Biochemical and Biophysical Research Communications 458 (2015) 663–666

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

The relationship between circulating kisspeptin and sexual hormones levels in healthy females

Fumihiko Katagiri [a, *, 1], Masato Kotani [b, c, 1], Tsuyoshi Hirai [d], Jiro Kagawa [e]

[a] Tokyo University of Pharmacy and Life Sciences, Horinouchi 1432-1, Hachioji 192-0392, Tokyo, Japan
[b] Asahina Shinryoujo, Nyufune 314-5, Okabecho, Fujieda, Shizuoka 421-1115, Japan
[c] Department of Internal Medicine, Fujieda Municipal General Hospital, Surugadai 4-1-11, Fujieda, Shizuoka 426-8677, Japan
[d] Department of Gynecology and Obstetrics, Fujieda Municipal General Hospital, Surugadai 4-1-11, Fujieda, Shizuoka 426-8677, Japan
[e] Department of Pediatrics, Fujieda Municipal General Hospital, Surugadai 4-1-11, Fujieda, Shizuoka 426-8677, Japan

ARTICLE INFO

Article history:
Received 28 January 2015
Available online 12 February 2015

Keywords:
Female
Kisspeptin
Menstruation

ABSTRACT

The kisspeptin (metastin) is an endogenous peptide, which regulates human reproduction by modulating gonadotropin-releasing hormone (GnRH) secretion. Kisspeptin was detected in peripheral blood, although GnRH was not. Previously, we measured plasma kisspeptin levels in male healthy subjects and patients with hypogonadism using enzyme immunoassay (EIA) to elucidate a normal range in healthy males and clinical implications of kisspeptin in male hypogonadism. We suggested that the plasma kisspeptin levels were received feedback from testosterone. In this study, we focused female subjects and elucidated the relationship between menstrual cycle and plasma kisspeptin levels to understand kisspeptin-hypothalamic-pituitary-gonadal axis. We measured plasma kisspeptin levels in eight female volunteers. The plasma kisspeptin levels in female are significantly higher than those in male. There are no significant correlation between plasma kisspeptin levels and sexual hormones. We revealed that the kisspeptin might stimulate a start of menstruation as a trigger, and progress menstruation covered for weakened ovarian function. We suggest that kisspeptin may be closely related with menstrual cycle and that the measurement of plasma kisspeptin levels is useful for understanding of reproductive system.

1. Introduction

Kisspeptin (metastin) has been identified as an endogenous ligand for orphan G protein-coupled receptor GPR54 [1,2]. Recently, the physiological activities come to be revealed that kisspeptin plays an important role for reproduction [3]. In the brain, kisspeptin is secreted by hypothalamus, and modulates gonadotropin-releasing hormone (GnRH) secretion, and known to be related with gonadal maturation [4]. Previously, we measured plasma kisspeptin levels in seven healthy male subjects and in four male cases with hypogonadism to understand the clinical implications of peripheral kisspeptin in hypogonadism [5]. The plasma kisspeptin levels were different in the each case with underlying illness [5-9]. Although the GnRH cannot be detected in the circulating blood from its short half-life, kisspeptin in plasma (serum) can be measured [10] and expected to be a biomarker for some of diseases [8,11,12]. The kisspeptin is known to be secreted from peripheral organs such as placenta, pancreas, testis, liver and small intestine [1,2]. The plasma kisspeptin levels in pregnant women are much higher than non-pregnant women [13]. However, the peripheral kisspeptin levels in female are not known well, especially for relationship with menstrual period. The purpose of this study is to measure plasma kisspeptin levels in healthy female subjects with normal menstrual cycle, in order to understand the physiological activities of peripheral kisspeptin and to identify normal range in healthy female.

2. Materials and methods

2.1. Subjects

Eight healthy female volunteers (subject A–H), aged 33–43 years old (median, 38 years old), with normal menstrual cycle (28–30 days) (Table 1), and 8 healthy post-menopausal female volunteers (subject a–h), aged 60–72 years old (median, 64 years

* Corresponding author. Fax: +81 42 676 5679.
E-mail address: katagiri@toyaku.ac.jp (F. Katagiri).
[1] The first two authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bbrc.2015.02.021
0006-291X/

Table 1
Characteristics of the healthy subjects.

Subject	Age [yr.]	BMI [kg/m^2]
A	33	18.1
B	33	20.6
C	35	20.0
D	36	20.7
E	38	23.4
F	41	27.0
G	42	19.3
H	43	17.6

old) (Table 2), participated in the study. The study was approved by the ethical committee of Fujieda Municipal General Hospital (Fujibyou 823) and registered in UMIN Clinical Trials Registry (000012579) [14]. Each subject received information on the scientific purposes of the study, and gave written informed consents. The subjects did not suffer from gynecological disorders.

