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Involvement of Central Metastin in the Regulation of Preovulatory Luteinizing Hormone Surge and Estrous Cyclicity in Female Rats

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Ovulation is caused by a sequence of neuroendocrine events: GnRH and LH surges that are induced by positive feedback action of estrogen secreted by the mature ovarian follicles. The central mechanism of positive feedback action of estrogen on GnRH/LH secretion, however, is not fully understood yet. The present study examined whether metastin, the product of metastasis suppressor gene KiSS-1, is a central neuropeptide regulating GnRH/LH surge and then estrous cyclicity in the female rat. Metastin had a profound stimulation on LH secretion by acting on the preoptic area (POA), where most GnRH neurons projecting to the median eminence are located, because injection of metastin into the third ventricle or POA increased plasma LH concentrations in estrogen-primed ovariectomized rats. Metastin neurons were immunohistochemically found in the arcuate nucleus (ARC) to be colocalized with estrogen receptors with some fibers in the preoptic area (POA) in close apposition with GnRH neuronal cell bodies or fibers. Quantitative RT-PCR has revealed that KiSS-1 and GPR54 mRNAs were expressed in the ARC and POA, respectively. The blockade of local metastin action in the POA with a specific monoclonal antibody to rat metastin completely abolished proestrous LH surge and inhibited estrous cyclicity. Metastin-immunoreactive cell bodies in the ARC showed a marked increase and c-Fos expression in the early proestrus afternoon compared with the day of diestrus. Thus, metastin released in the POA is involved in inducing the preovulatory LH surge and regulating estrous cyclicity. (*Endocrinology* 146: 4431–4436, 2005)

OVULATION IS CAUSED by a sequence of neuroendocrine events: GnRH and LH surges that are induced by positive feedback action of estrogen secreted by the ovarian mature follicles. The preoptic area (POA) has been considered to be a center for GnRH/LH surges because local implants of estrogen into the area induce LH surge in female rats (1). Estrogen receptor α is found in the POA but not colocalized in GnRH neurons (2). The mediobasal hypothalamus is another candidate for the site of positive feedback action of estrogen to induce GnRH/LH surge (3). The arcuate nucleus (ARC) has been suspected to be one of the sites for the estrogen-positive feedback effect on GnRH/LH release in rats (4, 5) and ewes (6). Thus, the central site of positive feedback action of estrogen on GnRH/LH secretion still remains to be determined.

Metastin was first isolated from human placenta and proposed to be the natural ligand for a G protein-coupled receptor, GPR54, also known as AXOR12 and hOT7T175 (7). It was recently reported that peripheral (8) or intracerebroventricular (icv) (9) injection of the peptide induces a profound stimulation of LH secretion in prepubertal rats, and that a genetic alteration leading to homozygous loss of function of GPR54 impairs pubertal development in mice (10). In adult males, icv (9) or peripheral (11) administration of metastin has been reported to stimulate LH secretion. In addition, metastin injection exerts no stimulation on LH release in GPR54 knockout mice, and central administration of metastin stimulates GnRH release in sheep (12). Thus, GPR54 and metastin may play a role in regulating the activity of the reproductive axis.

The present study sought to determine whether metastin plays a physiological role in inducing ovulation through stimulating the surge mode of GnRH/LH secretion to control estrous cyclicity in the female rat.

Materials and Methods

Animals and treatments

Adult Wistar-Imamichi strain female rats (230–280 g) were housed under controlled temperature and light conditions (lights on 0500–1900) and were provided with food and water *ad libitum*. Animals having shown at least two consecutive 4-d estrous cycles were used. Some animals were ovariectomized (OVX) for 2 wk before blood sampling. Other OVX animals immediately received sc estradiol (E2) implants to produce low (35.8 pg/ml) (13) or high (514.1 pg/ml) (14) E2 levels. The low level of E2 was previously confirmed to produce a negative feedback effect on LH pulses but not to induce LH surges in OVX rats (13). The high E2 level was reported to induce LH surges in OVX rats (14). Blood samples were collected through an indwelling atrial cannula that had been attached to the animals on the day before the blood sampling. Blood samples were collected every 6 min for 3 h to determine the acute effect

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Abbreviations: AM, Adrenomedullin; ANP, atrial natriuretic peptide; ARC, arcuate nucleus; CHO, Chinese hamster ovary; E2, estradiol; ER, estrogen receptor; FAM, 6-carboxy-fluorescein; FLIPR, fluorometric imaging plate reader; GPR, G protein-coupled receptor; icv, intracerebroventricular; MCH, melanin-concentrating hormone; ME, median eminence; NPY, neuropeptide Y; OVX, ovariectomized; POA, preoptic area; TAMRA, 6-carboxytetramethyl-rhodamine; 3V, third ventricle.

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of metastin and every 1 h from 1300–2000 h to detect LH surges. All surgeries were conducted under ether anesthesia and aseptic conditions.

All the animal experiments were approved by the Committee on Animal Experiments of the Graduate School of Bioagricultural Sciences, Nagoya University.

