The relationship between plasma levels of the endocannabinoid, anandamide, sex steroids, and gonadotrophins during the menstrual cycle

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Objective: To further investigate the relationship between plasma anandamide (AEA), sex steroids, and gonadotrophins to improve our understanding of how AEA may be involved in human fertility.
Design: Cross-sectional and longitudinal study.
Setting: University Hospital of Leicester NHS Trust, Leicester Royal Infirmary.
Patient(s): Healthy premenopausal and postmenopausal volunteers.
Intervention(s): UPLC-MS/MS–measured plasma AEA and ELISA-measured serum FSH, LH, estradiol, and progesterone levels at five different phases of the menstrual cycle and postmenopause.
Main Outcome Measure(s): Plasma AEA, serum steroids and gonadotrophins.
Result(s): Changes in AEA levels were similar in the two cohorts. The mean ± SEM levels in the early follicular phase (0.89 ± 0.06) for the cross-sectional cohort and the longitudinal cohort (0.73 ± 0.03) were higher than those in the late follicular phase (0.77 ± 0.09 cross-sectional; 0.63 ± 0.08 longitudinal). The highest AEA levels were measured at ovulation (1.38 ± 0.14 and 1.33 ± 0.16) and the lowest level was measured in the late luteal phase (0.66 ± 0.07 and 0.56 ± 0.06). There was a statistically significant positive correlation between AEA, estradiol (P=0.0015), LH (P<0.0001) and FSH levels but not progesterone (P=0.022).
Conclusion(s): Peak plasma AEA occurred at ovulation and positively correlated with estradiol and gonadotrophin levels suggesting that these may be involved in the regulation of AEA levels. (Fertil Steril® 2010;93:1989–96. ©2010 by American Society for Reproductive Medicine.)

Key Words: anandamide, endocannabinoid, gonadotrophin, menstrual cycle, sex steroid hormones

Anandamide (AEA) is an unsaturated fatty acid and is the most studied member of the endocannabinoid family (1). Endocannabinoids are an emerging class of lipophilic mediators that are generated in the body and have pleiotropic effects on many tissues, including the reproductive organs and the hypothalamus and pituitary (2). These effects are mediated through the cannabinoid receptors, which are the same receptors for the phytocannabinoids, such as Δ^9-tetrahydocannabinol and cannabidiol, that are the active molecules within marijuana (3). Because marijuana use is associated with infertility problems (4–6) and endocannabinoids act in a similar manner to phytocannabinoids (7), it is likely that AEA may also affect human fertility. It has been demonstrated, for example, that reduced plasma AEA levels are essential for implantation and early pregnancy success (4, 8, 9), that high AEA concentrations are embryotoxic (10–12), and elevated plasma levels are associated with miscarriage (13). We have also previously demonstrated in a cross-sectional cohort, in which a single plasma sample was taken in the early follicular (days 2–7) and late luteal (days 20–25) phase of the menstrual cycle and postmenopausal controls, that plasma AEA levels in the early follicular phase were higher than those of the late luteal phase and those in postmenopausal women (14). The demonstration that there is a higher expression of fatty acid amide hydrolase (FAAH), the enzyme responsible for the degradation of AEA, in peripheral lymphocytes after ovulation suggests a possible association of AEA with the factors controlling the menstrual cycle (15).

In follicular fluid obtained at egg collection in IVF cycles, AEA levels have been quantified (16) and suggest that the factors involved in folliculogenesis may also modulate AEA levels in the ovary. Despite these observations, little is known about the interplay between endocannabinoids and

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sex steroid hormones or gonadotrophins during the menstrual cycle, as well as the exact mechanisms controlling plasma AEA levels, which may be important for follicular development and appropriate endometrial priming for implantation.

Because our previous pilot study (14) was constrained by sample timing for AEA measurement (one sample in the early follicular phase and another in the late luteal phase), we sought in this study to investigate in further detail (cross-sectionally and longitudinally) the changes in plasma AEA levels throughout the menstrual cycle and how the levels might be related to those of the key ovarian sex steroid hormones and gonadotrophins involved in the regulation of the menstrual cycle. Understanding this relationship may eventually result in interventions that may improve outcomes in human reproduction and fertility.

