Plasma Substance-P and Substance-K and Gonadal Steroids in Relation to the Gonadotropin Surge in Normal Human Reproductive Cycles

D. L. MOHYI,¹⁻³ B. KERDELHUÉ,² V. LENOIR,² P. KOLM,¹ H. W. JONES, Jr.,¹ and G. S. JONES¹

Submitted: March 9, 1998

Accepted: May 7, 1998

Purpose: This study was designed to examine changes in peripheral plasma substance-P and -K levels, their association with follicle-stimulating hormone and luteinizing hormone release in normal reproductive cycles in humans, and their correlation with plasma estradiol and progesterone.

Methods: Fourteen healthy, normally menstruating women underwent daily blood sampling (cycle day 4, 4–14 days) for measurement of estradiol, progesterone, luteinizing hormone, and follicle-stimulating hormone, substances-P and -K, and daily transvaginal ultrasounds assessing follicular growth and documentation of ovulation.

Results: Estradiol peaked on day 13, luteinizing hormone and follicle-stimulating hormone peaked on day 14, and progesterone began an exponential increase on about day 13.

Conclusions: In contrast to other experimental designs using in vitro or in vivo rat or monkey tissue, peripheral levels of substances-P ($P = 0.8391$) and -K ($P = 0.3205$) reflected no modulation related to midcycle gonadotropin release in cycling woman.

KEY WORDS: substance-P; substance-K; gonadotropin surge.

INTRODUCTION

Substance-P (SP) is a neuropeptide consisting of 11 amino acids. It belongs to the family of tachykinins. This neurohormone is widely distributed through the brain, gastrointestinal tract, and urogenital tract and exhibits a wide variety of biological effects, including

regulation of blood flow, smooth-muscle activity, transmission of pain signals, and brain distribution consistent with a possible modulating action on regulation and release of gonadotropins from the pituitary and gonadal-hypothalamic feedback (1–4).

The structural characteristics, biological activity, and localization of substance-K (SK) are similar to those of SP. Although the decapeptide SK shares many of the biological effects of SP, there is very little information about the possible neuroendocrine role of SK (5,6).

Immunocytochemical studies indicate that tachykinin-containing neurons and fiber systems are coexistent with the luteinizing hormone-releasing hormone (LH-RH) neural pathways in the hypothalamus (7). Tachykinins may play a role in the control of hypothalamic LH-RH and pituitary LH secretion (8–11). SP was able to inhibit gonadotropin-releasing hormone (GnRH)-stimulated LH secretion from cultured human pituitary cells in a dose-dependent manner (4).

The possibility that brain SP may have a physiologic role in gonadotropin release was documented by Kerdelhué *et al.* (12), who observed that intracerebroventricular administration of SP to female rats completely blocked the preovulatory surge of LH and follicle-stimulating hormone (FSH).

Measuring SP concentration in the median eminence, the arcuate, and the medial preoptic nucleus in the female rat, significant estrous cycle-related fluctuations were recorded for SP. The concentration of SP was highest throughout the day of proestrus, compared to 3 other days of the estrous cycle (13). A similar observation was recently made at the blood level (14). In in vivo experiments in rats, a single subcutaneous injection of SP on the day of proestrus significantly decreased the preovulatory surge of LH. The stimulatory effect of progesterone (P_4) on GnRH-induced LH release was reduced by treatment with SP in vitro,

¹ The Jones Institute for Reproductive Medicine, Department of Obstetrics and Gynecology, Eastern Virginia Medical School, Norfolk, Virginia.

² Laboratoire de Neuroendocrinologie, Université Rene Descartes, Paris, France.

³ To whom correspondence should be addressed at Fertility Center of California, 845 West La Veta Avenue, Suite 104, Orange, California 92868.

using the anterior lobe of the pituitary gland placed in a perfusion chamber (15). In ovariectomized monkeys treated with estradiol benzoate, there is a sequential increase in the *in vitro* hypothalamic secretion of β -endorphin, GnRH, and SP, in that order. Furthermore, exposure to P_4 at 48 hr post-estradiol benzoate led to an acute augmentation of SP release (16–18). Furthermore, SP inhibits LH release in the ovariectomized monkeys.

In the present study, we sought to determine changes in plasma concentration of SP and SK in the human and their correlation with the midcycle gonadotropin surge, as well as estradiol (E_2) and P_4 levels, to elucidate the possible mechanisms of these neuropeptides in regulation of normal reproductive cycles.

MATERIALS AND METHODS

Fourteen healthy women between 26 and 35 years of age were studied. Each had regular ovulatory reproductive cycles, and none used hormonal medication for 3 months before the study. All women were of normal weight and height. The project was approved by the Institutional Review Board of the Eastern Virginia Medical School, and informed consent was obtained from each woman. The duration of the study was 4–14 days, which began as early as day 4 of the menstrual cycle.