Real-time RT-PCR

Expression of GPR54 (a) and KiSS-1 mRNA (b) was determined by quantitative RT-PCR in the POA, ARC-ME (median eminence) region and anterior pituitary obtained from cycling female rats at 0900, 1200, and 1500 h of proestrus or at 1400 h of estrus or diestrus d 1 and 2. The tissues were also collected from OVX rats with sc E2 implants to keep a low or high plasma level or without steroid treatment at 14:00 h. Real-time RT-PCR analysis (TaqMan) was performed using ABI PRISM 7900HT (PE Applied Biosystems, Foster City, CA). Briefly, DNA-free total RNA was purified from the punched-out hypothalamic tissues using RNeasy Mini kit and ribonuclease-free deoxyribonuclease Set (QIAGEN, Valencia, CA) following the manufacturer's instructions. cDNA from each RNA sample was synthesized with oligo (deoxythymidine) primer at 50 C using SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA). Forward primer, reverse primer, and TaqMan probe for rKiSS-1 were 5′-ATGATCTCGCTGGCTTCTTGG-3′, 5′-GGTTCACCACAGGTGCCATTTT-3′, and 5′-(FAM, 6-carboxy-fluorescein)-TGCTTCTCCTCTGTGTGGCCTCTTTTGG-(TAMRA, 6-carboxytetramethyl-rhodamine)-3′, respectively. Those for *β-Actin* were 5′-ATGAGCTGCCTGACGGTCAG-3′, 5′-GGAAGGCTGGAAGAGAGCCT-3′, and 5′-(FAM)-TTCCGATGCCCTGAGGCTCTTTTCCA-(TAMRA)-3′, respectively. Those for *rGPR54* were described elsewhere (15). The copy number of *rKiSS-1* and *rGPR54* transcript was normalized to the ratio to the copy number of the *β-actin* transcript for each sample.

Preparation of monoclonal antibodies

Rat metastin(1–52) comprising 52-amino acid residues with a disulfide bonding between Cys^4 and Cys^{18} and an amidated C terminus (15) was chemically synthesized using a solid-phase peptide synthesizer. For immunogens, keyhole limpet hemocyanin (10 nmol) was maleimidated with *N*-(γ-maleimidobutyryloxy) succinimide and then conjugated with [Cys^{36}]rat metastin(36–52) (CEKDMSAYNWNSFGLRY-NH2) (570 nmol). The immunogen (40 μg/mouse) together with complete or incomplete Freund's adjuvant was sc injected into BALB/c mice (female, 8 wk old) at 3-wk intervals. Four days after iv injection of immunogen (50 μg), spleen cells were fused with mouse myeloma cells P3-X63Ag8-U1 as described previously (16). The antirat rMet-3Ca monoclonal antibody (nos. 156 and 254) was then purified from ascites fluid with a protein A-immobilized column (IPA-300; Repligen, Cambridge, MA).

The antibody reactivity (No. 156) was investigated in the same manner described previously (17) using horseradish peroxidase-labeled [Cys^{36}] rat metastin (36–52) (15) in competitive enzyme immunoassay with rat metastin and human metastin. Because we found several hybridoma clones producing antibodies specific to the carboxyl-terminal RY-NH_2 structure, the reactivity was tested for the following peptides as well: neuropeptide Y (NPY) with RY-amide, adrenomedullin (AM) with GY-NH_2, atrial natriuretic peptide (ANP) with RY-OH, or melanin-concentrating hormone (MCH) without related structure as negative control.

To check the neutralizing activity of the antibody (No. 156), a stable Chinese hamster ovary (CHO) cell line that expressed rat GPR54 (OT7T175) was constructed by transfection of pAKKO expression vector harboring rat GPR54 to CHO $dhfr^-$ cells. Antirat prolactin-releasing peptide monoclonal antibody (P2L-1T) and antihuman metastin monoclonal antibody (KIS-1N) were prepared as described previously (16, 18). Rat metastin (at 30 nM) was preincubated with purified monoclonal antibodies, rMet-3Ca, P2L-1T, or KIS-1N (at 30, 300, or 3000 nM) at room temperature for 1 h. The mixture was then subjected to the fluorometric imaging plate reader (FLIPR) assay (7) with GPR54-expressing CHO cells at the final metastin concentration of 10 nM and antibody concentration of 10–1000 nM. Briefly, the cells were inoculated at 30,000 cells/well in 96-well black-walled plates, cultured overnight, and allowed to incorporate a calcium sensing dye, Fluo 3-AM, for 1 h in HEPES-buffered Hanks' solution (H-HBSS) including Pluronic F-127, fetal bovine serum, and Probenecid. After washing the cells with H-HBSS four times, the cells were challenged by the metastin-antibody mixture in an FLIPR device. The fluorescence intensity was monitored for 180 sec after metastin challenge.

The other antibody (No. 254), which was used for immunocytochemistry, showed a similar cross-reactivity and neutralizing activity (data not shown).