MATERIALS AND METHODS

Subjects

This study was undertaken in two parts. The first part was cross-sectional and designed to confirm our previous observations and furthermore to examine in greater detail (i.e., not only in the early follicular and late luteal phases) plasma changes during the menstrual cycle; the second part was longitudinally designed with the volunteers acting as their own controls to allow for further investigation of whether the changes in AEA levels observed cross-sectionally were mirrored in the same individuals. All the volunteers included in the study were healthy, not obese, menstruating in regular cycles (defined as occurring every 26–32 days) for 6 months before entry into the study, not taking any medication or hormonal contraception for at least six months before recruitment into the studies, and nonsmokers or smokers of fewer than 20 cigarettes per day. The postmenopausal women had all been amenorrheic for 2 years, had not been taking hormonal replacement therapy or any medication for six months before the study, had both ovaries and an intact uterus, and were recruited as controls for the premenopausal studies. Each volunteer gave a written signed informed consent before recruitment into the study, which had ethics approval from the Leicestershire and Rutland Local Research Ethics Committee (i.e., approval was obtained from the Institutional Review Board).

In both parts of the study, the menstrual cycle was divided into five phases based on the normal physiologic changes in the levels of sex steroid hormones and gonadotrophins that occur throughout the ovulatory menstrual cycle (17); early follicular (days 2–6), late follicular (days 8–12), ovulatory (days 13–16), early luteal (days 18–23), and late luteal (days 24–30).

Forty-two volunteers (35 premenopausal women aged 20–40 years, and seven postmenopausal women aged 52–68 years), with a body mass index (BMI) of 19–26 kg/m^2 took part in the cross-sectional part of the study. They were recruited for sample collection at defined periods of their menstrual cycle, based on their self-reported last menstrual period. For the pre-menopausal group, a single sample of blood was taken from each volunteer to measure the plasma AEA level for the particular period for which they were recruited. A single blood sample was obtained from the postmenopausal controls.

A total of 12 volunteers (seven premenopausal women aged 21–40 years and five postmenopausal women aged 54–68 years) with a BMI of 20–24 kg/m^2 were recruited into the longitudinal part of the study. The seven premenopausal volunteers kept a menstrual diary of their cycle length for 3 months before entering the study. Blood was collected serially for the measurement of plasma AEA, serum 17β-estradiol (E2), progesterone (P4), follicle stimulating hormone (FSH) and luteinizing hormone (LH). The start of menstruation was considered as day 1 of the cycle and the volunteers were asked to use a urine LH surge kit (Clear Blue Ovulation Test, Unipath Ltd., Bedford, United Kingdom) from day 8 of their cycle until the day of a positive urine test (14). Twenty-four hours after a positive urine LH surge test, ovulation blood samples were collected (i.e., LH+1) (18).

Because of variations in the lengths of the normal cycle it was necessary to define the various phases of the menstrual cycle for AEA measurement as early follicular phase (days 2–6 blood samples were collected between days 2–5 to ensure accuracy with a mean sampling day of 3.7 $\pm$ 1.11 [mean $\pm$ SD]), late follicular phase (sampling between day 8–11 with a mean sampling day of 10.3 $\pm$ 1.11 [mean $\pm$ SD]), ovulation (sampling done 24 hours after a positive urinary LH surge test that ranged between days 13–16 of the cycle, with a mean of 14.7 $\pm$ 1.25 days [mean $\pm$ SD]), early luteal phase (sampling between days 18–23; with a mean sampling day of 20.8 $\pm$ 2.11 [mean $\pm$ SD]) and the late luteal phase sample (sampling on day 24–30, with a mean sampling day of 26.3 $\pm$ 2.13 [mean $\pm$ SD]). The start day of the next menstrual period was noted so that the studied cycle length could be calculated. For this group, the mean length was 29.5 $\pm$ 1.98 (mean $\pm$ SD) and ranged between 26–32 days. The phases of the cycle were further confirmed using the results of serum sex steroid hormones and gonadotrophin levels, which have defined specific levels for each phase of the cycle (17). For the postmenopausal women, blood samples were taken weekly over a 4-week period for measurement of plasma AEA and serum hormones.