2.2. Study schedule

Venous blood samples were drawn from a forearm vein at the first day of menstrual period (Day 1), Day 2–11 (follicular phase), 12–16 (periovulatory period), 17–26 (luteal phase). The sampling was carried out in the afternoon.

2.3. Materials

Synthetic human kisspeptin-10 was purchased from the Peptide Institute (Osaka, Japan). Antiserum to kisspeptin-10 (G-048-56) was purchased from Phoenix Pharmaceuticals (Belmont, CA, USA). Goat affinity-purified antibody to rabbit IgG (whole molecule) (55641) was purchased from ICN Pharmaceuticals (Aurora, OH, USA). EMC-succinimide was purchased from Sigma (St. Louis, MO, USA). β-Galactosidase was purchased from Roche Diagnostics GmbH (Mannheim, Germany). All other reagents were analytical grade reagents from commercial sources. Serum sexual hormones were measured by widely-used methods.

2.4. Preparation of plasma extracts for kisspeptin measuring

After collecting blood, the plasma fraction was rapidly separated by a centrifuge at 4 °C and frozen at −20 °C until use. Each plasma aliquot was diluted five-folds with 4% acetic acid (pH 4.0), and loaded onto a C18 reversed-phase cartridge (Sep-Pak C18; Merck KGaA, Darmstadt, Germany). After washing with 4% acetic acid, peptides in plasma were eluted with 70% acetonitrile in 0.5% acetic acid (pH 4.0). The eluted samples were concentrated by spin-vacuum evaporation, lyophilized, and stored at −40 °C until assayed. Assays were performed within 72 h.

Table 2
Sexual hormones and kisspeptin levels in the post-menopausal subjects.

Subject	Age [yr.]	BMI [kg/m^2]	LH [mIU/mL]	FSH [mIU/mL]	Estradiol [pg/mL]	Progesterone [ng/mL]	Kisspeptin [fmol/mL]
a	60	21.6	38.0	102.2	5	<0.1	14.2
b	61	19.0	22.3	44.2	<5	<0.1	18.6
c	62	26.7	27.5	49.8	7	0.3	24.0
d	64	19.3	29.7	68.0	<5	<0.1	19.2
e	65	22.1	26.8	47.4	<5	0.2	27.4
f	65	25.8	22.5	56.8	<5	<0.1	18.6
g	71	19.7	35.8	59.2	<5	0.2	25.8
h	72	20.3	25.2	51.9	<5	<0.1	16.1

2.5. Enzyme immunoassay (EIA) procedures for kisspeptin

Peptide levels in plasma were measured using highly sensitive EIA for kisspeptin-like immunoreactive substance (LI) which was developed by us and has been described previously [10]. Assays were performed using a delayed-addition method. Separation of bound and free antigens was performed on anti-rabbit IgG-coated immunoplates (Nunc-Immuno Module Maxisorp F8; InterMed, Denmark). Human kisspeptin-10 was conjugated with β-D-galactosidase using EMC-succinimide according to the methods previously reported. The EIA for kisspeptin-LI were specific and highly sensitive. The detection limit was 0.52 fmol/mL and the coefficient of variance was not exceed 10%. The EIA showed complete cross-reactivity with synthetic kisspeptin-13, -14, and -54, and no cross-reactivity with neuropeptide FF (NPFF), NPAF, prolactin-releasing peptide (PrRP), and RFamide-related peptide-3 (RFRP).

2.6. Statistical analysis

The some results are expressed as mean ± SD. Comparison of the results was made by F test. $p < 0.05$ indicated statistical significance.

3. Results

3.1. The serum sexual hormones levels in female volunteers

The serum sexual hormones levels were shown in Fig. 1 and 2. All volunteers except subject F had normal sexual hormones cycles. Because serum FSH levels were high and serum estradiol levels were low in subject F, she was suspected to approach menopause. In general, LH surge is occurred just prior to ovulation, furthermore, serum estradiol levels were high before LH surge. We could not catch the elevation of estradiol and the LH surge in subject B that she seemed to have irregular menstrual cycle by overwork.