Immunocytochemistry

Ovariectomized or proestrous rats were perfused with 4% paraformaldehyde at 1400 h, and the brains were quickly removed. Frozen frontal sections (50 μm) obtained with a cryomicrotome were incubated with antirat metastin monoclonal antibody (No. 254) at 0.49 μg/ml for 24 h and then with Alexa488-conjugated goat antimouse IgG (Molecular Probes, Inc., Eugene, OR). No immunoreactivity was found with antirat metastin monoclonal antibody absorbed with 10^{-5} M synthetic C-terminal (36–52) of rat metastin for 2 h at 37 C. Rabbit anti-ER (estrogen receptor) α (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), rabbit anti-c-Fos (Santa Cruz) and rabbit anti-LH-RH (Sanbio, Uden, The Netherlands) polyclonal antibodies were used. We relied on the validation by the commercial suppliers for the specificity of the anti-ERα, c-Fos, and GnRH. For dual fluorescence immunocytochemistry of metastin and either ERα or c-Fos, ERα or c-Fos was visualized with Alexa594-conjugated goat antirabbit IgG (Molecular Probes, Inc.) in red. For the double immunostaining of metastin and GnRH, metastin and GnRH were visualized with diaminobenzidine in brown and 4-Cl-1-Naphthol in blue after the avidin-biotin peroxidase complex method.

Brain injection of metastin or antirat metastin

A guide cannula (Plastics One, Roanoke, VA) was stereotaxically implanted into the third ventricle (3V) (22G) or POA (26G) at least 1 wk before the drug injection according to a rat brain atlas (19). The stereotaxic coordinates were as follows: 3V, −0.8 mm posterior and 7.5 mm ventral to bregma at midline, POA; −0.3 mm posterior and 8.4 mm ventral to bregma and 0.4 mm lateral from midline. Metastin dissolved in ultra-pure water was injected through an internal cannula (28G for 3V and 33G for the POA), which was inserted through the guide cannula. Metastin solution was injected for 2 min with a microinfusion pump into the 3V at 1 μl/min or into the POA at 0.25 μl/min. Control animals were treated with PBS or nonimmunized mouse IgG in a similar manner.

To determine the role of endogenous metastin in regulating LH surges or estrous cyclicity, monoclonal antirat metastin antibody (No. 156) was infused into the POA through the internal cannula at 1 μl/h with an osmotic mini-pump (Alzet, Cupertino, CA) for 1 wk to determine the effect on estrous cycle or with an microinfusion pump on the proestrous day from 1000–1800 h to determine the effect on LH surge. PBS was administered in a similar manner in the corresponding control groups.

At the end of experiments, animals were injected with brilliant blue through the internal cannula, and the cannula placement was verified by the naked eye for 3V injection or histology for the POA injection.

RIA for LH

Plasma LH concentrations were determined with RIA using a rat LH RIA kit obtained from the National Hormone and Pituitary Program and are expressed in terms of NIADDK rat LH RP-3. The least detectable LH concentration was 0.16 ng/ml for 50 μl plasma, and the intra- and interassay coefficients of variation were 5.6% at 2.3 ng/ml and 6.4% at 1.4 ng/ml, respectively.

Statistics

The effect of metastin injection into the 3V or POA and effect of antimetastin injection into the POA were analyzed by one-way ANOVA with repeated measures. Significant differences in the numbers of cells between diestrus and proestrus were determined by Student's *t* test. Statistical differences in the levels of mRNA expression of each brain area between various treatments were determined by one-way ANOVA followed by Bonferroni test.

Results

Specificity and neutralizing activity of monoclonal antibody

The antibody reacted with rat and human metastin in the same manner, but not with NPY, AM, ANP, and MCH (Fig. 1A). These results indicate that the antibody recognized the specific region of rat metastin(43–51). As indicated in Fig. 1B, the monoclonal antirat metastin antibody was found to have a potent effect to neutralize the action of rat metastin on GPR54-expressing CHO cells.

Effect of metastin injection in OVX or E2-treated OVX rats

The injection of metastin at 2 nmol into the 3V caused a sustained surge-like LH secretion in low-level E2-primed female rats but not in OVX animals (Fig. 2A). The stimulatory effect of metastin on LH secretion was maintained for more than 3 h in all E2-treated OVX animals. Local injection of metastin (0.5 nmol) into the POA induced a sustained surge-like increase in plasma LH level in E2-primed OVX rats (Fig. 2B).