All samples were collected between 10:00 and 12:00 AM to avoid any diurnal variation. The volunteers in the cross-sectional study had 4 mL of blood obtained for plasma AEA alone, whereas volunteers in the longitudinal study had 8 mL of blood collected for both plasma and serum. The blood for plasma was collected into ethylenediaminetetraacetic acid (EDTA) tubes, whereas blood for serum was collected into serum gel tubes. The blood in the serum tubes was left to clot for 15 minutes before being centrifuged at 1200 $\times$ g for 30 minutes, and separated serum was stored at –20°C for later hormone measurements. Separated plasma was processed within 2 hours of blood collection for AEA measurement by the first author to minimize assay variation.

TABLE 1

Age and BMI of the volunteers in the cross-sectional and longitudinal studies.

Distribution	Cross-sectional arm (n = 42)	Longitudinal arm (n = 12)
Premenopausal women	n = 35	n = 7
Postmenopausal women	n = 7	n = 5
Premenopausal age in years [mean (range)]	30 (20–42)	28.8 (21–40)
Postmenopausal age in years [mean (range)]	60 (52–68)	59.4 (54–68)
Premenopausal BMI in kg/m^2 [mean (range)]	22 (19–26)	21.7 (20–23)
Postmenopausal BMI in kg/m^2 [mean (range)]	23 (21–25)	23.8 (22–24)

Note: Data are expressed as mean (range). As expected, the women in the postmenopausal group were significantly older than the premenopausal women ($P<0.05$, Student's t-test). The BMI for all groups of women was not significantly different.

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Measurement of Plasma Anandamide

Plasma AEA levels were measured using UPLC-MS/MS as we have previously described (19). Briefly, 4 mL of blood were collected in EDTA tubes and transported to the laboratory on ice. The blood was then transferred to polypropylene tubes and centrifuged at 1200 × *g* for 30 minutes at 22°C to separate plasma from cells. Two mL of separated plasma was transferred to a 7-mL Kimble scintillation vial and 2.5 pmol of deuterium-labeled AEA (AEA-d8; Cayman Chemicals, Nottingham, United Kingdom) were added to estimate the efficiency of the lipid extraction, which was found to be 10.4 ± 1.17% (mean ± SD; n = 4). Protein was precipitated by adding 2 mL of ice cold acetone followed by centrifugation at 1200 × *g* for 10 minutes at 22°C. The supernatant was transferred to a fresh Kimble vial and the acetone was evaporated under a stream of nitrogen gas until the volume had halved. An equal volume of methanol:chloroform (1:2 vol:vol) was added, followed by gentle inversion to extract lipids/followed by another centrifugation step at 1200 × *g* for 10 minutes at 22°C to separate layers. The lower organic layer was recovered and dried completely under a gentle stream of nitrogen before reconstitution in acetonitrile (80 μL). Reconstituted samples were injected into the UPLC-MS/MS and analyzed in triplicate.

The UPLC-MS/MS system is composed of an Acquity UPLC system connected in-line with a Quattro Primer tandem mass spectrometer (Waters Ltd, Hertfordshire, United Kingdom). The column that was used was an Acquity UPLC BEH C_{18} (2.1 × 50 mm, 1.7 μm) maintained at 40°C. Mobile phases were A (2mM ammonium acetate containing 0.1% formic acid) and B (acetonitrile containing

TABLE 2

Plasma AEA levels in premenopausal and postmenopausal women in the two studies.

