Short term changes in melatonin and cortisol serum levels after a single administration of estrogen to menopausal women

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Abstract

OBJECTIVES: It has been well-documented that serum melatonin levels are insensitive to estrous or menstrual ovarian steroid variations in the female rat or the human. However, a negative coupling has been already demonstrated between the nocturnal serum melatonin peak and serum E_2 concentration during the late premenopausal period in the woman. The objection of the present study was designed to determine if diurnal serum melatonin values can be also lowered by a single administration of estrogen. **METHODS:** We performed a detailed analysis of variations of serum estradiol, LH, FSH, melatonin and cortisol after one single I.M. injection of 2 mg of a conjugated estrogen, delestrogen (estradiol valerate) in 0.1 ml of oil. A 15 ml blood collection was done at 8:00 a.m. before the injection, and at 8:30 a.m., 9:00 a.m., 10:00 a.m., 12:00 noon, and 4:00 p.m. 17β-estradiol, LH and FSH were determined by microparticle enzyme immunoassays kits. Melatonin determination was made using a RIA kit and cortisol was assayed by a RIA method. **RESULTS:** A significant rise in serum 17β-estradiol was already seen by one hour after the injection of estradiol valerate. Then, an almost linear increase was observed up to at last eight hours after the injection of estradiol valerate. A significant decrease in serum LH was not seen before four hours after the injection of estradiol valerate. Overall, there was a trend toward a decline in serum melatonin and cortisol concentration. The decreasing trend of cortisol serum level was tested as significant over time (p< 0.001). However, the decrease in serum concentration did not reach a significant level for melatonin. **CONCLUSION:** Overall, these results show that after menopause an acute administration of estrogen during the early diurnal period of the day leads to a significant rapid decrease in cortisol serum values, but to only a partial non significant decrease in melatonin serum values.

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Introduction

The relationship between the steroidal environment and the circadian secretion of melatonin and cortisol has been the subject of many studies in the human.

For serum melatonin, despite well-characterized menstrual phases and ovarian hormone variations characteristic of ovulatory cycles, consistent variations in the plasma melatonin circadian rhythm by menstrual phase has not been found in the human [4,5,8,18]. Similarly, pineal melatonin synthesis and release are not altered throughout the estrous cycle in female rats [19]. These findings suggest that circadian melatonin secretion is not modulated by physiological variations of sex steroids and that melatonin secretion may not be necessary for estrogen cyclicity.

Nevertheless, a significant negative correlation was observed between the peak of serum melatonin concentration and the serum estradiol concentration in premenopausal women aged 40–50 years, but not before [18]. Also, daily oral administration of conjugated estrogen to postmenopausal women markedly suppressed nocturnal melatonin secretion [17,18]. However, it has been reported that both transdermal and oral estradiol treatment led to an increase, as well as a decrease of melatonin secretion in different postmenopausal women [2]

However, the influence of the hypothalamic-pituitary-adrenal axis on the menstrual cycle has been well-established [13], as well as the influence of ovarian steroids on hypothalamic-pituitary-adrenal regulation [1,6,14].

Serum cortisol values exhibited, during each phase of the menstrual cycle, a marked circadian rhythm, with values 2–3 fold higher during the morning than during the afternoon. Maximum cortisol values occurred between 4:00 a.m. and 8:00 a.m. and there was a strong coupling between the onset of the surge and the acrophase of the cortisol circadian rhythm. Maximal plasma cortisol values were seen at 4:00 a.m. when the preovulatory LH surge started at 4:00 a.m. and at 8:00 a.m. when it started at 8:00 a.m. [15]. However, morning maximal plasma cortisol values were higher during the follicular phase than during the luteal phase [15]. Furthermore, the activity of the adrenocortical axis appears to be more pronounced, with higher morning maximal values and higher evening minimal values, during the follicular phase than during the luteal phase. High cortisol values can be observed up to the day of the LH peak; then there is a decrease in the activity of the adrenocortical axis [16].

The present study was undertaken to establish the short term kinetics of the change in melatonin and cortisol secretion after one single administration of estradiol to postmenopausal women.

Materials and methods

Subjects

The experimental study was performed in 10 menopausal women aged 52±1.3 years. Each had had regular ovulatory reproductive cycles before menopause, and none had used hormonal medication for at least six months before the study, nor were they on other medications known to interact with the neuroendocrine reproductive or adrenal axis. All women were of normal weight and height. Their body mass index was 28.2±1.7. The project was approved by the Institutional Review Board of the Eastern Virginia Medical School, and a written informed consent was obtained from each woman.

