Substance P plasma concentration during the LH preovulatory surge of the menstrual cycle in the human

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Abstract

OBJECTIVES: In order to determine the exact pattern of plasma Substance P (SP) concentration during the LH preovulatory surge and the functional correlates which could exist between plasma SP, LH, 17 β-estradiol (E2) and Progesterone, we performed a detailed analysis of changes in plasma SP concentration, during the critical phases of the LH preovulatory surge in the Human.

METHODS: The experimental study was performed in 21 women between the ages of 26 and 35 years. For each subject, blood samples were taken every 15min, between 07:00 a.m. and 09:00 a.m. for 3 consecutive days when E2 plasma values reached at last 125 *p*g/ml. Then, each subject, according to the mean LH value of each day, was classified into one of the following groups: 1) the day before the day of the ascending phase, 2) the day of the ascending phase, 3) The day of the LH surge, 4) the day of the descending phase, 5) the day after the day of the descending phase.

RESULTS: Mean SP plasma values for the day of the LH peak, the day of the descending phase and the day after the day of the descending phase were all significantly higher than the values of the day of the ascending phase. Overall, there was an almost linear increase for plasma SP values between the day before the day of the ascending phase and the day after the day of the descending phase Also, this linear increase in plasma SP concentration exhibited a positive correlation ($p = 0.016$) with plasma progesterone concentrations which also started to increase on the day of the ascending phase of the LH surge.

CONCLUSIONS:Taken together with previous results which have shown that the administration of a SP antagonist reduces both the amplitude and the duration of the preovulatory LH surge in the monkey, the increase in plasma SP concentrations, possibly driven by the rise in serum progesterone concentration, which take place at the time of the preovulatory LH surge, is certainly an important element of the Hypothalamo-Pituitary-Gonadal interactive network necessary for the full development of the preovulatory LH surge in the Human.

ORIGINAL ARTICLE

Introduction

Substance P (SP), a factor discovered in 1931 both in intestine and brain [36], has been later characterized as an undecapeptide widely distributed in the gastrointestinal tract and in the peripheral and central nervous system. [32,3,37,38,16,7]

There is now evidence that SP is a regulatory peptide involved in the control of the Hypothalamic-Pituitary-Gonadal (HPG) axis.

In the rat, an SP-containing hypothalamic neuronal system located in the arcuate nucleus projects to the median eminence [30] and SP-immunoreactive boutons contact the capillaries of the hypothalamo-pituitary portal system. 17β-estradiol (E2) up-regulates hypothalamic SP and neurokinin A (NKA) content [1], and increases preprotachykinin (PPT) gene expression [2, 34]. Physiological SP and NKA variations in the female hypothalamus during the estrous cycle have also been described [31, 10]. More recently, variations in levels of substance P-encoding α-γ-PPT and SP receptor [neurokinin$_1$ (NK_1)] transcripts were documented in the rat hypothalamus throughout the estrous cycle [13].

The concentrations of SP in the brain, median eminence, arcuate nucleus, medial preoptic nucleus, and anterior pituitary (AP) of rats [31, 17, 8] appear to be mainly modulated by E2 and Progesterone. Furthermore, the number of SP-binding sites in the AP varies in an opposite way with the number of GnRH-binding sites during the rat estrous cycle [23].

In the monkey hypothalamus, numerous SP-containing cells have been found in the arcuate nucleus as well as in the periventricular area of the dorsal tuberal region [35]. Several studies have investigated the role of SP in the control of the HPG axis in the monkey during the induction of an E2- induced preovulatory-like LH surge. A depletion of the SP content of the AP is noted in the OVX monkey [19] 48 h after *in vivo* treatment with Estradiol Benzoate (E2B) at the time of the LH surge. On the other hand, exposure of the OVX monkey to E2B leads to an almost linear increase in the *in vitro* hypothalamic secretion of SP [21] up to 48 h after *in vivo* E2B treatment. When hypothalami were excised and put in culture 48 h after E2B treatment, an acute release of SP occurred following *in vitro* treatment with progesterone [21].