3.2. The plasma kisspeptin levels in female volunteers

The plasma kisspeptin levels were shown in Fig. 3. The plasma kisspeptin levels at Day1 indicated a significant correlation with age ($p = 0.030$, $R^2 = 0.573$). The plasma kisspeptin levels in younger volunteers (subject A–D) did not vary enormously, while the plasma kisspeptin levels in volunteers over age 38 (subject E–H) showed an elevation corresponding to LH surge. The ovarian function was weakened with increasing age. The stimulation from the hypothalamus might come to be strong in women approached menopause, as a result of weakened ovarian function. Therefore, the stimulation by a large amount of kisspeptin might be required for start of menstruation as a trigger, and the profile of plasma kisspeptin levels in volunteers over age 38 might be sharpened. On the other hand, menstrual cycle in younger women with normal ovarian function might make progress in a straightforward manner without strong hypothalamus stimulation.

3.3. The serum sexual hormones levels and the plasma kisspeptin levels in post-menopausal female volunteers

The serum sexual hormones levels and the plasma kisspeptin levels in post-menopausal female volunteers were shown in Table 2. Serum gonadotropins levels were high (LH < FSH), and serum estradiol and progesterone levels were very low, caused by suppression of ovarian function. Plasma kisspeptin levels showed between 14.2 and 27.4 fmol/mL (20.5 ± 4.7 fmol/mL).

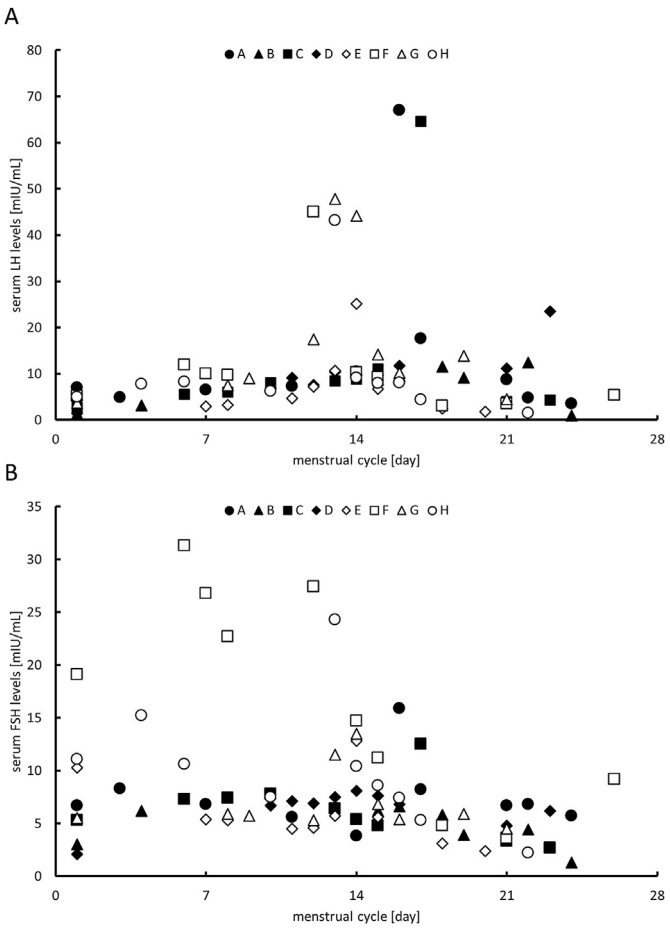

Fig. 1. Serum LH (A) and FSH (B) levels in eight healthy female volunteers.