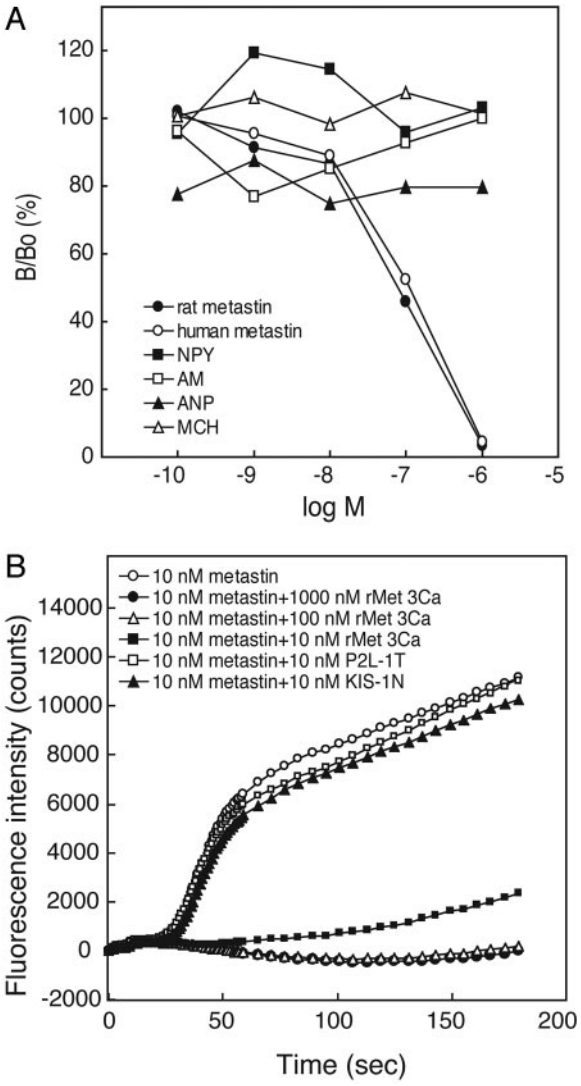

FIG. 1. A, Reactivity of monoclonal antirat metastin antibodies with metastin and other peptide. Monoclonal antibodies were examined for reactivity with rat metastin (*closed circle*), human metastin (*open circle*), NPY (*closed square*), AM (*open square*), ANP (*closed triangle*), and MCH (*open triangle*). B, Neutralizing effect of antirat metastin antibody. Response of rat GPR54-expressing CHO cells to 10 nM rat metastin was determined using FLIPR assay in the absence (*open circle*) and presence of rMet-3Ca antibody (*closed circle*, 1000 nM; *open triangle*, 100 nM; *closed square*, 10 nM), 10 nM P2L-1T antibody (*open square*), or 10 nM KIS-1N antibody (*closed triangle*).

KiSS-1 or GPR54 mRNA expression in hypothalamus

The quantitative RT-PCR analysis demonstrated that GPR54 mRNA is highly expressed in the POA and ARC in female rats without obvious changes throughout the estrous cycle or between steroid treatments (Fig. 3A). The metastin receptor mRNA expression was low in the pituitary in all groups. KiSS-1 mRNA is highly expressed in the ARC, and the expression varies during the estrous cycle and is modified by the steroidal milieu (Fig. 3B). The expression was highest in diestrus 2 in the estrous cycle and was increased by ovariectomy and suppressed by the high level of E2 implants (one-way ANOVA followed by Bonferroni test). KiSS-1 mRNA expression was low in the POA and pituitary.

Immunocytochemistry of metastin neurons in hypothalamus

Immunocytochemistry of metastin with a specific monoclonal antibody revealed metastin-immunopositive cell bodies in the ARC region (Fig. 4A) but not in other hypothalamic nuclei, such as the POA or amygdala (data not shown). No staining was found with preabsorbed antibody (Fig. 4B). Dual immunocytochemistry showed ERα immunoreactivities in most metastin neurons in the ARC (Fig. 4C). Metastin-immunoreactive fibers were found in the POA in close apposition to GnRH neuronal cell bodies or fibers (Fig. 4D). Metastin neurons also showed c-Fos immunoreactivities in the early afternoon of proestrus (Fig. 4E) but not at diestrus (Fig. 4F). Numbers of visualized metastin neurons and c-Fos-immunoreactive metastin neurons were significantly ($P < 0.05$, Student's t test) higher at proestrus compared with diestrus (Fig. 4G).

Effects of immunoneutralization of endogenous local metastin release in POA on estrous cyclicity and proestrous LH surge

Infusion of a specific monoclonal antibody (4.68 mg/ml) at 1 μl/h with a microinfusion pump from 1000–1800 h completely blocked the proestrous LH surge (Fig. 5A). Control animals with POA infusion of vehicle showed LH surges in the late afternoon of proestrus with a peak at 1600–1700.

Estrous cycles were disrupted in female rats receiving continuous POA infusion of monoclonal antirat metastin with mini osmotic pump at 1 μl/h for 6 d starting at diestrous d 1 (Fig. 5B). Some rats showed persistent estrus but others exhibited diestrus. Vehicle- or nonimmunized mouse IgG-treated controls showed normal estrous cyclicity of 4 or 5 d.

