ACTH, β-endorphin, substance P, and corticotrophin releasing hormone in plasma and follicular fluid in hormonally stimulated menstrual cycles for in-vitro fertilization in the human

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Changes in plasma concentrations of ACTH, β-endorphin (β-EP) and cortisol have been found to be associated during the human menstrual cycle. Changes in hypothalamic levels of gonadotrophin releasing hormone (GnRH), β-EP and substance P (SP) have also been associated with the oestrous cycle in the rat. Therefore, an attempt was made to measure the activity of the corticotrophic axis and SP by measuring blood and follicular fluid concentrations of ACTH, β-EP, SP and corticotrophin releasing hormone (CRH) during the hormonal ovarian stimulation phase for in-vitro fertilization (IVF), in a series of 19 patients. At the plasma level, there was significant change over treatment days in ACTH (P = 0.1550), β-EP (P = 0.1137), or SP concentrations (P = 0.5625). CRH was not detectable over treatment days. In addition, there was no significant change in neuropeptide over treatment days between those women who became pregnant and those who did not (P = 0.17 for all). In the follicular fluid, ACTH was not detectable, β-EP concentration was three times higher than in the plasma, CRH was detectable, and SP concentration was similar to that of plasma. There was no apparent correlation, however, between β-EP or SP concentrations in the plasma and follicular fluid from a given patient. In conclusion, the absence of changes in the activity of the corticotrophic axis during the hormonal ovarian stimulation suggests that there was no major stress component associated with the stimulation phase of IVF or the occurrence of a pregnancy.

Key words: ACTH/CRH/β-endorphin/IVF/SP

Introduction

There is increasing experimental evidence which demonstrates interactions between the adrenal and gonadal endocrine axes in cycling female rats and in normally menstruating women, and it has also been documented that an abnormal activation of the corticotrophic axis may play a role in stress-related reproductive dysfunctions. An increase has been reported in the activity of various components of the corticotrophic axis around the time of ovulation. Serum concentrations of ACTH and corticosterone are elevated prior to the start of the pre-ovulatory luteinizing hormone (LH) surge on the afternoon of pro-oestrus in the rat (Raps et al., 1971; Buckingham et al., 1978; Brann and Mahesh, 1991), and a mid-cycle elevation of ACTH and cortisol has also been reported in women (Genazzani et al., 1975). Also, previous studies have shown that plasma β-endorphin (β-EP) concentrations have pre- or post-ovulatory peaks, independent of the induction of ovulation (Vrbicky et al., 1982; Petraglia et al., 1986). We recently reported an activation of the hypothalano–anterior pituitary corticotropin releasing hormone (CRH), and ACTH systems during the oestradiol 17β-induced plasma LH surge in the ovariectomized monkey (Kerdelhué et al., 1995).

The rapid fall of LH at the mid-cycle surge has been demonstrated to be related to an inhibitory effect of CRH on LH, but not of ACTH or cortisol (Rivier et al., 1986; Oster and Ferin, 1987; Petraglia et al., 1987; Williams et al., 1990; Feng et al., 1991). Also, i.v. infusion of ACTH in ovariectomized monkeys does not lead to the same decrease in LH secretion as does CRH (Xiao and Ferin, 1988). Additionally, in female rats (Petraglia et al., 1987), CRH decreases plasma LH concentrations by inhibiting gonadotrophin releasing hormone (GnRH) release into the hypophyseal–portal blood.

Substance P (SP), a tachykinin produced in the peripheral and central nervous system, plays an inhibitory role in gonadotrophic function. When administered at noon on the day of pro-oestrus it reduced the amplitude of the afternoon preovulatory LH surge (Battmann et al., 1991) in the rat. Substance P also reduced the amplitude of the GnRH-induced LH release from perfused isolated rat and monkey anterior pituitaries, or human dispersed anterior pituitary cells (Kerdelhué et al., 1978, 1992a; Wormald et al., 1989; Battmann et al., 1991). Changes in hypothalamic concentrations of SP are associated with the oestrous cycle in female rat, as are GnRH and β-EP (Parnet et al., 1990). Oestradiol 17β regulates the SP content in the anterior pituitary (Kerdelhué et al., 1993), and the mRNA encoding for preprotachykinins (Brown et al., 1990), the SP precursors. It also stimulates the release of hypothalamic SP in the monkey (Kerdelhué et al., 1992b). Changes in related reproductive hormones, i.e. LH, follicle stimulating hormone (FSH) and oestradiol, have also been documented throughout the hormonally stimulated human cycle (Jones, 1986). Taken together, the available results may
Materials and methods

Subjects

The profiles of the 19 study patients are shown in Table I.

Study protocol

These 19 patients underwent 9 days of venepuncture taken at the same time of day (8:00 a.m.), to allow for the diurnal rhythm in ACTH and β-EP. A 15 ml blood serum sample was assayed for oestradiol 17β. An additional 10 ml of blood taken from the same needle (to avoid the stress of sampling) was collected in EDTA-containing tubes, placed on crushed ice and centrifuged for 10 min at 4°C at 1500 g. The plasma samples were stored at −20°C until assayed for plasma ACTH, β-EP, and SP. Human chorionic gonadotrophin (HCG) 10 000 units was administered to induce final oocyte maturation and oocytes were aspirated at 36 h post-HCG exposure. The residual follicular fluid was then centrifuged for 10 min at 4°C and the supernatant was frozen at −28°C until ACTH, β-EP and SP assays were performed.

