding regulation and circadian rhythm mentioned above, its physiological role in the SON is unknown.

On the other hand, the PVN and SON are known to be common sites synthesizing arginine–vasopressin (AVP) and oxytocin (OXT) [7,8]. Recently, NMU2R was detected in AVP-positive neurons in the PVN, indicating a possible role of NMS in the secretion of this hormone [9]. However, the relationship between NMS and AVP remains to be elucidated. AVP synthesized in the magnocellular region of the SON and PVN is exported to the posterior pituitary gland and secreted into the peripheral blood, subsequently acting on the kidney through specific receptors in the distal renal tubule to decrease urine volume. In the present study, we examined the possible involvement of central NMS in AVP secretion and urinary output in rats.

Materials and methods

Animals and icv injection. Male Wistar rats, weighing 300–350 g, were housed in individual Plexiglas cages in an animal room maintained under a constant light–dark cycle (lights on from 7:00 to 19:00 h) and temperature (22 ± 1 °C) for at least one week. Food and water were provided ad libitum. Icv cannulae were implanted into the lateral cerebral ventricles by a method that has been described previously [5]. After surgery, all rats were housed individually in Plexiglas cages. During a 6-day postoperative recovery period, the rats became accustomed to the handling procedure. Rat NMS or rat neuromedin U (Peptide Institute Inc., Osaka, Japan) was dissolved in saline, and 10 μl of the solution was injected into each free-moving rat through a 27-gauge injection cannula connected to a 50-μl Hamilton syringe. All procedures were performed in accordance with the Japan Physiological Society's guidelines for animal care.

Measurement of plasma AVP. Whole blood was collected by decapitation at 5, 60, and 180 min after icv injection of 0.02, 0.2, and 2 nmol NMS at 18:45 h into a tube containing EDTA and the proteinase inhibitor, Aprotinin (Sigma–Aldrich Inc.). Each group consisted of 8 rats. After centrifugation at 4 °C, the plasma was stored at −80 °C until measurement of AVP. AVP concentration was measured using an EIA kit (Assay designs Co., Ann Arbor, MI, USA) following the manufacturer's protocol.

Measurement of urinary volume and water intake. Before measurement, rats ($n = 4$/each group) were maintained individually in metabolic cages for four days to allow them to habituate. Twelve-hour urinary volume and water intake were measured every day in the dark phase, because each was very slight during the light period. After habituation, icv injection of 0.02, 0.2, and 2 nmol of NMS and neuromedin U was performed at 18:45 h, and then urinary volume and water intake were measured at 07:00 on the following morning. The control rats were injected with the same volume of saline. These experiments were repeated twice, and a total of 8 samples were collected in each group.

Immunohistochemistry for FM-4. Immunohistochemical analyses for the NMUR2 in the PVN and SON were performed using a modification of a method described previously [10]. The brain was placed in fixative for 5 days at 4 °C and then transferred to 0.1 M phosphate buffer containing 20% sucrose. Each brain was cut into serial, 18-μm-thick sections at −20 °C with a cryostat. The sections were incubated for 2 days with a rabbit anti-NMUR2 antibody (Abcam Ltd., Cambridge, UK) at 4 °C. Slides were then incubated with Alexa-546-labeled goat–anti-rabbit IgG antibody (Molecular Probes Inc., OR, USA, dilution 1:400). Samples were observed with the aid of an Olympus AX-70 fluorescence microscope (Olympus, Tokyo, Japan).

RT-PCR of FM-3 and FM-4 mRNA in the PVN and SON. The PVN and SON were punched out from the frozen brain slices using a method described previously [11]. Spinal cord was used as a control tissue, because it shows abundant expression of NMUR1 and NMUR2 [12]. Total RNA was extracted from the samples using Trizol reagent (Invitrogen Co., Carlsbad, CA) as described previously [13]. First-strand cDNA was synthesized from 2 μg of total RNA by random primer reverse transcription using a SuperScript III First-strand cDNA synthesis kit (Invitrogen Co.). The resulting cDNA was subjected to PCR amplification using sense and antisense primers specific for FM-3 and FM-4 by using iCycler (582BR: Bio-Rad Laboratories, Tokyo, Japan). The primer sets used for rat NMUR1and NMUR2 were as follows: rat NMUR1 primer set: 5′-CACGACTCCCATAGCCA-3′ (sense), 5′-TCACACCCTGGATCCCTGTT-3′ (antisense); rat NMUR2 primer set: 5′-GATGAATCCCTTGAGGCGAA-3′ (sense), 5′-ATGGCAAACACGAGGACCAA-3′ (antisense). PCR products were electrophoresed on a 2% agarose gel. GAPDH was used as a control housekeeping gene, as reported previously.

Immunofluorescence double staining for AVP and Fos in the PVN and SON. Immunohistochemical staining for AVP and cFos was performed 90 min after icv injection of 0.5 nmol NMS. Frozen brain sections were cut with a cryostat at a thickness of 18 μm. The sections were pretreated with blocking solution comprising 1.5% donkey serum and 3% bovine serum albumin for 1 h, and then incubated for 2 days at 4 °C with rabbit antiserum against rat AVP (Progen Biotechnik, Inc., Heidelberg, Germany) together with goat antiserum against rat cFos (Santa Cruz Biotechnology Inc. Cal., USA). After washing, the sections were incubated with a second antibody solution comprising Alexa-488-labeled anti-rabbit IgG antibody and Alexa-546-labeled donkey anti-goat IgG antibody solution (Molecular Probes, Inc.) for 30 min, followed by observation with a fluorescence microscope (Akisoskope 2plus, Zeiss, Germany).

Statistical analysis. The data (means ± SEM) were analyzed statistically by ANOVA and Student's t test. Differences at $P < 0.05$ were considered statistically significant.

Results and discussion

Plasma AVP levels after icv injection of NMS were significantly increased at a concentration of 0.2 and 2.0 nmol compared with the saline group (Fig. 1). The increase was observed at 5 min after icv injection, and continued for

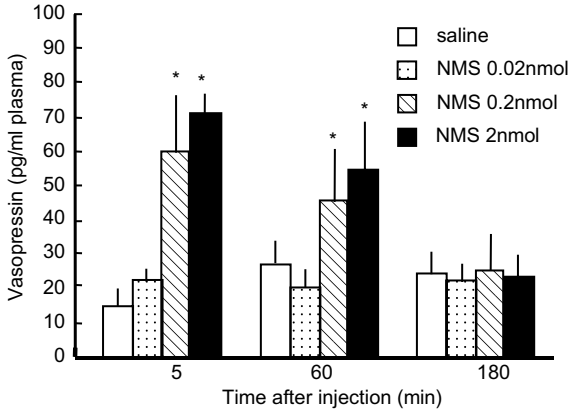

Fig. 1. Effect of icv administration of NMS on plasma AVP levels in rats. Whole blood was collected by decapitation at 5, 60, and 180 min after icv injection of 0.02, 0.2, and 2 nmol NMS or saline at 18:45 h. Each bar and vertical line represent means ± SEM ($n = 8$). Asterisks indicate significant differences from saline group ($^*P < 0.05$).