Phase of the cycle	Cross-sectional arm (n = 42)	Longitudinal arm (n = 7)	*P* value
Early Follicular	0.89 ± 0.06 nM (n = 7)	0.73 ± 0.03 nM (n = 7)	0.038
Late Follicular	0.77 ± 0.09 nM (n = 7)	0.63 ± 0.08 nM (n = 7)	0.281
Ovulation	1.38 ± 0.14 nM (n = 7)	1.33 ± 0.16 nM (n = 7)	0.815
Early Luteal	1.09 ± 0.13 nM (n = 7)	0.96 ± 0.10 nM (n = 7)	0.453
Late Luteal	0.66 ± 0.07 nM (n = 7)	0.56 ± 0.06 nM (n = 7)	0.308
Postmenopausal	0.98 ± 0.07 nM (n = 7)	1.05 ± 0.07 nM (n = 5)	0.441

Note: Data are expressed as mean ± sem. *P* values were calculated using unpaired Student's t-test with Welch's correction for unequal variances.

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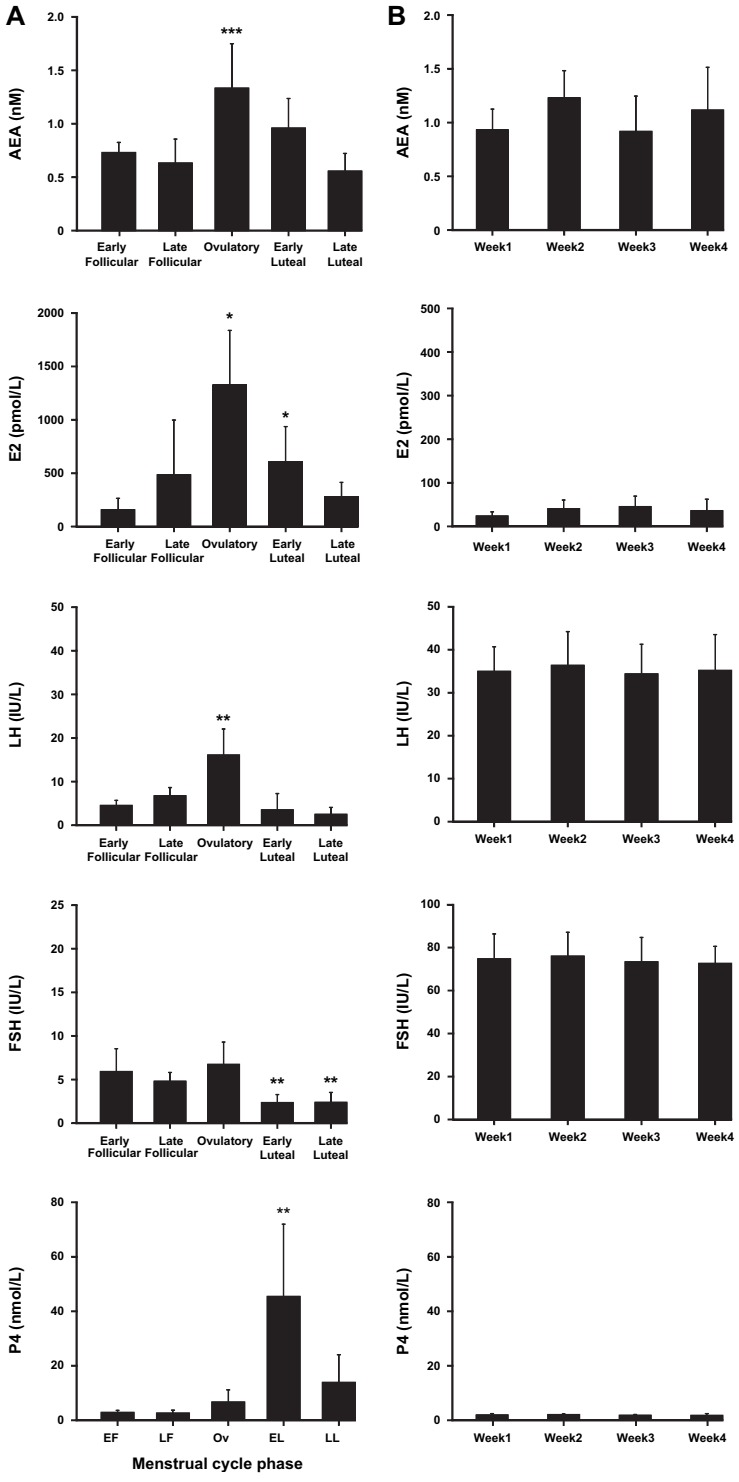
FIGURE 1