ACTH, β-endorphin, substance P and CRH assays

ACTH, β-EP and SP determinations were performed by specific radioimmunoassays (RIAs) following Sep-Pak extractions. Sep-Pak columns (Waters Associates, Milford, MA), filled with a non-polar stationary phase (Sep-Pak C18), were used for the preparation of samples for RIA of ACTH, β-EP and SP. Briefly, the cartridges were prepared by successive washings with 5 ml ethanol, 5 ml 8 M urea, and 10 ml H₂O before the plasma samples were introduced to the column. The columns were then washed with 10 ml H₂O and 10 ml 4% acetic acid. The peptides which were retained were eluted with 5 ml of a mixture of ethanol (90%) and acetic acid (4%). After overnight roto-evaporation of the eluate in a Savant SpeedVac Concentrator System (Savant Instruments, Inc., Farmingdale, NY), the residue was reconstituted in RIA buffer and ACTH, β-EP, SP and CRH measured. The recovery of each peptide from Sep-Pak was 85%, 1995). The limit of detection of the assay was 1.5 pg/tube. These 19 patients underwent 9 days of venepuncture taken at the same time of day (8:00 a.m.), to allow for the diurnal rhythm in ACTH and β-EP. A 15 ml blood serum sample was assayed for oestradiol 17β. An additional 10 ml of blood taken from the same needle (to avoid the stress of sampling) was collected in EDTA-containing tubes, placed on crushed ice and centrifuged for 10 min at 4°C at 1500 g. The plasma samples were stored at −20°C until assayed for plasma ACTH, β-EP, and SP. Human chorionic gonadotrophin (HCG) 10 000 units was administered to induce final oocyte maturation and oocytes were aspirated at 36 h post-HCG exposure. The residual follicular fluid was then centrifuged for 10 min at 4°C and the supernatant was frozen at −28°C until ACTH, β-EP and SP assays were performed.

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The rabbit ACTH antiserum was a generous gift from Dr Charles Oliver (INSERM, Marseille, France). This antiserum does not cross-react with β-EP corticotrophin-like intermediate peptide (CLIP) or ACTH 23–29, but does cross-react with ACTH 7–38 to the extent of 30% (Melik Parsadanianz et al., 1993). The limit of detection of the ACTH assay was 15 pg/tube. The intra- and inter-assay coefficients of variation were 8.2 and 9.0% respectively.

β-Endorphin was measured according to a previously described method (Kerdelhüé et al., 1982). The limit of detection of the assay was 8 pg/tube. The intra- and inter-assay coefficients of variation were 8.0 and 10.0% respectively. Substance P was measured according to a previously described method (Cheramy et al., 1978). The limit of detection of the assay was 1 pg/tube. The intra- and inter-assay coefficients of variation were 7.0 and 9.0% respectively. CRH was measured according to a previously described method (Kerdelhüé et al., 1995). The limit of detection of the assay was 1.5 pg/tube. The intra- and inter-assay coefficients of variation were 6.0 and 8.0% respectively.

Oestradiol 17β assay

Oestradiol 17β assay was performed by microparticle enzyme immunoassay using the IMX analyser (Abbott Laboratories, Abbott

Table I. Patient characteristics and response to ovarian stimulation

<table>
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<tr>
<th>Patients</th>
<th>Age</th>
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*In-vitro fertilization (IVF) for all patients, except for 4, 5 and 6 (in the pregnant group) and 19 (in the non-pregnant group) who had intrauterine insemination (IUI).

Endomet = endometriosis; LP = luteal phase; administration of Lupron during the luteal phase; number of (75 mIU FSH) ampoules; number of (75 mIU FSH + 75 mIU LH) human menopausal gonadotrophin (HMG) ampoules; days of stimulation.
ACTH, β-EP, SP and CRH in plasma and follicular fluid for IVF

Figure 1. Pattern of mean (±SEM) plasma concentration of 17β oestradiol in blood samples taken from day –9 to day +1. Day 0 is the day of human chorionic gonadotrophin (HCG) injection.

Figure 2. Pattern of mean (±SEM) plasma concentration of ACTH in blood samples taken from day –9 to day +1. Day 0 is the day of human chorionic gonadotrophin (HCG) injection.

Figure 3. Pattern of mean (±SEM) plasma concentration of β-endorphin in blood samples taken from day –9 to day +1. Day 0 is the day of human chorionic gonadotrophin (HCG) injection.

Figure 4. Pattern of mean (±SEM) plasma concentration of substance P in blood samples taken from day –9 to day +1.

Statistical analysis
Repeated measures analysis of variance (ANOVA) was used to assess whether there was a change in neuropeptide concentrations over treatment days.

Results
Plasma 17β oestradiol
Figure 1 shows the typical rise in plasma 17β oestradiol, following ovarian stimulation, as a function of the day (0) when HCG was administered to induce ovulation. The rise became markedly significant by day –3 before HCG was given.