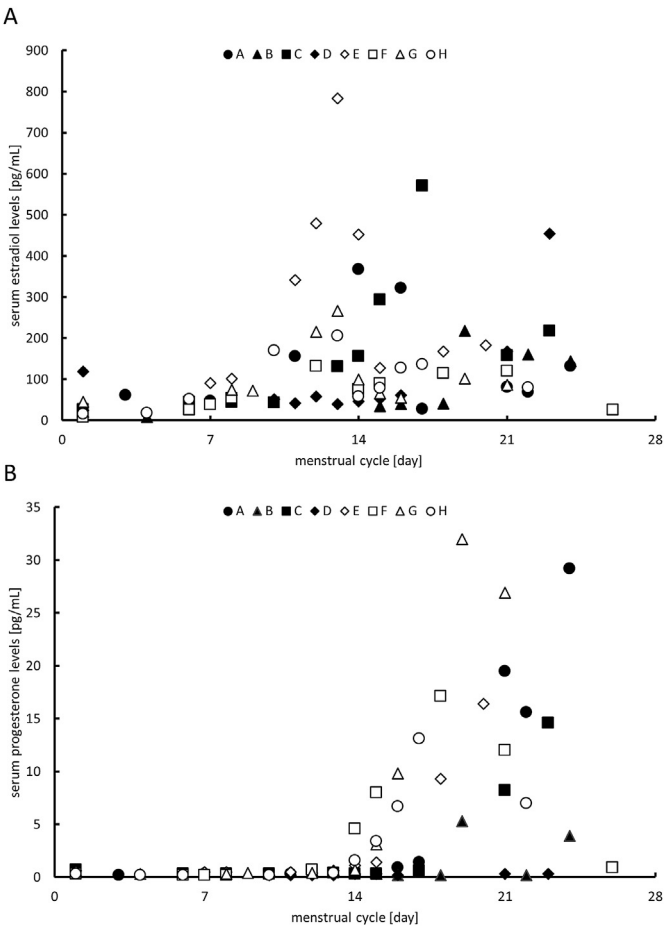

Fig. 2. Serum estradiol (A) and progesterone (B) levels in eight healthy female volunteers.

4. Discussions

Previously, we reported that the plasma kisspeptin levels in healthy male were 12.3 ± 2.5 fmol/mL [5]. The plasma kisspeptin levels in post-menopausal female (20.5 ± 4.7 fmol/mL) were not significantly different from those in male ($p = 0.062$). The plasma kisspeptin levels in follicular phase female (17.4 ± 6.1 fmol/mL) were significantly higher than those in male ($p = 0.015$), however, not significantly different from those in post-menopausal female ($p = 0.244$). The plasma kisspeptin levels in luteal phase female (23.9 ± 10.9 fmol/mL) were significantly higher than those in follicular phase female ($p = 0.002$), post-menopausal female ($p = 0.015$), and male ($p < 0.001$). In general, the serum progesterone levels in luteal phase shows high value. However, there is no significant correlation between plasma kisspeptin levels and serum progesterone levels ($p = 0.542$, $R^2 = 0.018$). The administration of kisspeptin increases serum gonadotropins concentrations [15]. Since the estrogen receptor α is located in kisspeptin neurons [16], the kisspeptin secretion is suggested to be regulated by estrogen. However, there are no significant correlation between plasma kisspeptin levels and either serum LH, FSH, estradiol, or progesterone levels.

In conclusion, we revealed that the plasma kisspeptin levels in female were significantly higher than those in male. Especially in female approached menopause, the kisspeptin might stimulate next menstrual cycle as a trigger, and progress menstruation covered for weakened ovarian function. Further study is needed, but we suggest that kisspeptin may be closely related with menstrual cycle and that the measurement of plasma kisspeptin levels is useful for understanding of reproductive system.

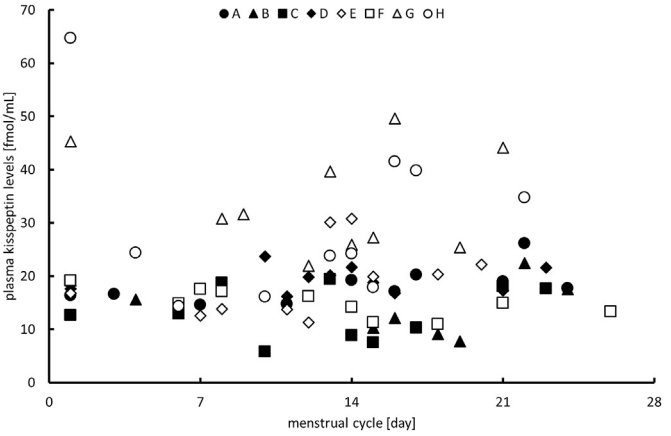

Fig. 3. Plasma kisspeptin levels in eight healthy female volunteers.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

We thank our colleagues in Fujieda Municipal General Hospital, Shimada Municipal Hospital, and Asahina Shinryoujo. This study

was supported in part by Asahina Shinryoujo, and Grant-in-Aid for Scientific Research (to FK; 22790172) from the JSPS (22790172).

Transparency document

Transparency document associated with this article can be found in the online at http://dx.doi.org/10.1016/j.bbrc.2015.02.021.

References

[1] T. Ohtaki, Y. Shintani, S. Honda, H. Matsumoto, A. Hori, K. Kanehashi, Y. Terao, S. Kumano, Y. Takatsu, Y. Masuda, Y. Ishibashi, T. Watanabe, M. Asada, T. Yamada, M. Suenaga, C. Kitada, S. Usuki, T. Kurokawa, H. Onda, O. Nishimura, M. Fujino, Metastasis suppressor gene KiSS-1 encodes peptide ligand of a G-protein-coupled receptor, Nature 411 (2001) 613–617.