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0.1% formic acid). The liquid chromatography gradient conditions were as follows: 80% A for 0.5 minutes; linear to 100% B over the next 1 minute, changing to 80% A over the next 1 minute; then the column was equilibrated again at 80% A until 3.5 minutes had elapsed. Samples were quantified using tandem electrospray mass spectrometry in positive ion mode (ES+). Source parameters including capillary voltage of 0.6 kV, cone voltage of 18 V, source

FIGURE 1 Continued

Comparison of plasma AEA serum hormone levels through the menstrual cycle in the longitudinal study with levels found in postmenopausal women over the same period. Plasma AEA levels (nM) and E2 (E2; pmol/L), P4 (nmol/L), LH (IU/L) and FSH(IU/L) from premenopausal women were measured longitudinally over a single cycle (**A**) and are compared with the longitudinal measurements in a cohort of postmenopausal women measured over the same 4-week period (**B**). Data are presented as mean ± SEM for seven women in each group. One-way ANOVA with Tukey's honestly significance difference test when compared with early follicular period or the first measurement in week 1 for the postmenopausal women. All the data for the postmenopausal women were not significantly different. The laboratory references ranges for E2, P4, LH, and FSH ovulatory samples were 520–1470pmol/L, >30nmol/L, 14–70IU/L, and 2–20IU/L, respectively. *$P < 0.01$; **$P < 0.001$.

temperature of 120°C, desolvation temperature of 440°C, cone gas flow of 49 L/hour, and desolvation gas flow of 800 L/hour. Subsequently, MS/MS conditions for monitoring each precursor $[M+H]^+$ ion comprised entry, collision, and exit energies of –2, 17 and –17 eV, respectively. Product ions were monitored in multiple-reaction-monitoring mode.

The injection volumes for samples and standards were 7 μL with needle overfill. Eight-point calibration curves were performed in triplicate for up to 19 pmol/mL of AEA. Detected peaks were integrated using the Masslynx software version 4.1 (Waters Corporation, Milford, MA) and Quanlynx software (Waters Corporation, Milford, MA) was used to calculate the concentration of AEA using calibration curves of concentration against relative response. The ratio of the area under the curve for AEA-d0 to AEA-d8 was determined, and quantification of the amount of AEA in plasma samples containing the same amount of AEA-d8 was made directly from a plot of area ratios versus known AEA-d0 amounts (Cayman Chemicals) injected into the system. Standard curves were derived from the quantification of known and increasing quantities of the labeled AEA. The recovery of the AEA was constant over the range 1.66–133 fmol on the column

FIGURE 2

Pearson correlations for the measurements of plasma AEA, serum LH, FSH, E2, and P4 for the premenopausal women. Correlations for the levels of plasma AEA with serum LH, FSH, E2, and P4 were constructed for the premenopausal women and the R^2 values and significance of the correlations are summarized in Table 3.