In vitro, an inhibitory effect of SP was documented at the AP level in the rat [22], monkey [20], and human [42].

To date, three categories of SP-binding neurokinin receptors have been described: NK_1 with primary affinity for SP; NK_2 with primary affinity for NKA; and NK_3 with primary affinity for neurokinin B. NK_1 receptors have been characterized on lactotrops and gonadotropic cells at the AP level [28,29]. NK_1 mRNA encodes a seven-transmembrane domain tachykinin receptor, with preferential affinity to SP [15,14] and is widely expressed in the central and peripheral nervous system [26] and in the AP [41].

Recently, the SP/NK1 Receptor system has been shown to be involved in depression and anxiety [4,29,11].

In the present study, in order to determine the exact pattern of plasma SP concentration and the functional correlates which could exist between plasma SP, LH, E2 and Progesterone, we performed a detailed analysis of changes in plasma SP concentration during the critical phases of the preovulatory LH surge in the Human.

Material and Methods

Subjects

The experimental study was performed in 21 women between the ages of 26 and 35 years. Each had regular ovulatory reproductive cycles, and none had 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.

Blood samples were taken every 15 min, between 07:00 a.m. and 09:00 a.m. for 3 consecutive days when 17β-estradiol (E2) values reached at least 125 pg/ml, i.e. around days 8–11 of the menstrual cycle.

Each subject, according to the mean LH value of each day, was classified into one of the following groups:

1) Day before the ascending phase,
2) Day of the ascending phase,
3) Day of the LH Peak,
4) Day of the descending phase,
5) Day after the descending phase.

All subject had their 3 consecutive days within a period of 5 days.

This procedure allows us to have a significant number of time points from the 48 h which precede the initiation of the LH surge to the 48 h which follow.

The experimental study was carried out during the months of July and November. All subjects were medical technologists, nurses or wives of hospital personnel and were thus familiar with the surroundings. They were allowed to carry on their normal duty or pursue normal function during the intervals between blood draws.

Study Protocol

When blood was drawn, two tubes were taken. A 7-ml blood sample was collected for FSH, LH, E2 and progesterone determination. An additional 5 ml of blood was immediately collected in EDTA-containing tubes, placed on crushed ice and spun 10 min at 4°C at 1,500 g. The plasma samples were stored at –20°C until assayed for SP.

Serum E2, LH, FSH and Progesterone Assay

E2, LH and FSH concentrations were determined by microparticle enzyme immunoassays using the IMX analyzer (Abbott Laboratories, Abbott Park, III, USA). Progesterone (P4) determination was made using a radioimmunoassay kit (Pantex, Santa Monica, Calif.,