Discussion

The present study clearly demonstrated that metastin has a potent effect on LH release in the presence of estrogen in rats. One of the sites of metastin action on LH release is the POA because local metastin injection induced a surge-like

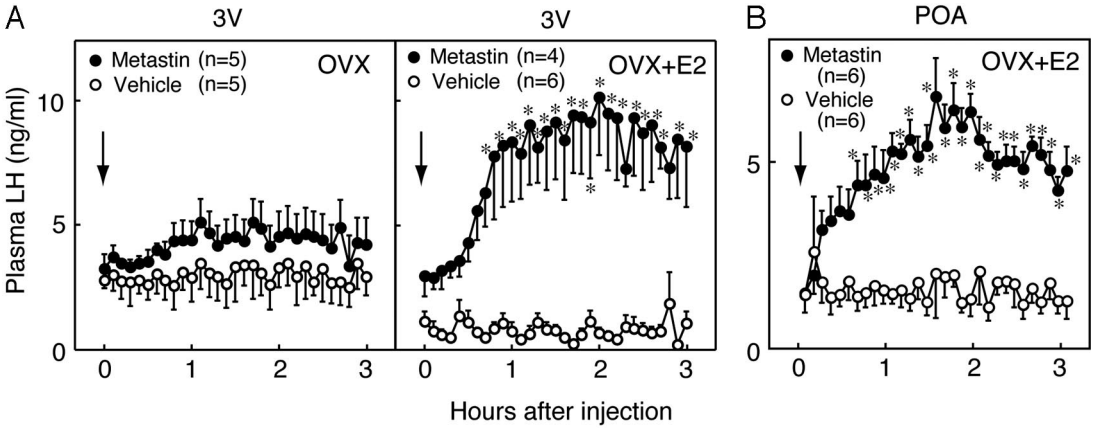

FIG. 2. Mean plasma LH levels in OVX or E2-primed OVX rats receiving 3V (A) or POA (B) injection of human metastin. OVX rats received sc E2 implants to produce a negative feedback effect on LH pulse. Blood samples were taken every 6 min for 3 h from 1300 h. Values are means ± SEM; *, $P < 0.05$ *vs.* PBS-treated control (one-way ANOVA with repeated measures).

increase in plasma LH concentration. In the present study, the RT-PCR analysis of GPR54, a gene encoding metastin receptor (20), showed that GPR54 mRNA is highly expressed in the POA. The previous (12) and present studies showed that metastin-immunoreactive fibers are in close apposition with GnRH cell bodies in the POA. In addition, GPR54 mRNA was recently found to be expressed in GnRH cell bodies in the POA (21). These findings strongly suggest that endogenous metastin is released in the POA to stimulate GnRH secretion in female rats.

The present study is the first to demonstrate that endogenous metastin could play a critical role in regulating GnRH/LH surge, ovulation and then estrous cycle, because the POA infusion of a specific antimetastin antibody into the POA to neutralize the locally released metastin in the POA, served to block preovulatory LH surge and disrupted estrous cyclicity. It is likely that metastin neurons located in the ARC are associated with endogenous metastin release to induce proestrous GnRH/LH release because the ARC metastin neurons express c-Fos at proestrous afternoon. The brain mechanism mediating estrogen-positive feedback action on GnRH/LH has been a central but controversial issue in reproductive physiology. Goodman (1) first reported that an E2 microimplant in the POA-induced LH surge in OVX rats and suggested that the site of estrogen-positive feedback action resides in the POA. On the other hand, there are several lines of evidence indicating that the positive feedback action site resides in the MBH in sheep (3) and monkeys (22).

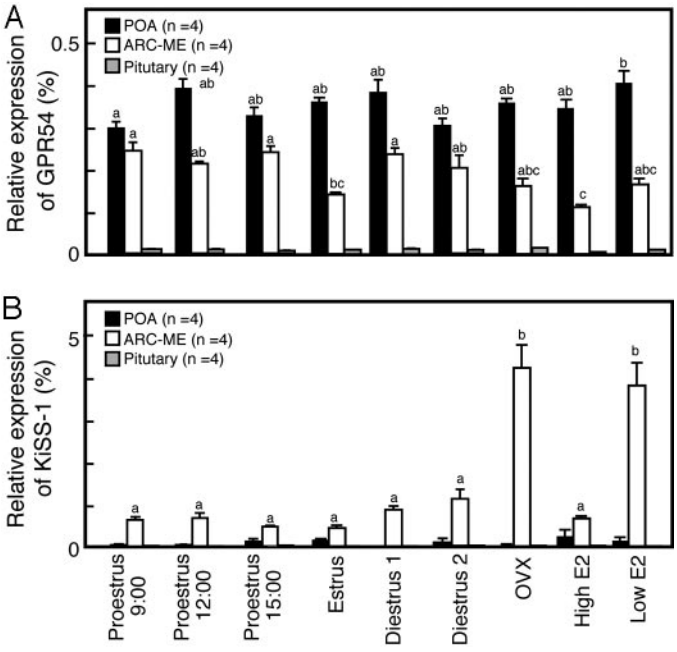

FIG. 3. Localization of GPR54 (A) and KiSS-1 mRNA (B) by quantitative RT-PCR. The POA, ARC-ME, and anterior pituitary were obtained from cycling female rats at 0900, 1200, and 1500 h of proestrus or at 14:00 h of estrus or diestrus d 1 and 2. The tissues were also collected from OVX rats with sc E2 implants to keep a low (35.8 pg/ml) or high (514.1 pg/ml) plasma level or without steroid treatment at 1400 h. Values with *same letters* within each brain area are not significantly different (one-way ANOVA followed by Bonferroni test).