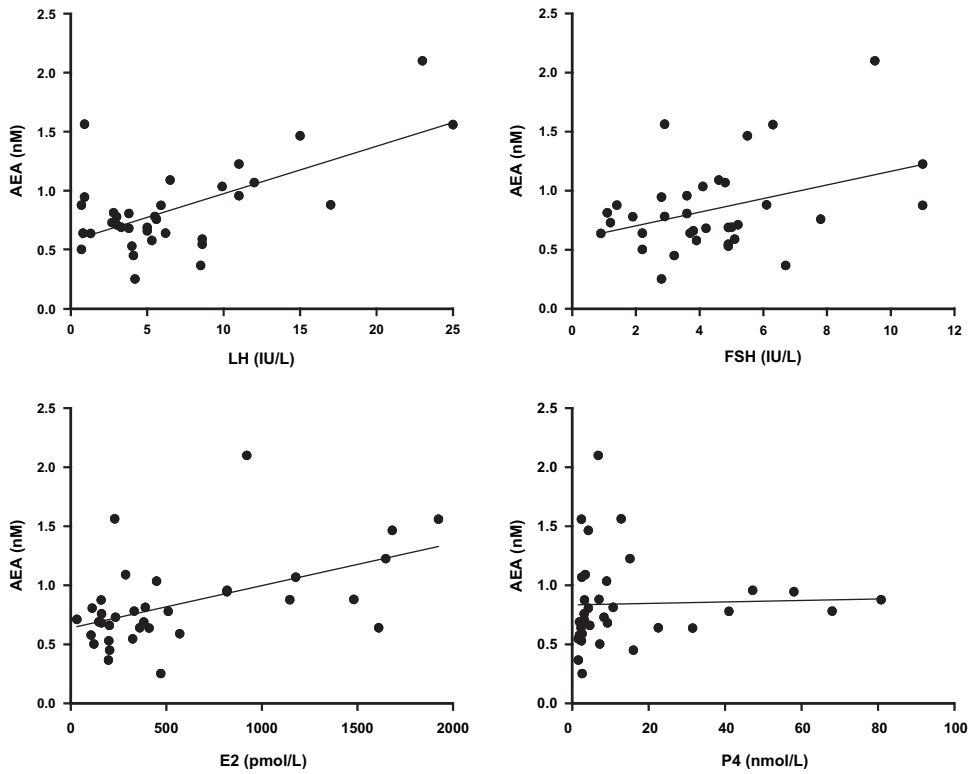

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TABLE 3

Correlations between AEA, estradiol, progesterone, luteinizing hormone and follicle stimulating hormone in pre-and post-menopausal women.

Correlations	Premenopausal women	Postmenopausal women
AEA and LH	$R^2 = 0.399$ (0.378–0.797) $P < 0.0001$	$R^2 = 0.0002$ (–0.454–0.430) $P = 0.949$
AEA and FSH	$R^2 = 0.148$ (0.060–0.637) $P = 0.0221$	$R^2 = 0.143$ (–0.703–0.076) $P = 0.099$
AEA and E2	$R^2 = 0.265$ (0.220–0.724) $P = 0.0015$	$R^2 = 0.017$ (–0.329–0.543) $P = 0.5782$
AEA and P4	$R^2 = 0.001$(–0.301–0.364) $P = 0.8418$	$R^2 = 0.036$ (–0.274–0.584) $P = 0.4194$

R^2 values are presented with 95% confidence intervals in parentheses. The *n* values are shown in Table 1.

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(0.23–19nM). The standard curves were linear beyond 133 fmol AEA-d0, with a lower limit of detection of 0.055 fmol AEA-d0 injected on the column. The intraassay and interassay coefficients of variation for the range of detection (1.66–133 fmol on the column) were 3.7 and 6.2%, respectively.

Hormone Measurements

Serum luteinizing hormone, follicle stimulating hormone, estradiol, and progesterone All stored samples were processed at the same time on an automated ADVIA Centaur Assay System (Bayer Diagnostics, Newbury, Berkshire, United Kingdom) to prevent any interassay variation. The range of detection was 0.07–200 IU/L for LH, 0.3–200 IU/L for FSH, 36.7–3670 pmol/L for estradiol, and 0.48–190.8 nmol/L for progesterone and similar to normal reference values (20).

Statistical Analysis

Data were normally distributed and are therefore expressed as mean ± SD or mean ± SEM, where appropriate. Parametric tests were used for statistical analysis and comparison between groups was performed with a one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference test or Student's t-test. Pearson correlation was used to evaluate the relationship between different variables, with $P<0.05$ considered to be significant.

RESULTS

Subjects

There were no statistically significant differences in the ages and BMIs of the volunteers in the premenopausal and postmenopausal cohorts (Table 1). As expected, the women in the postmenopausal group were significantly older than the premenopausal women ($P<0.05$).

Plasma AEA Levels

Plasma AEA levels in the premenopausal and postmenopausal women in the cross-sectional and longitudinal arms of the study were comparable (Table 2). Plasma AEA levels were significantly lower ($P=0.038$) in the early follicular phase of the cycle in the longitudinal arm when compared with the cross-sectional arm (Table 2). There were, however, no significant differences in AEA levels in the other phases of the menstrual cycle or in the postmenopausal women between the two arms of the study.