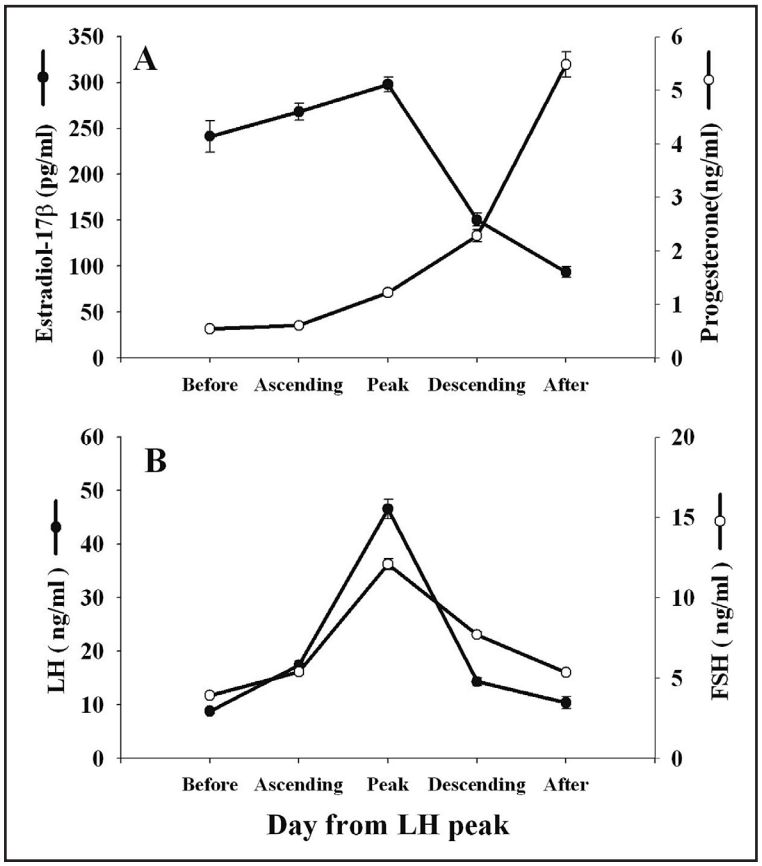

Figure 1. Pattern of mean daily (±SEM) serum concentration of Estradiol-17β and Progesterone (part A) and LH and FSH (part B) in blood samples taken at 15 min intervals from 07:00 a.m. to 09:00 a.m. Blood samples were taken during 3 consecutive days for each subject. The number of subjects per experimental day are given into brachets: Before (4); Ascending (11); Peak (17); Descending (10); After (5).From Kerdelhué et *al* [24].

USA). The intra- and interassay coefficients of variation were 6.3 and 6.7% respectively for E2 ; 3.1 and 5.6% respectively for LH ; 4.7 and 7.6% respectively for FSH, and 5.6 and 7.9% respectively for P4.

Plasma SP Assay

Sep-Pack columns (Waters Associates Milford, Mass., USA) filled with a stationary nonpolar phase (Sep-Pack C18) were used for the preparation of the samples prior to the SP assay, as previously described [21]. Briefly, cartridges were prepared by successive washed with 5 ml methanol, 5 ml 8 M urea and 10 ml of double-distilled water before the introduction of 2 ml of plasma on the column. The column was then washed with 10 ml of H_2O and 10 ml of 4% acetic acid. Peptides retained on the column were eluted with 5 ml of a mixture of 90% ethanol and 4% acetic acid. After a speed vac evaporation (Savant Instruments, Farmingdale, N.Y., USA) for 12 h the residuum was reconstituted in 0.5 ml of the RIA buffer and aliquots of 0.1 and 0.3 ml were used for the assay. This procedure allows the recovery of 85% of added ^{125}I-SP or unlabelled SP. The SP assay was performed as previously described [5]. There was no cross-reactivity with GnRH, TRH, or β-endorphin LH, FSH, nor with neurokinins NKA, neuromedin A or B. The intra- and inter-assay CVs were 10 and 12% at binding levels of 40–60%, respectively. The lower limit of detection was 1 pg per tube.

Statistical Analysis

Cycle days were standardized in reference to the time of the LH peak.

Data analysis was performed with two statistical softwares, SigmaStat (SPSS Inc, Chicago, IL, USA) and RS1 (Brooks automation Inc, Chelmsford, MA, USA) as a complement; in addition, the latter allows an easy data and table handling and provides a complete environment for developing personal programs. Most of the methods or tests they used refer to Zar [43]; the references of particular ones are given below.

The normality of the sample distribution-parents was checked with Kolmogorov-Smirnov (Lilliefors) or Shapiro-Wilk test according to data number and homoscedasticity was tested according to Conover [6].

Two factors could affect hormone concentrations: the day and the hour of blood collection. The group of subjects whose blood was collected on the same day was designated by the name of this day, i.e, day before the ascending phase, day of the ascending phase, day of the LH peak, day of the descending phase, day after the descending phase.

