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Short communication

Estradiol suppresses NMU mRNA expression during sexual maturation in the female rat pituitary

Fumi Shimizu, Toshiya Matsuzaki *, Takeshi Iwasa, Masahiro Minakuchi, Akira Kuwahara, Toshiyuki Yasui, Minoru Irahara

Department of Obstetrics and Gynecology, Institute of Health Biosciences, The University of Tokushima Graduate School, 3-18-15 Kuramoto-cho, Tokushima 770-8503, Japan

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Abstract

Neuromedin U (NMU) suppresses food intake and gonadotropin secretion. However, the developmental transition of NMU expression in the pituitary gland and the regulation of NMU expression are unclear. The objective of this study was to examine the transition of the expression of NMU mRNA in the pituitary glands of female rats from the juvenile period to the mature period of development. Furthermore, factors such as estradiol, insulin, leptin, and inhibin A, whose expressions change throughout puberty and which affect gonadotropin secretion in pituitary cell culture, were examined. In the pituitary gland, the expression of NMU mRNA was significantly lower in 8-week-old rats than in 4- and 6-week-old rats. In the pituitary cell culture, the expressions of NMU mRNA in the estradiol- and insulin-treated groups were significantly lower than in the control group. These results suggest that the expression of NMU mRNA in the female rat pituitary is reduced as the rats develop from the pubertal to the mature period. Additionally, the reduction of NMU expression in the pituitary may be related to the increases in serum estradiol and insulin levels that occur during the pubertal period, which may negate NMU suppression of gonadotropin secretion.

Keywords: Pituitary; Estrogen; Neuromedin U; Puberty; Gonadotrophin

1. Introduction

Neuromedin U (NMU) was first isolated from the porcine spinal cord in 1985 and named for its potent contractile effect on the uterus (Minamino et al., 1985). NMU is widely distributed in the gastrointestinal tract, spinal cord, pituitary gland, and hypothalamus (Domin et al., 1987; Ballesta et al., 1988; Howard et al., 2000; Graham et al., 2003). The primary effects of NMU are to suppress food intake and stimulate energy expenditure. Secondary effects include regulating smooth muscle contraction, increasing blood pressure, and modifying intestinal ion transport (Howard et al., 2000; Kojima et al., 2000; Nakazato et al., 2000).

The effects of NMU on the pituitary gland and the reproductive system are unclear. While one experiment reported that intracerebroventricular NMU administration did not affect luteinizing hormone (LH) plasma levels in male rats (Gartlon et al., 2004), others showed that NMU had some effects on the pituitary gland and the reproductive system. For example, administration of NMU into the third ventricle inhibited pulsatile LH secretion in adult ovariectomized rats (Quan et al., 2003). NMU has also been shown to suppress LH secretion from cultured rat anterior pituitary cells. Furthermore, vaginal opening occurred earlier in NMU-KO mice than in wild type mice (Fukue et al., 2006). These experiments demonstrated the negative effect of NMU on reproductive function in pubertal or mature animals, but NMU expression in juvenile animals was not examined.

In this study, the transition of NMU mRNA expression in the pituitary glands of female rats was examined from the juvenile to the mature period of development. Furthermore, factors such as estradiol, insulin, leptin, and inhibin A, whose expressions change throughout puberty and are thought to affect gonadotropin secretion in the pituitary, were examined.

2. Materials and methods

2.1. Animals

Female Wistar rats, aged 4 (juvenile), 6 (pubertal), and 8 (adult) weeks, were purchased from Charles River Japan Co. (Yokohama, Japan). The body weights

* Corresponding author. Tel.: +81 88 633 7177; fax: +81 88 631 2630.
E-mail address: mat@clin.med.tokushima-u.ac.jp (T. Matsuzaki).

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of juvenile, pubertal, and adult female rats were 75–95 g, 130–180 g, and 170–200 g, respectively.

2.2. Total RNA isolation, reverse transcription of RNA

The animals were decapitated under anesthesia using pentobarbital sodium (40 mg/kg body weight, i.p.), and the cerebrum and pituitary gland were removed immediately. The total RNA was isolated using a TRIzol® reagent kit (Invitrogen Corp., Carlsbad, CA, USA) and an RNeasy® mini kit (Qiagen Gmbh., Hilden, Germany). Reverse transcription was performed using SuperScript™ III (Invitrogen Corp., Carlsbad, CA, USA) in accordance with the manufacturer's instructions.