Previous studies (2, 23) have revealed that GnRH neurons in the POA do not contain ERα, and the positive feedback action of estrogen on GnRH neurons has been suggested to be conveyed by ERα-containing neurons other than GnRH neurons (24). Taken together with the present results, the proestrous increase in circulating estrogen may induce metastin release in the POA to induce proestrous LH surge and regulate estrous cyclicity. Thus, metastin neurons in the ARC may be a target of estrogen feedback action during the preovulatory period to induce metastin release in the POA and then GnRH/LH surge in female rats. It still remains to be determined whether the POA is the metastin action site to induce preovulatory LH surge because the diffusion of metastin or antimetastin injected into the POA was not assessed in the present study.

Metastin production is negatively regulated by estrogen because the expression of KiSS-1 mRNA in the ARC was lowest at proestrous afternoon, highest at diestrus 2, and was increased by ovariectomy and decreased by estrogen treatment. This is consistent with a previous study showing that the KiSS-1 mRNA level is lower at proestrus or estrus than at diestrous d 1 during the rat estrous cycle (9). The change in metastin mRNA expression seems inconsistent with the change in the number of ARC metastin neurons or c-Fos

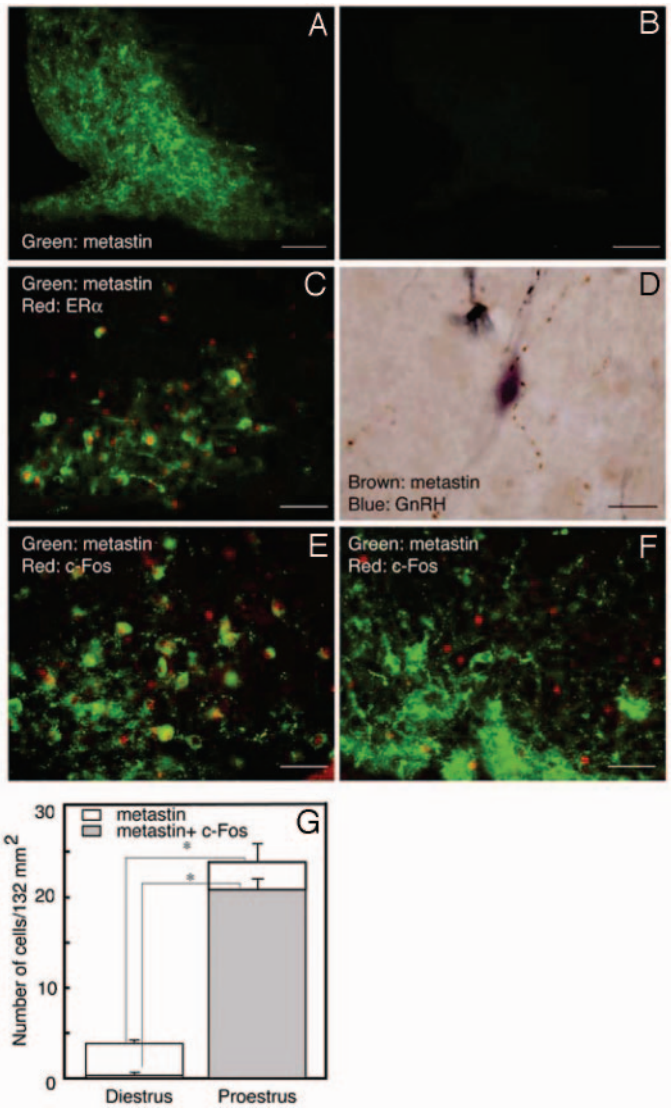

FIG. 4. Immunocytochemistry of metastin in the ARC-ME and POA. Metastin-immunopositive cells were found in the ARC (A; *green*). No staining was found with preabsorbed antibody (B). ERα (C; *red*) was localized in metastin-immunoreactive cells in the ARC. Metastin-immunoreactive fibers were in close apposition with GnRH cell bodies in the POA (D: *brown*, metastin; *blue*, GnRH). Expression of c-Fos was found in metastin neurons in the ARC at proestrus (E) but not found at diestrus (F). Numbers of metastin neurons and c-Fos-immunoreactive metastin neurons were significantly (*, $P < 0.05$; Student's *t* test) higher at proestrus compared with diestrus (G).

