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Neuromedin U precursor-related peptide (NURP) exerts neuromedin U-like sympathetic nerve action in the rat

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ABSTRACT

It has been suggested that novel peptide that is produced from the neuromedin U (NMU) precursor may exist, as this precursor contains multiple consensus sequences for proteolytic processing. Recently, we identified two mature novel peptides comprising 33 and 36 residues in the rat brain, which were designated neuromedin U precursor-related peptide (NURP) 33 and 36. In the present study, we compared the roles of NURP33 and 36 with that of NMU, as neither activates the NMU receptors. Immunoreactivity for NMU and NURPs was widely present in the central nervous system and showed a similar distribution. Intracerebroventricular (icv) injection of NURP33 in rats increased locomotor activity, energy expenditure, heart rate and back surface temperature (BS-T), similarly to NMU or NURP36. NMU treatment reduced food intake, but NURP33 did not. Pretreatment with the β3 blocker, SR59230A, and the cyclooxygenase blocker, indomethacin, inhibited the NURP33- or NMU-induced increase of BS-T. In addition, icv injection of NURP33 or NMU increased the expression of mRNA for cyclooxygenase 2 in the hypothalamus and for uncoupling protein 1 in the brown adipose tissue. These results suggest that although NURP33 and 36 do not activate the NMU receptors, they might exert NMU-like sympathetic nerve action in the brain.

1. Introduction

Neuromedin U (NMU) was originally isolated from the porcine spinal cord and described as a potent stimulator of uterine contraction [1,2]. In 2000, several groups demonstrated that NMU was the endogenous ligand for two orphan G-protein-coupled receptors, FM-3/GPR66 and FM-4/TGR-1, which were subsequently renamed NMU receptor type 1 (NMU-R1) and 2 (NMU-R2), respectively. NMU-R1 is widely distributed in peripheral tissues such as intestine, testis, pancreas, uterus, lung, and kidney [3–5], whereas NMU-R2 is located mainly in the brain [3,6]. Intracerebroventricular (Icv) administration of NMU affects food intake [3,7–9] and circadian rhythm [10]. In addition, NMU increases energy expenditure, locomotor activity, heart rate and thermogenesis through sympathetic nerve-like action [8,11–13]. NMU-knockout mice have a lower body temperature than wild-type mice [14].

In 2005, Mori et al. suggested the existence of another novel peptide other than NMU that is produced from NMU precursor protein [15], since the NMU precursor protein has four proteolytic processing sites (Fig. 1A). Recently, we identified this additional peptide and designated it neuromedin U precursor-related peptide (NURP) [16]. Two mature NURP peptides comprising 33 and 36 residues were subsequently purified by immunoaffinity chromatography from rat brain. In addition, we showed that NURP did not activate either NMU-R1 or R2. These results suggest that the physiological role of NURP differs from that of NMU [16]. However, the receptor for NURP has yet to be clarified.

In our preliminary studies have indicated that after icv administration of NURP, cFos expression is widely distributed in areas of the central nervous system, such as the cerebral cortex, dorsomedial hypothalamus, posteromedial cortical amygdala, and solitary nucleus. This induced distribution of cFos is similar to that after treatment with NMU. In the present study, therefore, we compared

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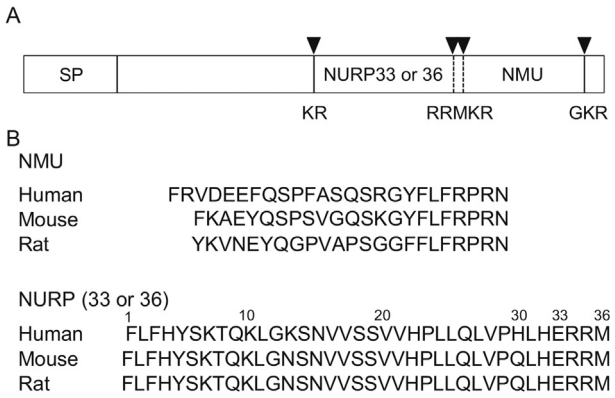

Fig. 1. Schematic representation of the precursor of NMU and NURP (A). The filled arrowheads indicate cleavage sites by proprotein convertase. The sequences of proteolytic processing sites are shown. The amino acid sequences of NMU and NURPs (B).

the effects of icv administration of NURP on food intake, locomotor activity, heart rate, energy consumption and – in particular – thermoregulation with those of NMU, having recently clarified the thermoregulatory mechanism of the latter [12]. Icv administration of NMU increased the back surface temperature (BS-T) through an increase in the expression of mRNAs for prostaglandin E synthase and cyclooxygenase 2 (COX2) in the hypothalamus, and an increase of heat production in brown adipose tissue (BAT) via the β3 adrenergic receptor.