In the longitudinal arm of the study, the plasma AEA level (0.73 ± 0.03 nM) in the early follicular phase (day 2–5) of the menstrual cycle were higher than those (0.63 ± 0.08 nM) in the late follicular (day 8–11) phase (Fig. 1), but this was not statistically significant ($P>0.05$). There was a statistically significant ($P<0.0001$) rise in plasma AEA level from 0.63 ± 0.08 nM in the late follicular (day 8–11) phase to 1.33 ± 0.16 nM at the time of ovulation (LH+1) followed by a statistically nonsignificant decrease to 0.96 ± 0.10 nM in the early luteal (day18–23) phase. The lowest plasma AEA level (0.56 ± 0.06 nM) was observed in the late luteal (day 24–30) phase and was significantly lower ($P<0.05$) than that of the early luteal (day 18–23) phase. When the highest plasma AEA level measured at the time of ovulation (LH+1) was compared with the lowest level observed in the late luteal (day 24–30) phase, there was a statistically significant difference ($P<0.0001$).

In the postmenopausal women of the longitudinal arm of the study, plasma AEA levels fluctuated slightly, but there was no statistically significant difference over the four-week sampling period ($P=0.329$) (Fig. 1). The mean AEA level was 1.05 ± 0.07 nM, which was comparable to the levels in postmenopausal controls of the cross-sectional arm of the study (Table 2).

Ovarian Steroids and Gonadotrophin Levels

Figure 1 shows that the measured serum levels of E2, P4, LH and FSH in premenopausal and postmenopausal women were in keeping with the classically described patterns for these hormones in premenopausal ovulating women and postmenopausal women (21).

Serum E2 levels in the premenopausal women were highest around the time of ovulation (1,326 ± 193.2 pmol/L). In the postmenopausal women, there were statistically insignificant minimal fluctuations in E2 levels over the 4-week period (P=0.445); the mean E2 level over the 4 weeks was 36.4 ± 4.55 pmol/L. Serum P4 levels were highest (45.5 ± 10.01 nmol/L) in the early-luteal phase of the cycle (Fig. 1). In contrast, P4 levels in postmenopausal women remained stable over the 4 weeks (mean value of 1.920 ± 0.06 nmol/L). The peak serum LH level in the premenopausal women at the time of ovulation was 16.13 ± 2.24 IU/L. The mean postmenopausal serum LH level was 35.25 ± 0.42 IU/L, with no significant change over the four-week period (P=0.976) (Fig. 1). The highest serum FSH level (6.76 ± 0.96 U/L) in premenopausal women was observed at the time of ovulation. In the postmenopausal women, the mean serum FSH level was 74 ± 0.75 IU/L, with no significant fluctuations in the levels over the 4-week period (P=0.958). This finding was as expected—higher than the level in the pre-menopausal women.

The relationship between plasma AEA and serum sex steroid hormones and gonadotrophin levels in premenopausal women is shown in Figure 2. There were highly statistically significant positive correlations between plasma AEA levels and serum LH (P<0.0001), FSH (P=0.022) and E2 levels (P=0.0015), but not with serum P4 levels (P=0.841). In the postmenopausal women, no such correlations were demonstrated (Table 3).

DISCUSSION

To our knowledge, this is the first study to examine the relationship between plasma AEA levels, ovarian sex steroids, and gonadotrophins throughout the normal menstrual cycle and the postmenopausal period. Using both cross-sectional and longitudinal data, we corroborated and extended our previous cross-sectional study that plasma AEA levels are higher in the follicular (proliferative) phase than in the luteal (secretory) phase of the menstrual cycle (14, 19).

In our previous study (14), we focused on the early follicular (days 2–7) and late luteal (days 20–25) phases of the menstrual cycle only. By further investigating the late follicular, ovulatory, and early luteal phases of the menstrual cycle, and measuring the levels of the hormones controlling the menstrual cycle, we were able to relate plasma AEA concentrations to the levels of the sex steroid hormones and gonadotrophins.