Following a brief office visit, daily pelvic ultrasound was performed for assessment of follicular growth and documentation of follicular rupture to confirm ovulation during each day of participation. Two test tubes of blood were drawn each day. A 15-ml blood serum sample was collected for FSH, LH, E_2 -17 β , and P_4 . An additional 10 ml of blood was collected in ethylenediaminetetraacetic acid-containing tubes placed on crushed ice and spun for 10 min at 4°C at 1500g. The plasma samples were stored at –2°C until assayed for plasma SP and SK. E_2 , FSH, and LH determinations were performed by microparticle enzyme immunoassays using the IMX analyzer (Abbott Laboratories, Abbott Park, IL). Progesterone determinations were made using a commercially available radioimmunoassay kit (Pantex, Santa Monica, CA).

SP and SK determinations were performed by specific radioimmunoassays following Sep-Pak extractions (Sep-Pak columns; Waters Associates, Milford, MA), filled with a nonpolar stationary phase. Sep-Pak C18 was used for the preparation of samples for radioimmunoassays of SP and SK. Briefly, the cartridges were prepared by successive washings with 5

ml of ethanol, 5 ml of 8 M urea, and 10 ml of H_2O before the samples were introduced to the column. The columns were then washed with 10 ml of H_2O and 10 ml of 4% acetic acid. The retained peptides were eluted with 5 ml of a mixture of ethanol (90%) and acetic acid (4%), after overnight rotoevaporation of the eluted solution in a Savant Speed Vac Concentrator System (Savant Instrument, Inc., Farmingdale, NY). The residuum was reconstituted in radioimmunoassay buffer and SP and SK measured (19).

Cycle days were standardized to LH surge on day 14 of reproductive cycles. Repeated-measures analysis of variance was used to test for trends over cycle days for each variable. To determine whether there was a relationship between changes in SP or SK and changes in E_2 , LH, FSH, or P_4 over standardized cycle days, each subject's SP/SK data were plotted along with E_2 , LH, FSH, or P_4 data from day 6 to day 17. Data at days 4, 5, 18, 19, and 20 were sparse and were omitted from the analyses. Figure 1 is a composite pattern of mean ($\pm$ standard error of the mean) serum concentration of E_2 , LH, FSH, P_4 , SP, and SK of 14 normal cycling women, standardized to peak LH at day 14.

RESULTS

There were significant trends ($P < 0.001$ for all) for E_2 , LH, FSH, and P_4 . Estradiol peaked on day 13, LH and FSH peaked on day 14, and P_4 began an exponential increase on approximately day 13. There was no significant trend for SP ($P = 0.8391$) or for SK ($P = 0.3205$). The trend for both was virtually a horizontal line. The mean of SP over days 6–17 was 6.24 pg/ml, and the mean of SK over days 6–17 was 0.72 pg/ml. Individual changes from day to day can be viewed as simply random fluctuations about these means and are not related to changes in E_2 , FSH, LH, or P_4 (Fig. 1).

DISCUSSION

The classic theory behind the initiation of the mid-cycle surge of LH is that when ovarian E_2 secretion reaches critical levels, it elicits positive feedback on the hypothalamus and pituitary, which results in increased output of GnRH and LH. It is established that the sensitivity of the pituitary to GnRH varies during the menstrual cycle in synchrony with changes in circulating E_2 levels (20). E_2 has opposite effects on SP mRNA levels in the hypothalamus compared with the anterior

Fig. 1. Composite pattern of mean ($\pm$ standard error of the mean) serum concentration of E₂, LH, FSH, P₄, SP, and SK of 14 normal cycling women, standardized to peak LH at day 14.

pituitary in rodents. This may indicate that SP has different functional roles in the hypothalamus and in the anterior pituitary. SP found locally in the anterior

pituitary is probably involved in the regulation of anterior pituitary secretion. SP in the hypothalamus may play a role in intrahypothalamic regulation. Gonadal

steroids alter SP mRNA expression in the anterior pituitary, in that estrogen decreases pituitary SP-encoding transcripts, compared with the increase in the hypothalamus (1).