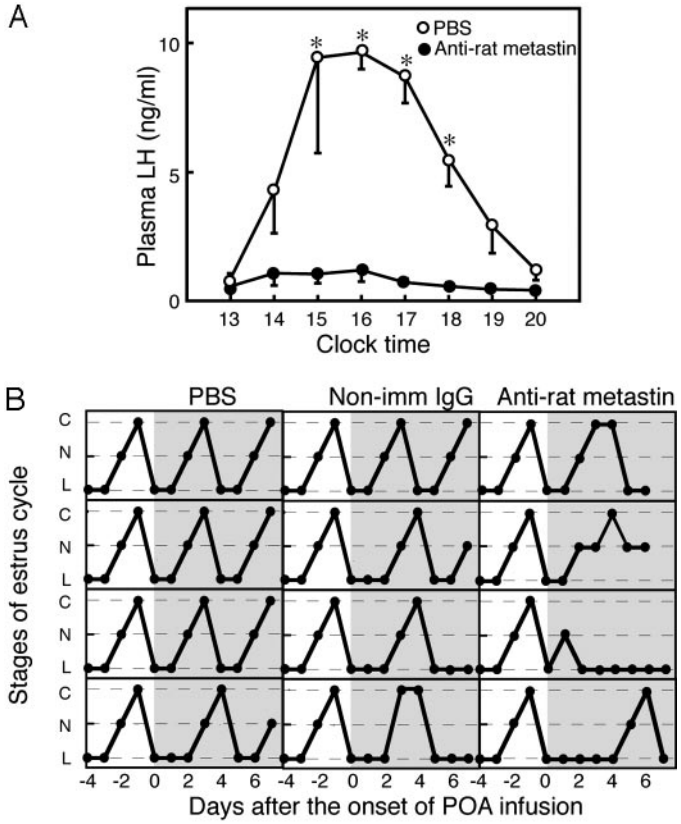

FIG. 5. Effect of POA infusion of antirat metastin monoclonal antibody on proestrous LH surge (A) or estrous cyclicity (B). A, PBS (n = 6) or antirat metastin monoclonal antibody (n = 5) was infused into the POA at 1000–1800 h. Blood samples were collected every hour at 1300–2000 h through an indwelling atrial cannula. Values are means ± SEM; *, $P < 0.05$ *vs.* vehicle-treated control (one-way ANOVA with repeated measures). B, Phase of the estrous cycle was determined by the major cell population in the vaginal smear. Shaded areas represent the period of POA infusion of PBS, nonimmunized mouse IgG (non-imm IgG) or antirat metastin monoclonal antibody. N, Nucleated cells; C, cornified cells; L, leukocytes.

expression in the metastin neurons. One explanation is that metastin is accumulated during the diestrus until the proestrus and that the metastin neurons are activated by estrogen to release metastin in proestrous afternoon to stimulate GnRH in the POA because the neurons express c-Fos in proestrous afternoon at 1400 h. The inhibitory action of estrogen on metastin production could be important because it might trigger a shut-down of LH release in the proestrous afternoon. It is also possible that estrogen facilitates metastin-metastin connections within the ARC for an excitation of the metastin neurons during proestrus. In the present study, the number of metastin neurons increased at proestrus with many fibers in the ARC. Estrogen may cause a synaptic remodeling of the ARC metastin neuronal network. Results from the group of Naftolin (5) demonstrated that estrogen causes synaptic remodeling in the ARC before the preovulatory LH surge. Further studies will be needed to clarify the relationship between metastin mRNA and estrogen feedback action on LH release.

In conclusion, the present study suggests that metastin neurons located in the ARC and projecting to the POA are one of the targets of estrogen-positive feedback action to release metastin into the POA, stimulate GnRH/LH surge during proestrus, and regulate estrous cycle in the female rat.