In both arms of the study, plasma AEA levels varied throughout the menstrual cycle with a similar pattern, and both were characterized by peak plasma AEA levels at the time of presumptive ovulation (cross-sectional arm; days 13–16) and validated ovulation (longitudinal arm; LH+1). The peak plasma AEA level observed at the time of ovulation in both arms suggests a possible association between AEA and folliculogenesis and/or ovulation. In a previous report (16), the AEA levels in follicular fluid obtained at the time of egg collection in stimulated cycles in women undergoing IVF-ET treatment were similar to the levels obtained at the time of ovulation in this study; this is not surprising because the oocyte retrieval stage in IVF cycles corresponds to the time of ovulation in the natural menstrual cycle. This led us to speculate that either AEA may be produced locally in the ovarian follicle or its production elsewhere in the body may be intimately related to folliculogenesis or ovulation. Indeed, in a small pilot study, in which we quantified the AEA levels in follicular fluid obtained at oocyte retrieval, we demonstrated a direct correlation between AEA levels in follicular fluid and follicle size (22), supporting the suggestion that AEA may be involved in folliculogenesis. We are currently investigating whether increased AEA levels in follicular fluid are related to ovarian physiological events, such as follicle development and oocyte maturation.

The observation that plasma AEA levels declined slightly during the early luteal phase of the menstrual cycle, coinciding with the "window of implantation," is in keeping with studies in animals which suggest that low AEA levels are beneficial to implantation. Lazzarin et al. (15) demonstrated that at this key point in the menstrual cycle the peripheral mononuclear cells increase production and activity of FAAH, the principle enzyme involved in the degradation of AEA. This activity of FAAH is thought to be under the control of progesterone, which is elevated in the midluteal phase of the menstrual cycle. As a result, it is reasonable to speculate that once ovulation occurs, the increase in progesterone-induced FAAH expression and activity leads to a decrease in plasma AEA levels.

If peripheral FAAH activity was the only determinant of plasma AEA levels, then there should be a strong inverse correlation between plasma AEA and serum progesterone levels. We observed no such correlation and the patterns of AEA and P4 levels did not appear to be related; this suggests that in the normal menstrual cycle, P4 is unlikely to be the main controlling factor of plasma AEA levels. The fact that serum E2 levels and plasma AEA levels are significantly correlated suggests a closer association between these two molecules. Maccarrone et al. (23) showed that AEA release from endothelial cells was stimulated by E2, supporting the possibility that E2 and AEA levels are closely linked.

Recently, we reported that plasma AEA levels below 2nM were predictive of live birth in women with a threatened miscarriage (13), suggesting that plasma AEA levels above this value were not conducive to successful pregnancy. In this study, the level of plasma AEA in the early luteal phase of the menstrual cycle, which coincides with the implantation window, were all lower than 1 nM. Although there is evidence that high levels of AEA are embryotoxic and have effects on periimplantation events in the mouse (10, 11) and sheep (12), the exact relationship between AEA and human implantation has not yet been studied. We are currently investigating the possible relationship between plasma AEA and serum hormones with implantation in women using IVF.

The findings of positive correlations between plasma AEA and serum FSH, LH and E2 levels in premenopausal women,

suggest that these hormones may be involved in the regulation of AEA or vice versa. The gonadotrophins are, however, unlikely to be the sole regulators of AEA, as there was no corresponding increase in the levels of plasma AEA in the postmenopausal women, where serum FSH and LH levels were grossly elevated (Fig. 1). However, because reproductive/endocrine physiology in premenopausal and postmenopausal women is different, it is possible that the AEA-regulating mechanisms are different in these two periods or there are other factors involved that are yet to be thoroughly understood. Further studies will need to be undertaken to explore in details precisely how this regulation may occur.

Our observations suggests that AEA may be controlled by either gonadotrophins or most probably by estradiol, or by both during the menstrual cycle, and are important to further our understanding of the role of endocannabinoids (especially AEA) in human fertility and will form the basis for further studies investigating the precise controlling mechanisms that influence successful implantation and other facets of human reproduction, such as folliculogenesis, ovulation, early embryo development, and oviductal transport.

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