2. Materials and methods

2.1. Animals

Male Wistar rats (Charles River Japan, Inc., Yokohama, Japan) weighing 300–350 g were housed in individual Plexiglas cages (420 × 250 × 200 mm) in an animal room maintained under a constant light-dark cycle (lights on 07:00–19:00 h) and temperature (22 ± 1 °C). Food and water were provided *ad libitum*. All procedures were performed in accordance with the Japanese Physiological Society's guidelines for animal care, and the experiments were authorized by Animal Experiment Committee of University of Miyazaki (authorization number: 2012-006-5).

2.2. Peptides

Rat NURP33 and rat NURP36 were chemically synthesized using a model 431A peptide synthesizer (Applied Biosystems, CA, USA). The synthesized peptides were purified to a single peak by reversed-phase high-performance liquid chromatography. The structures of these synthetic peptides were confirmed by sequencing analysis and mass spectrometry. The amino acid sequences of NURP33 and NURP36 are shown in Fig. 1B. The amounts of the peptides were determined by amino acid analysis. Rat NMU was purchased from the Peptide Institute, Inc. (Osaka, Japan).

2.3. Measurement of NMU and NURP contents in the central nervous system

NMU and NURP in the rat brain at 2 and 18 months of age were quantified by radioimmunoassay (RIA). On the basis of a previous study in which the levels of NMU precursor mRNA expression in central and peripheral tissues were quantified by real-time PCR [17], we selected the midbrain, hypothalamus, brainstem and spinal cord for measurement, since these brain areas expressed high levels of mRNA. Details of the RIA method have been described elsewhere [16]; [^{125}I-Tyr^{34}]-rat NURP33 and radioiodinated rat NMU were used as the tracer ligands, and antisera for rat NURP (#24–6) and NMU (#14–4) were used at final concentrations of 1/14,000 and 1/380,000, respectively. Known amounts of rat NURP and rat NMU were used to obtain the standard curves.

2.4. Intracerebroventricular injection

Using a method that has been described previously [12], icv cannulae were implanted into the lateral cerebral ventricles, and after surgery all rats were housed individually in Plexiglas cages. During a 4-day postoperative recovery period, the rats became accustomed to the handling procedure. NURP or NMU was dissolved in saline, and then injected into each freely moving rat through a 27-gauge injection cannula connected to a 50-μl Hamilton syringe. All manipulations under dark conditions were performed using night vision infrared binoculars.

2.5. Measurement of locomotor activity

Locomotor activity was measured using a rat locomotor activity recording system (Muromachi Co. Ltd., Tokyo, Japan) comprising infrared sensors, an interface, and a computer. The infrared sensors were placed above the cages and measured all locomotor activity (e.g. eating, walking and grooming). After the icv cannulation, rats were housed in the cage in order to habituate to the test chamber for four days. Each cage with its infrared sensor was placed in an isolated chamber. After the icv injection of NURP, NMU or saline, data were collected at 30-min intervals for 2 h and analyzed using CompactACT AMS software (Muromachi Co.).

2.6. Measurement of heart rate

Heart rate was measured non-invasively using a BP-98A instrument (Softron Co., Ltd., Tokyo, Japan) starting from 10:00 h. Each rat was gently fixed in the instrument holder, and measurements were performed once every 7 days to allow habituation before the experiments. The temperature of the holder was set at 37–39 °C. For data collection, 5 consecutive measurements were performed and then the average value was taken.

2.7. Measurement of oxygen consumption and carbon dioxide emission

After icv canulation, rats were placed individually in Oximax (Columbus Instrument, Columbus, OH, USA) recording cages, and oxygen consumption and carbon dioxide emission were measured continuously at 10-min intervals for 4 days. NURP, NMU or saline was then injected icv at 09:00 h. The recording was continued sequentially for 2 h, and calorie consumption thereafter was calculated using the volume of oxygen consumed (VO_2) and the volume of carbon dioxide production (VCO_2) (Calorie consumption = $VO_2 \times (3.815 + 1.232 \times VO_2/VCO_2)$.

2.8. Measurement of BS-T

BS-T in freely moving animals was recorded using infrared thermographic imaging (FLIR SC620, FLIR Systems, Danderyd, Sweden) as described in our previous paper [18]. We started infrared thermographic imaging of the BS-T from 19:30 h. Images taken at 1-min intervals were saved during the following 30 min. Thereafter, NURP, NMU or saline was administered icv, and measurements were conducted for the following 2 h. The FLIR SC620 has a thermal resolution lower than 0.04 °C, an accuracy of ±2%,

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