The regulation of pituitary gonadotropin secretion in mammals is believed to involve a balance between GnRH input from the hypothalamus and feedback regulation from the gonads. Both LH and FSH are subject to the negative feedback of ovarian steroids. Changes in the frequency of the GnRH pulse from the hypothalamus can account for some of the changes in the LH:FSH ratio (21). The action of GnRH is modulated by several factors at the pituitary level, thereby resulting in differential production and secretion of the LH and FSH, and has self-potentiating effects on the gonadotropins (22). Estradiol enhances the self-priming effects of GnRH on the pituitary and potentiates pituitary responsiveness to GnRH by increasing the number of GnRH receptors through direct stimulation of protein synthesis required for receptor formation (23). At the same time, E₂ decreases the number of SP receptors at the pituitary level (2). FSH and LH secretions were stimulated after immunoneutralization of endogenous SP in the cyclic rat, indicating physiologically significant inhibitory effect on LH release (24). SP inhibits GnRH-stimulated LH and FSH release from rat anterior pituitary cell in primary culture (24). SP apparently does not act through an inhibitory effect on hypothalamic GnRH release but, rather, on GnRH-induced LH release directly at the anterior pituitary level. Secretion of LH at midcycle is of physiological significance because it is responsible for the completion of oocyte maturation and ovulation and for conversion of the preovulatory follicle into a corpus luteum (25). Progesterone stimulates SP release from the hypothalamus at the appropriate time to terminate GnRH stimulation of LH surge (16). Therefore, SP may modulate initiation and termination of the mid-cycle LH surge (4). Secretion of FSH and LH is controlled through release of GnRH from the hypothalamus into the pituitary portal circulation (26). There is a poor correlation between peripheral LH-RH and LH activity. This may be because of dilution of spillover hypothalamic LH-RH in the peripheral circulation (27). SP within the hypothalamus or anterior pituitary may function as a local or paracrine substance to facilitate synaptic regulation of GnRH release or may be released into hypophyseal portal circulation to modulate LH release.

In the present work, blood samples from one time point per day (morning) failed to provide evidence for a human menstrual cycle-related fluctuation in blood SP and SK, contrary to what was seen during the

estrous cycle in the female rat. However, in the rat the estrous cycle variation of SP and SK was evidenced using the sampling of five time points per day. Nevertheless, there is a point that must be emphasized: there is two times more SP than SK in the cycling woman during the menstrual cycle, contrary to the situation in the cycling rat, where equivalent concentrations of SP and SK were observed. Furthermore, SP and SK concentrations stay almost in parallel in the human but evolved in an opposite way throughout the rat estrous cycle. This could represent a major difference in the synthesis and release of SP and SK between the human and the rat and emphasizes the major role of SP in both species.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Ms. Pauline M. Clynes, editor of the *Jones Institute*, for her critical editorial review and manuscript preparation; Elizabeth Throneberry, R.N., for her nursing assistance; and Beverly Cox, B.S., M.T., for her assistance with hormone assays.

REFERENCES

1. Brown ER, Harlan RE, Kruase JE: Gonadal steroid regulation of substance P (SP) and SP-encoding messenger ribonucleic acids in the rat anterior pituitary and hypothalamus. *Endocrinology* 1990;126:330-340
2. Kerdelhué B, Tartar A, Lenoir V, El Abed A, Hublaur P, Millar RP: Binding studies of Substance P anterior pituitary binding sites: changes in Substance P binding sites during the rat estrus cycle. *Regul Peptides* 1985;0:133-144
3. Maggio JE: Tachykinins. *Annu Rev Neurosci* 1988;11:13-28
4. Wormald PJ, Millar RP, Kerdelhué B: Substance P receptors in human pituitary: A potential inhibitor of luteinizing hormone secretion. *J Clin Endocrinol Metab* 1989;69:612-615
5. Debeljuk L, Ghosh P, Bartke A: Neurokinin A levels in the hypothalamus of rats and mice: effects of castration, gonadal steroids and expression of heterologous growth hormone genes. *Brain Res Bull* 1990;25:717-721
6. Debeljuk L, Bartke A: Immunoreactive substance P and neurokinin A in the hypothalamus and anterior pituitary gland of Siberian and Syrian hamsters and of rats. *J Reprod Fertil* 1994;101:427-444
7. Tsuruo Y, Hisano S, Okamura Y, Tsukamoto N, Diakoku S: Hypothalamic substance P-containing neurons. Sex dependent topographic differences and ultrastructural transformation associated with stages of the estrous cycle. *Brain Res* 1984;305:331-341
8. Cheramy A, Michelot R, Levie V, Nieoullon A, Glowinski J, Kerdelhué B: Effect of the immunoneutralisation of Substance P in the cat *substantia nigra* on the release of dopamine from dendrites and terminals of dopaminergic neurons. *Brain Res* 1978;155:404-408