[2] M. Kotani, M. Detheux, A. Vandenbogaerde, D. Communi, J.M. Vanderwinden, E. Le Poul, S. Brezillon, R. Tyldesley, N. Suarez-Huerta, F. Vandeput, C. Blanpain, S.N. Schiffmann, G. Vassart, M. Parmentier, The metastasis suppressor gene KiSS-1 encodes kisspeptins, the natural ligands of the orphan G protein-coupled receptor GPR54, J. Biol. Chem. 276 (2001) 34631–34636.

[3] J. Roa, M. Tena-Sempere, Connecting metabolism and reproduction: roles of central energy sensors and key molecular mediators, Mol. Cell. Endocrinol. 397 (2014) 4–14.

[4] S. Messager, E.E. Chatzidaki, D. Ma, A.G. Hendrick, D. Zahn, J. Dixon, R.R. Thresher, I. Malinge, D. Lomet, M.B. Carlton, W.H. Colledge, A. Caraty, S.A. Aparicio, Kisspeptin directly stimulates gonadotropin-releasing hormone release via G protein-coupled receptor 54, Proc. Natl. Acad. Sci. U.S.A. 102 (2005) 1761–1766.

[5] M. Kotani, F. Katagiri, T. Hirai, J. Kagawa, Plasma kisspeptin levels in male cases with hypogonadism, Endocr. J. 61 (2014) 1137–1140.

[6] H. Kanasaki, I.N. Purwana, A. Oride, T. Mijiddorj, U. Sukhbaatar, K. Miyazaki, Circulating kisspeptin and pituitary adenylate cyclase-activating polypeptide (PACAP) do not correlate with gonadotropin serum levels, Gynecol. Endocrinol. 29 (2013) 583–587.

[7] Y.E. Jeon, K.E. Lee, J.A. Jung, S.Y. Yim, H. Kim, S.K. Seo, S. Cho, Y.S. Choi, B.S. Lee, Kisspeptin, leptin, and retinol-binding protein 4 in women with polycystic ovary syndrome, Gynecol. Obstet. Invest. 75 (2013) 268–274.

[8] J.J. Logie, F.C. Denison, S.C. Riley, T. Ramaesh, S. Forbes, J.E. Norman, R.M. Reynolds, Evaluation of kisspeptin levels in obese pregnancy as a biomarker for pre-eclampsia, Clin. Endocrinol. (Oxf.) 76 (2012) 887–893.

[9] D. Panidis, D. Rousso, G. Koliakos, A. Kourtis, I. Katsikis, D. Farmakiotis, E. Votsi, E. Diamanti-Kandarakis, Plasma metastin levels are negatively correlated with insulin resistance and free androgens in women with polycystic ovary syndrome, Fertil. Steril. 85 (2006) 1778–1783.

[10] F. Katagiri, K. Tomita, S. Oishi, M. Takeyama, N. Fujii, Establishment and clinical application of enzyme immunoassays for determination of luteinizing hormone releasing hormone and metastin, J. Pept. Sci. 13 (2007) 422–429.

[11] C.N. Jayasena, A.N. Comninos, A. Januszewski, H. Gabra, A. Taylor, R.A. Harvey, M.A. Ghatei, S.R. Bloom, W.S. Dhillo, Plasma kisspeptin: a potential biomarker of tumor metastasis in patients with ovarian carcinoma, Clin. Chem. 58 (2012) 1061–1063.

[12] F. Katagiri, K. Nagai, A. Kida, K. Tomita, S. Oishi, M. Takeyama, R. Doi, N. Fujii, Clinical significance of plasma metastin level in pancreatic cancer patients, Oncol. Rep. 21 (2009) 815–819.

[13] Y. Horikoshi, H. Matsumoto, Y. Takatsu, T. Ohtaki, C. Kitada, S. Usuki, M. Fujino, Dramatic elevation of plasma metastin concentrations in human pregnancy: metastin as a novel placenta-derived hormone in humans, J. Clin. Endocrinol. Metab. 88 (2003) 914–919.

[14] https://upload.umin.ac.jp/cgi-open-bin/ctr/ctr.cgi?function=brows&action=brows&recptno=R000014714&type=summary&language=E.

[15] Y.M. Chan, Effects of kisspeptin on hormone secretion in humans, Adv. Exp. Med. Biol. 784 (2013) 89–112.

[16] S.L. Dubois, M. Acosta-Martinez, M.R. DeJoseph, A. Wolfe, S. Radovick, U. Boehm, J.H. Urban, J.E. Levine, Positive, but not negative feedback actions of estradiol in female mice require estrogen receptor alpha (ERalpha) in kisspeptin neurons, Endocrinology (2015) (in press).