As data were unbalanced and most of them deviate from the requirements of norrmality and homoscedasticity, despite logarithmic Ln transform, and as the sample sizes were large enough, a two way analysis of variance (ANOVA 2), based on a general linear model [36], was performed for determining the role of each factor. The results were compared with those given by an ANOVA 2, after converting concentrations to ranks and those com-

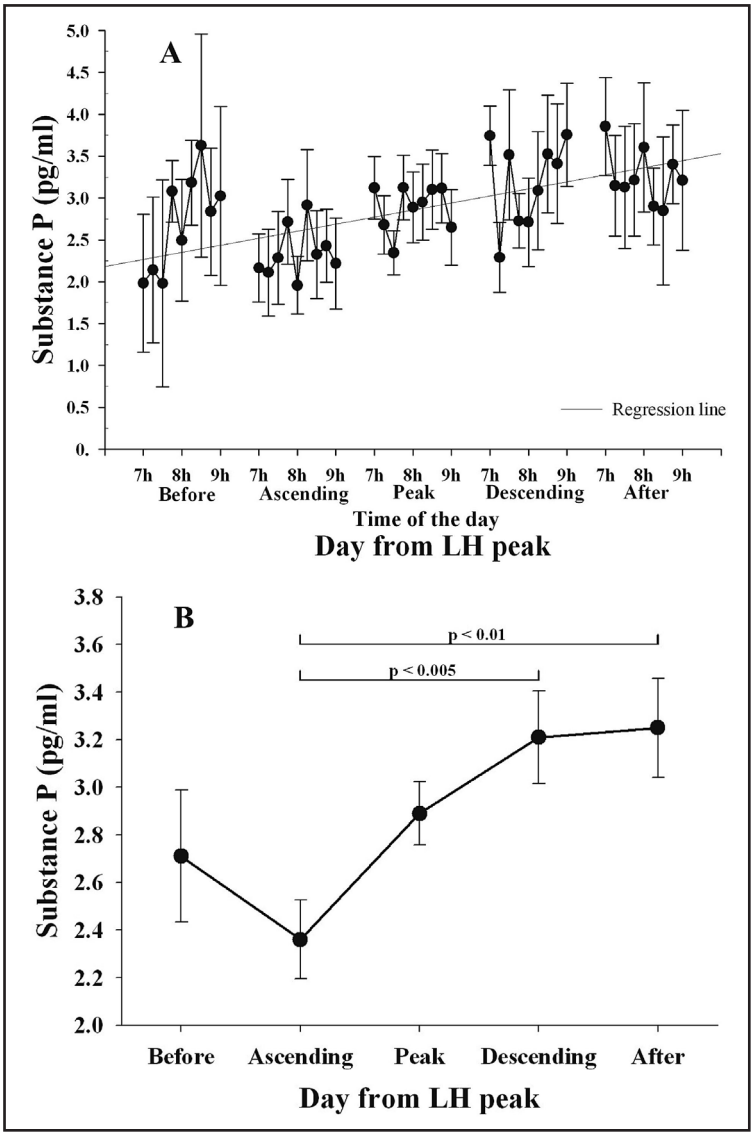

Figure 2. Pattern of mean (±SEM) plasma concentration of Substance P in blood samples taken at 15 min intervals from 07:00 a.m. to 09:00 a.m., with the regression line from the beginning to the end of study. (part A) – Pattern of mean daily (±SEM) plasma concentration of Substance P(part B). Blood samples were taken during 3 consecutive days for each subject. The number of subjects per experimental day are given into brachets: Before (4); Ascending (11); Peak (17); Descending (10); After (5).

ing from an ANOVA 2 using an additive model based on a comparison of weighted means[36].

When factor Time did not affect hormone concentration, a Kruskal-Wallis ANOVA on ranks was run with pairwise multiple comparisons according to Dunn's method. Linear and nonlinear least squares regressions were used to model SP, as a function of Time, LH or Progesterone.

When statistical assumptions of linear correlation analysis were not satisfied, the rank correlation coefficient ρ of Spearman was calculated.

Results

LH, FSH, 17 β-Estradiol, and Progesterone serum values were the same as those previously published [24]. The data were also synchronized according to the day of the LH peak of each subject.

Figure 1 shows the pattern of mean 17β-Estradiol (E2) and Progesterone serum concentration (Fig.1A) and LH and FSH serum concentration (Fig.B) for each experimental day.