2.3. Real-time quantitative RT-PCR analysis

Real-time PCR was performed using a PCR System 9700 (PE Applied Biosystems, Foster City, CA, USA) with SYBR® Green PCR Master Mix (PE Applied Biosystems, Foster City, CA, USA) in accordance with the manufacturer's instructions. Primer pairs for rat NMU were used as previously reported (Graham et al., 2003). The primer sequences for rat NMU were sense 5′-TGC TGC TCG CCT GCT GTG C-3′ and antisense 5′-CCG TTG CGT GGC CTG AAT AAA A-3′. The primer sequences for β-actin were sense 5′-TCA TGA AGT GTG ACG TTG ACA TCC GT-3′ and antisense 5′-CTT AGA AGC ATT TGC GGT GCA CGA TG-3′ (Promega Co., Madison, WI, USA). Real-time PCR conditions were as follows: an initial denaturation stage at 95 °C for 10 min was followed by a 50-cycle amplification stage, consisting of a 15-s denaturing step at 95 °C, a 30-s annealing step at 58 °C for rat NMU and at 65 °C for β-actin, and a 1-min extension step at 72 °C. For dissociation curve analysis, samples were subjected to 95 °C for 15 s, 60 °C for 1 min, and 95 °C for 15 s. Beta-actin was used as an internal control.

2.4. NMU mRNA expression in primary culture of rat anterior pituitary cells

Rat anterior pituitary cells were cultured as previously reported (Kanematsu et al., 1991; Tezuka et al., 2002). Four-week-old female rats were decapitated, and their pituitaries were removed. The pituitaries were cut into small pieces and washed in Dulbecco's modified Eagle's medium (DMEM; Nissui Co., Tokyo, Japan). These pieces were subjected to enzymatic dispersion for 40 min at 37 °C using 0.25% trypsin, and then dissociated by pipetting with 0.2% pancreatin at 37 °C for 1 min (Tezuka et al., 2002). The cells were seeded in DMEM containing 10% fetal bovine serum, plated on 96-well culture dishes (Falcon Plastics, Los Angeles, CA, USA) at a density of 10^6 viable cells/well, and incubated for 48 h in the culture medium alone (control) or in a culture medium containing 10^{-7} mol/l of 1 of 5 test hormones: estradiol (Wako Pure Chemical Industries, Ltd., Osaka, Japan), insulin (Wako Pure Chemical Industries, Ltd., Osaka, Japan), leptin (Wako Pure Chemical Industries, Ltd., Osaka, Japan), GnRH (Wako Pure Chemical Industries, Ltd., Osaka, Japan), and inhibin A (National Institute for Biological Standard and Control, Hertfordshire, UK). All hormones were dissolved in a PBS buffer and thinned down to 10^{-6} mol/l before being put into their respective culture medium. The hormones made up one-tenth of the volume of each well, giving the culture mediums a final concentration of 10^{-7} mol/l. Culture medium was added to the control wells. The cell cultures were maintained at 37 °C under a mixture of 95% air and 5% CO_2 at 100% humidity.

After being cultivated for 48 h, cells were collected using trypsin, and total RNA was isolated using RNeasy® microkits (Qiagen Gmbh., Hilden, Germany). Reverse transcription was done using SuperScript™ III (Invitrogen Corp., Carlsbad, CA, USA) in accordance with the manufacturer's instructions. The expression of NMU mRNA was analyzed quantitatively using the real-time PCR method described above. Beta-actin was used as an internal control. For comparison purposes, the mean value of the control's expression was arbitrarily set at 1.

2.5. Statistical analysis

Differences in mean values among the groups were analyzed using Student's unpaired *t*-test. *p*-Values less than 0.05 were defined as indicating statistical significance. Data are reported as the mean ± S.E.M. for each group.

3. Results

3.1. mRNA expressions in the pituitary gland and cerebrum

The NMU mRNA levels in the pituitary gland and cerebrum of female rats of different ages were investigated (Fig. 1). In the pituitary gland, the expression of NMU mRNA was significantly higher in 4-week-old rats ($1.23 \pm 0.09, p < 0.05$) and 6-week-old rats ($1.32 \pm 0.13, p < 0.05$) than in 8-week-old rats (1.01 ± 0.05). In the cerebrum, the expression of NMU mRNA did not differ significantly among the groups.

3.2. NMU mRNA expression of cultured pituitary cells

Fig. 2 shows the NMU mRNA levels in the primary culture of rat anterior pituitary cells. The NMU mRNA levels in the estradiol- (0.57 ± 0.04, $p < 0.01$) and insulin-treated (0.55 ± 0.04, $p < 0.01$) groups were significantly lower than in the control group (1.00 ± 0.09). In the other groups, the NMU mRNA expression levels were not significantly different from the expression level in the control group.

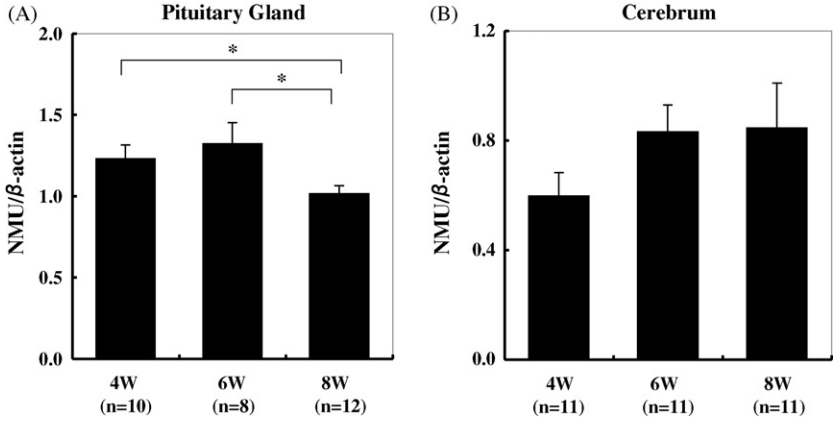

Fig. 1. Expression of NMU mRNA in the female rat pituitary gland (A) and cerebrum (B). Data are presented as the mean ± S.E.M. *$p < 0.05$ vs. 8-week-old rats.