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References

1. **Goodman RL** 1978 The site of the positive feedback action of estradiol in the rat. Endocrinology 102:151–159
2. **Herbison AE, Pape JR** 2001 New evidence for estrogen receptors in gonadotropin-releasing hormone neurons. Front Neuroendocrinol 22:292–308
3. **Caraty A, Fabre-Nys C, Delaleu B, Locatelli A, Bruneau G, Karsch FJ, Herbison A** 1998 Evidence that the mediobasal hypothalamus is the primary site of action of estradiol in inducing the preovulatory gonadotropin releasing hormone surge in the ewe. Endocrinology 139:1752–1760
4. **Horvath TL, Garcia-Segura LM, Naftolin F** 1997 Lack of gonadotropin-positive feedback in the male rat is associated with lack of estrogen-induced synaptic plasticity in the arcuate nucleus. Neuroendocrinology 65:136–140
5. **Naftolin F, Mor G, Horvath TL, Luquin S, Fajer AB, Kohen F, Garcia-Segura LM** 1996 Synaptic remodeling in the arcuate nucleus during the estrous cycle is induced by estrogen and precedes the preovulatory gonadotropin surge. Endocrinology 137:5576–5580
6. **Clarke IJ, Pompolo S, Scott CJ, Rawson JA, Caddy D, Jakubowska AE, Pereira AM** 2001 Cells of the arcuate nucleus and ventromedial nucleus of the ovariectomized ewe that respond to oestrogen: a study using Fos immunohistochemistry. J Neuroendocrinol 13:934–941
7. **Ohtaki T, Shintani Y, Honda S, Matsumoto H, Hori A, Kanehashi K, Terao Y, Kumano S, Takatsu Y, Masuda Y, Ishibashi Y, Watanabe T, Asada M, Yamada T, Suenaga M, Kitada C, Usuki S, Kurokawa T, Onda H, Nishimura O, Fujino M** 2001 Metastasis suppressor gene KiSS-1 encodes peptide ligand of a G-protein-coupled receptor. Nature 411:613–617
8. **Matsui H, Takatsu Y, Kumano S, Matsumoto H, Ohtaki T** 2004 Peripheral administration of metastin induces marked gonadotropin release and ovulation in the rat. Biochem Biophys Res Commun 320:383–388
9. **Navarro VM, Castellano JM, Fernandez-Fernandez R, Barreiro ML, Roa J, Sanchez-Criado JE, Aguilar E, Dieguez C, Pinilla L, Tena-Sempere M** 2004 Developmental and hormonally regulated messenger ribonucleic acid expression of KiSS-1 and its putative receptor, GPR54, in rat hypothalamus and potent luteinizing hormone-releasing activity of KiSS-1 peptide. Endocrinology 145:4565–4574
10. **Seminara SB, Messager S, Chatzidaki EE, Thresher RR, Acierno Jr JS, Shagoury JK, Bo-Abbas Y, Kuohung W, Schwinof KM, Hendrick AG, Zahn D, Dixon J, Kaiser UB, Slaugenhaupt SA, Gusella JF, O'Rahilly S, Carlton MB, Crowley Jr WF, Aparicio SA, Colledge WH** 2003 The GPR54 gene as a regulator of puberty. N Engl J Med 349:1614–1627
11. **Gottsch ML, Cunningham MJ, Smith JT, Popa SM, Acohido BV, Crowley WF, Seminara S, Clifton DK, Steiner RA** 2004 A role for kisspeptins in the regulation of gonadotropin secretion in the mouse. Endocrinology 145:4073–4077
12. **Messager S, Chatzidaki EE, Ma D, Hendrick AG, Zahn D, Dixon J, Thresher RR, Malinge I, Lomet D, Carlton MB, Colledge WH, Caraty A, Aparicio SA** 2005 Kisspeptin directly stimulates gonadotropin-releasing hormone release via G protein-coupled receptor 54. Proc Natl Acad Sci USA 102:1761–1766
13. **Cagampang FR, Maeda KI, Tsukamura H, Ohkura S, Ota K** 1991 Involvement of ovarian steroids and endogenous opioids in the fasting-induced suppression of pulsatile LH release in ovariectomized rats. J Endocrinol 129:321–328
14. **Tsukamura H, Maeda KI, Yokoyama A** 1988 Effect of the suckling stimulus on daily LH surges induced by chronic oestrogen treatment in ovariectomized lactating rats. J Endocrinol 118:311–316
15. **Terao Y, Kumano S, Takatsu Y, Hattori M, Nishimura A, Ohtaki T, Shintani Y** 2004 Expression of KiSS-1, a metastasis suppressor gene, in trophoblast giant cells of the rat placenta. Biochim Biophys Acta 1678:102–110
16. **Matsumoto H, Murakami Y, Horikoshi Y, Noguchi J, Habata Y, Kitada C, Hinuma S, Onda H, Fujino M** 1999 Distribution and characterization of immunoreactive prolactin-releasing peptide (PrRP) in rat tissue and plasma. Biochem Biophys Res Commun 257:264–268
17. **Suzuki N, Matsumoto H, Kitada C, Masaki T, Fujino M** 1989 A sensitive sandwich-enzyme immunoassay for human endothelin. J Immunol Methods 118:245–250
18. **Horikoshi Y, Matsumoto H, Takatsu Y, Ohtaki T, Kitada C, Usuki S, Fujino M** 2003 Dramatic elevation of plasma metastin concentrations in human pregnancy: metastin as a novel placenta-derived hormone in humans. J Clin Endocrinol Metab 88:914–919
19. **Paxisons G, Watson C** 1997 The rat brain. 3rd ed. San Diego: Academic Press
20. **Muir AI, Chamberlain L, Elshourbagy NA, Michalovich D, Moore DJ, Calamari A, Szekeres PG, Sarau HM, Chambers JK, Murdock P, Steplewski K, Shabon U, Miller JE, Middleton SE, Darker JG, Larminie CG, Wilson S, Bergsma DJ, Emson P, Faull R, Philpott KL, Harrison DC** 2001 AXOR12, a novel human G protein-coupled receptor, activated by the peptide KiSS-1. J Biol Chem 276:28969–28975
21. **Irwig MS, Fraley GS, Smith JT, Acohido BV, Popa SM, Cunningham MJ, Gottsch ML, Clifton DK, Steiner RA** 2004 Kisspeptin activation of gonadotropin releasing hormone neurons and regulation of KiSS-1 mRNA in the male rat. Neuroendocrinology 80:264–272
22. **Yamaji T, Dierschke DJ, Hotchkiss J, Bhattacharya AN, Surve AH, Knobil E** 1971 Estrogen induction of LH release in the rhesus monkey. Endocrinology 89:1034–1041
23. **Hrabovszky E, Shughrue PJ, Merchenthaler I, Hajszan T, Carpenter CD, Liposits Z, Petersen SL** 2000 Detection of estrogen receptor-β messenger ribonucleic acid and ^{125}I-estrogen binding sites in luteinizing hormone-releasing hormone neurons of the rat brain. Endocrinology 141:3506–3509
24. **Goubillon M, Delaleu B, Tillet Y, Caraty A, Herbison AE** 1999 Localization of estrogen-receptive neurons projecting to the GnRH neuron-containing rostral preoptic area of the ewe. Neuroendocrinology 70:228–236

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