Plasma ACTH
Figure 2 shows plasma ACTH values from day –9 to day +1, in relation to the day of HCG injection. Although values tended to be higher at the beginning of the stimulatory process, with a fall in ACTH concentrations between day –4 and day –3, this was not statistically significant (P = 0.1550).

Plasma β-endorphin
Figure 3 shows plasma β-EP values from day –9 to day +1 in relation to the day of HCG injection. Although values tended to be higher between day –8 to day –3 than during the last period of the stimulation, there was no significant change in β-EP concentrations (P = 0.1137).

Plasma substance P
Figure 4 shows plasma SP values from day –9 to day +1 in relation to the day of HCG injection. There was no significant change in SP concentrations (P = 0.5625).

Plasma CRH
Plasma CRH values were undetectable (<1 pg/ml) in the plasma of any patient.

Follicular fluid ACTH, β-endorphin, substance P, and CRH concentrations
ACTH was not detectable in the follicular fluid of any patient. β-Endorphin was detectable in follicular fluids of all patients. The β-EP fluid concentration varied between 5 and 50 pg/ml among patients, but values were similar in different specimens of follicular fluid from individual patients. Interestingly, the mean β-EP concentration was three times higher in the follicular fluid (20 ± 2 pg/ml follicular fluid) than in the plasma (7 ± 0.3 pg/ml plasma, P < 0.001) of the same patients.

Substance P was detectable in follicular fluids of all patients. The concentration varied from 2 to 8 pg/ml among patients and, with few exceptions, was in the same range in different
specimens of follicular fluid of individual patients. Follicular fluid SP and plasma SP concentrations were similar (3.7 ± 0.09 pg/ml versus 3.6 ± 0.1 pg/ml).

CRH was detectable in follicular fluids of all patients. The concentration varied from 5 to 15 pg/ml and, with few exceptions, was in the same range in different specimens of follicular fluid from individual patients.

**Neuropeptides and pregnancy**

There was no significant difference \( P = 0.17 \) for all in neuropeptide concentrations following stimulation between those women who became pregnant (six of 19) and those who did not.

**Discussion**

The findings described in the present study represent the first attempt to evaluate ACTH, β-EP, SP plasma and CRH concentrations, and to correlate the plasma concentrations of these neuropeptides with follicular fluid concentrations in relation to various hormonal and biological parameters during the ovarian stimulatory phase of the IVF process. Some other infertility parameters of the patient have also been correlated.

The results obtained from plasma show that although there was a trend for ACTH and β-EP concentrations to change, these changes did not reach statistical significance.

It appears that, under the clinical conditions described, the rising concentration of 17β oestradiol does not have a significant stimulatory or inhibitory effect on ACTH, β-EP or SP concentration. Obviously, changes in the activity of various components of the corticotrophic axis which occur during the menstrual cycle become blunted during the hormonally stimulated menstrual cycle.

Also, as the majority of patients had received GnRH agonist treatment during the preceding luteal phase (‘LL’), this may interfere with the normal interaction between corticotrophic and gonadotrophic axes. This may, in part, explain the negative findings.

Although ACTH was not detected in the follicular fluid and β-EP values were three times higher in the follicular fluid than in the plasma, no specific relationship was noted between the SP concentration in follicular fluid and plasma. The presence of β-EP, in concentrations several times higher than those in plasma, has already been reported in human follicular fluid (Facchinetti et al., 1986). In addition, β-EP binding sites were identified in the porcine ovary, using \(^3\)H-naloxone, on granulosa cells (Hamada et al., 1995). These results suggest a relationship between folliculogenesis and ovarian β-EP.

The differences found between β-EP and ACTH concentrations in follicular fluid and plasma may suggest the operation of a local pro-opioid–melanocortin (POMC) system at the ovarian concentration. This could be orientated toward the production of β-EP, as in the hypothalamus, or toward the production of ACTH, as in the anterior pituitary. Such a possibility is further substantiated by the fact that CRH, which controls the synthesis of POMC and release of β-EP and/or ACTH, is also present in the follicular fluid, but not in the serum.

However, no relationship was seen between β-EP concentrations in individual specimens of follicular fluid and the morphology of oocytes and their outcome following IVF.

The present results show no major changes in the secretion rates of ACTH, β-EP and SP in the plasma of women undergoing ovarian stimulation for IVF, suggesting that there is no major stress associated with this process.

Similarly, Harlow et al. (1996), demonstrated that neither cortisol concentrations, nor the amount of stress experienced, were significantly changed in women undergoing hormonally stimulated menstrual cycles for IVF.

In conclusion, even though it has been well documented that psychological stress factors undoubtedly stimulate the activity of the hypothalamo–pituitary–adrenal axis, perhaps inducing different concentrations of activity of the axis, the high oestradiol concentrations seen after ovarian stimulation may normalize variations in the activity of the corticotrophic axis and blunt possible psychological influences. Therefore, the activity of the gonadotrophic axis, among patients undergoing IVF, appears to some extent to be normalized and less susceptible to environmental or endogenous influences.

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**References**


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