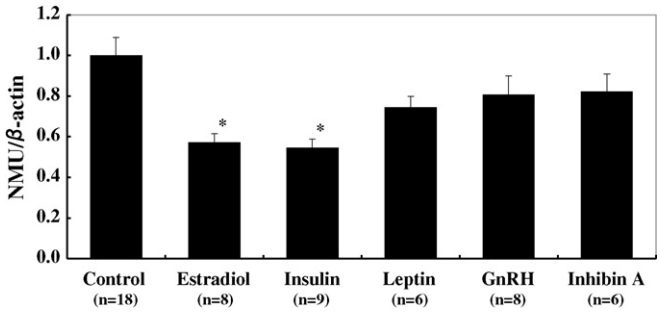

Fig. 2. Effects of various factors on the expression of NMU mRNA in cultured rat anterior pituitary cells. The relative value of NMU mRNA expression in the control group was arbitrarily set at 1. Data are presented as the mean ± S.E.M. *$p < 0.01$ vs. control group.

4. Discussion

There have been many studies on the role of NMU in the physiological regulation of feeding and body weight, but there have been only a few studies dealing with the effect of NMU on reproductive functions. This is the first study to have found that, in female rat pituitary glands, NMU mRNA expression was significantly higher in the juvenile and pubertal periods than in the mature period. Furthermore, the present study showed that insulin and estradiol suppressed the expression of NMU mRNA in cultured female rat pituitary cells.

On the other hand, although it is already well known that serum leptin and GnRH concentrations increase throughout pubertal development, and that Inhibin A interferes with pituitary gland FSH secretion (Rivier and Vale, 1991; Ge et al., 1992), in the present study, none of these factors was found to affect NMU mRNA expression in the cultured pituitary cells.

Fukue et al. reported that vaginal opening was delayed in female NMU knockout mice and suggested that NMU suppresses the onset of puberty. Indeed, NMU has been reported to have suppressive effects on gonadotropin secretion in both the hypothalamus and the pituitary. NMU slightly decreased pulsatile GnRH release in fasted ovariectomized rats and suppressed LH secretion in cultured rat pituitary cells (Quan et al., 2003; Fukue et al., 2006). The suppressive effect of NMU may have affected both of these sites. To the best of our knowledge, the present study is the first to have examined the developmental transition of NMU mRNA expression in any species. In the present study, NMU mRNA expression was stable in the cerebrum and decreased in the pituitary. To date, the onset of puberty has been believed to be triggered by the onset of hypothalamic GnRH release, and several factors, such as leptin, kisspeptin, NMU, and melatonin, have been identified as potential candidates for this phenomenon. Unfortunately, the present data do not support the notion that NMU plays a key role as the trigger of GnRH release in puberty. Melatonin also has suppressive effects on pulsatile GnRH release and the onset of puberty (Kennaway et al., 1986; Rivest, 1987; Batmanabane and Ramesh, 1996). Furthermore, melatonin has been found to stimulate a newly discovered peptide, gonadotropin inhibitory hormone (GnIH), which suppresses both GnRH expression in the hypothalamus and LH in the pituitary of birds (Tsutsui et al., 2000; Ubuka et al., 2005; Kriegsfeld et al., 2006). Further research would be needed to identify the role of these peptides with respect to pubertal GnRH secretion and their relationship.

The present study also demonstrated that estradiol and insulin suppressed NMU mRNA expression. Increased estradiol secretion may reduce NMU mRNA expression, which may, in turn, lead to an increase in gonadotropin secretion that would accelerate sexual maturation. Furthermore, there has already been a large body of research showing that insulin increases at the onset of puberty and enhances gonadotropin secretion (Adashi et al., 1981; Davoren and Hsueh, 1984; Laron et al., 1988; Nobels and Dewailly, 1992), as well as gonadotropin's stimulation of estradiol and progestin production. This suggests that insulin may have an effect similar to that postulated for estradiol above.

In summary, the pubertal increase in estradiol and insulin may lead to the suppression of NMU mRNA expression in the pituitary, which may negate the suppression of gonadotropin secretion by NMU. However, since this is the first study to have found that NMU mRNA expression in female rat pituitary glands was significantly higher in the juvenile and pubertal periods than in the mature period, further studies are needed to clarify the relationship between estradiol, insulin, and NMU during pubertal development.

Conflict of interest

None.

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