The LH values were statistically ($p < 0.001$) higher on the day of the LH peak than on others days. Also, values on the ascending phase were higher than those on the day before and the day after ($p < 0.001$) and values on the descending phase were higher than those on the day before ($p = 0.02$). Serum E2 values (Fig. 1A) were at their highest the day of the LH surge.Serum progesterone values (Fig 1A) exhibited a continuous rise throughout the entire experimental period. A significant rise ($p < 0.001$) was already observed the day of the peak of the LH surge.

Figure 2 shows the pattern of means of SP plasma concentrations from the day which precedes the ascending phase to the day which follows the descending phase of the preovulatory LH peak. No trend could be found within each group because the parameters of every regression line were not significant. On the contrary, there exists a linear relationship between individual concentrations and hours, from the time of the first blood sampling: SP = 2.279 + 0.001032*Hour of which the parameters are significantly different from 0 ($p \leq 0.0009$) (fig.2 A).

An ANOVA 2 showed that only factor Day affected SP concentrations. Therefore, a Kruskal-Wallis ANOVA on ranks was run: the role of factor Day was very significant ($p = 0.001$) and according to Dunn's method, values from the day of the ascending phase were lower than those of the day of the descending phase and of the day after the descending phase(fig.2B).

As the shape of SP and P4 curves according to time (fig.1A and fig.2B) where nearly similar, the ρ correlation was calculated and found equal to 0.261 ($p = 0.0001$). However, this correlation could be attributed to the relationship of each variable with the time factor; therefore, a partial correlation, with the elimination of the time effect on these two variables, was calculated: this correlation ($\rho_{PP4.t} = 0.169$) was significantly different from zero ($p = 0.0008$).

Last, if episodic pulses were recorded for concentrations of blood LH and SP, with a periodicity of 60–90 min, no significant relationships was found between pulses of LH and SP.

Discussion

Overall, our results show that there is a linear increase in SP plasma concentration from the day of the ascending phase of the LH surge to the day after the descending phase of the LH surge.

In the present series of observations, the continuous increase in serum progesterone concentration, which occurs between the ascending day of the LH surge to the day after the day of the descending phase of the LH surge, might be responsible for the continuous increase in plasma SP during the same period of time. In the monkey, progesterone has been shown to stimulate the release of SP, after a necessary E2 exposure, from the hypothalamus *in vitro* [20]. It needs to be noted that in the time period of the study the E2 surge had been almost maximized as early as before the first day of the study. A biphasic effect of E2 on the secretion of SP has been well documented.

During the rat estrous cycle, there was a positive correlation between plasma levels of SP from the beginning of the day of diestrous I to late afternoon on the day of proestrous when SP plasma values starts to fall, in correlation with highest E2 serum values, just before the initiation of the LH surge[10].

During the monkey menstrual cycle, there is a positive correlation between blood concentrations of E2 and SP during the early follicular phase, but a negative correlation during the late follicular phase[25].

The increase in plasma SP concentration, controlled by changes in ovarian steroids, is certainly a constitutive part of the myriad of hypothalamic events underlying the preovulatory LH surge.

In that view it was already shown that the administration of a SP Receptor specific antagonist, which cross the blood brain barrier, markedly reduces the amplitude and the duration of the LH preovulatory surge in the monkey [25].

Although it is difficult to prove it directly, it is very likely that SP plasma concentration reflects at least the hypothalamic SP activity. At the hypothalamic level, a close juxtaposition between GnRH immunoreactive neurons and Substance P immunoreactive axons has been seen in the human[9] and a stimulatory effect of SP on GnRH release, and consequently a stimulation of LH and FSH release, has been documented[40]. Also, an inhibitory action of SP on CRF release[12], which stimulates ACTH release, but is also inhibitory to GnRH release[33], has been described. Interestingly, there is a fall in the ACTH-Cortisol activity righ after the LH preovulatory peak during the menstrual cycle in the Human[24].

In summary, the increase in plasma SP concentrations which take place at the time of the LH preovulatory surge is certainly an important element among others [25,16] of the HPG integrative network necessary for the full development of the preovulatory LH surge in the Human.

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