9. Makara GB, Kakucska I, Lenoir V, Kerdelhué B, Palkovits M: A substance P containing hypothalamic neuronal system projects to the median eminence. *Brain Res* 1986;374:399–401
10. Morel G, Chayvialle JA, Kerdelhué B, Dubois PM: Ultrastructural evidence for endogenous substance-P like immunoreactivity in the rat pituitary gland. *Neuroendocrinology* 1982; 35:86–92
11. Sahu A, Kalra SP: Effects of tachykinins of luteinizing hormone release in female rats: Potent inhibitory action of neuropeptide K. *Endocrinology* 1992;130:1571–1577
12. Kerdelhué B, Valens M, Langlois Y: Substance P inhibits the secretion of gonadotropin in female rats. *Proceedings 60th Annual Meeting, Endocrine Society, Miami, FL, 1978, abstract 186, p 167*
13. Parnet P, Lenoir V, Palkovits M, Kerdelhué B: Estrous cycle variations in gonadotropin-releasing hormone, substance P and β -endorphin contents in the median eminence, the arcuate nucleus and the medial preoptic nucleus in the rat: a detailed analysis of proestrus changes. *J Neuroendocrinol* 1990; 2:291–296
14. Duval P, Lenoir V, Moussaoui S, Garret C, Kerdelhué B: Substance P and neurokinin A variations throughout the rat estrous cycle; comparison with ovariectomized and male rats: I. Plasma, hypothalamus, anterior and posterior pituitary. *J Neurosci Res* 1996 (in press)
15. Battman T, Parsadaniantz SM, Jeanjean B, Kerdelhué B: In-vivo inhibition of the preovulatory LH surge by substance P and *in-vitro* modulation of gonadotrophin-releasing hormone-induced LH release by substance P, oestradiol and progesterone in the female rat. *J Endocrinol* 1991;130:169–175
16. Kerdelhué B, Jeanjean B, Lenoir V, Jones G, Hodgen GD: Progesterone induces the release of hypothalamic substance P by a direct time-dependent mechanism in a primate perfusion system. *Proceedings, 70th Annual Endocrine Society Meeting, 1988 (abstract)*
17. Kerdelhué B, Jones GS, Gordon K, Seltman H, Lenoir V, Williams RF, Hodgen GD: Effects of Substance P and progesterone on the pituitary during the estradiol-induced LH surge in primates. *Neuroendocrinology* 1992;11:81–90
18. Kerdelhué B, Jones G, Gordon K, Seltman H, Lenoir V, Millar R, Williams R, Hodgen G: Hypothalamo-anterior pituitary gonadotropin-releasing hormone and substance P systems during the 17- β -estradiol-induced plasma luteinizing hormone surge in the ovariectomized female monkey. *Endocrinology* 1993;132:1151–1157
19. Kerdelhué B, Jones GS, Gordon K, Seltman H, Lenoir V, Williams RF, Hodgen GD: The interaction between hypothalamic GnRH, β -endorphin and Substance P neuronal systems as key regulators of the LH surge: an hypothesis. *Neuroendocr (Life Sci Adv)* 1992;11:91–100
20. Wang CF, Lasley BL, Lein A, Yen SSC: The functional changes of the pituitary gonadotrophs during the menstrual cycle. *J Clin Endocrinol Metab* 1976;42:718–728
21. diZerega GS, Nixon WE, Hodgen GD: Intercycle serum FSH elevations: Significance in recruitment and selection of the dominant cycle. *J Clin Endocrinol Metab* 1980;50:1046–1048
22. Pickering AJMC, Fink G: Priming effect of luteinizing hormone releasing factor: in-vitro and in-vivo evidence consistent with its dependence upon protein and RNA synthesis. *J Endocrinol Metab* 1976;69:373–379
23. Menon M, Peegel H, Katta V: Estradiol potentiation of gonadotrophin releasing hormone responsiveness in the anterior pituitary is mediated by an increase in gonadotrophin releasing hormone receptors. *Am J Obstet Gynecol* 1985;151:534–540
24. Kerdelhué B, Khar A, Nay D, Langlois Y, Bernardo T, Liwska J, Jutisz M: Inhibition in vitro par la substance P de l'excrétion des gonadotropines induite par le LH-RH a partir de cellules adénohypophysaires de rat en culture. *CR Acad Sci (Paris)* 1070;287:879–882
25. Katz E: The luteinized unruptured follicle and other ovulatory dysfunctions. *Fertil Steril* 1988;50:839–89
26. Yen SSC, Tsai CC, Naftolin F, Vandenberg G, Ajabor L: Pulsatile patterns of gonadotropin release in subjects with and without ovarian function. *J Clin Invest* 1972;34:671
27. Elkind-Hirsch K, Ravnkar V, Tulchinsky D, Schiff I, Ryan KJ: Episodic secretory patterns of immunoreactive luteinizing hormone-releasing hormone (IR-LH-RH) in the systemic circulation of normal women throughout the menstrual cycle. *Fertil Steril* 1984;41:56–61