Study Protocol

A 15 ml blood collection was done at 8:00 a.m. immediately followed by an intramuscular injection of 2 mg of delestrogen (estradiol valerate) in 0.1 ml of oil. Then, additional blood samples of 15 ml were collected at 8:30 a.m., 9:00 a.m., 10:00 a.m., 12:00 noon and 4:00 p.m. Blood samples were spun at room temperature and at 1,500 g for E_2, LH, FSH, melatonin, and cortisol determination.

Serum E_2, LH and FSH Assay

E_2, LH and FSH concentrations were determined by microparticle enzyme immunoassays using the IMX analyzer (Abbott Laboratories, Abbot Park, III, USA). The intra- and interassay coefficients of variation were 6.3 and 6.7% respectively for E_2; 3.1 and 5.6% respectively for LH; and 4.7 and 7.6% respectively for FSH.

Serum Melatonin

Melatonin determination was made using a radioimmunoassay (RIA) kit. The detection limit was 1 pg/ml and the intra- and interassay coefficients of variation were less than 3 and 4% respectively.

Serum Cortisol Assay

Plasma samples were assayed by a previously published RIA method [16]. Briefly, plasma samples (50 μl) were extracted with 1 ml of absolute ethanol. The medium was vortexed and 10 minutes later centrifuged at 4°C for 15 minutes. Then, 50 and 100 μl of the supernatant were removed and evaporated using a Speed Vac concentrator. The dry residue from the 50 and 100 μl extracts was dissolved in 300 μl of the RIA buffer. The rabbit polyclonal cortisol antiserum was a generous gift from Drs. C. Delarue and H. Vaudry (INSERM u413, Mont Saint-Aignan, France). The specificity of the antibody was previously published. The antiserum was used at a final dilution of 1:40,000 in a total volume of 500 μl. Cortisol used as standard was from Sigma. The detection limit was 6 ng/ml (with the 50 μl extract) and the intra- and interassay coefficients of variation were less than 4 and 6% respectively.
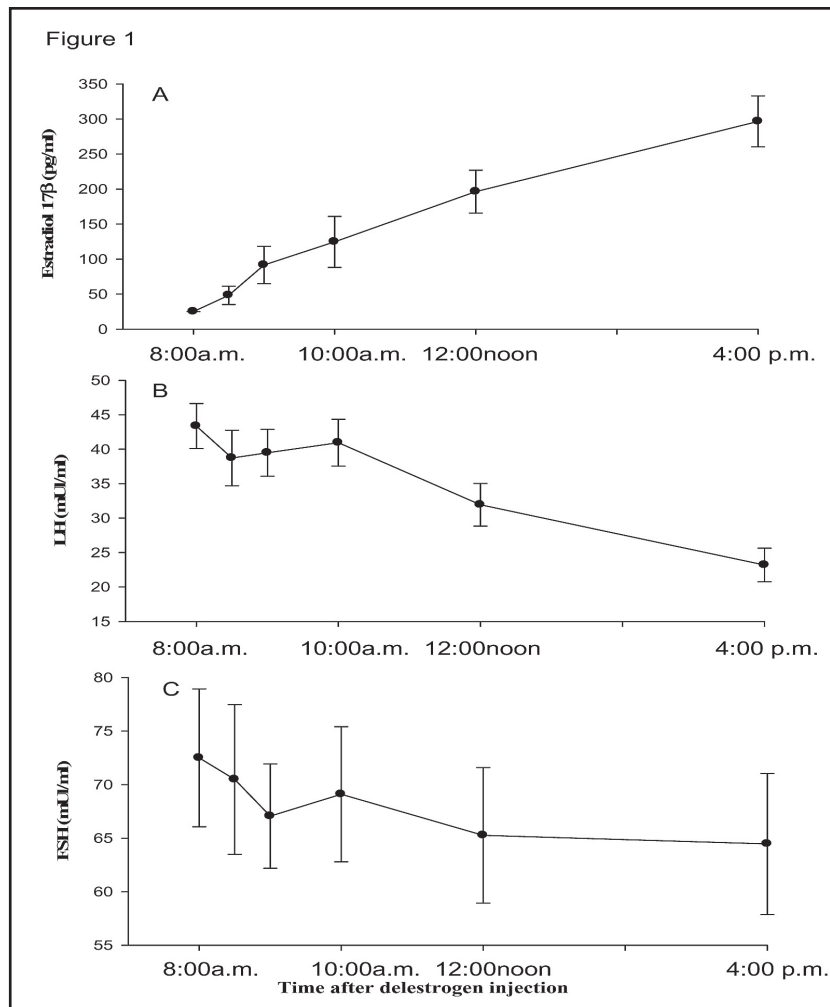
Figure 1. Pattern of mean of eight subjects (±SEM) of estradiol-17β (part A), LH (part B) and FSH (part C) in blood samples taken at 8:00 a.m. before the administration of 2 mg of delestrogen in 0.1 ml of oil, and at 8:30 a.m., 9:00 a.m., 10:00 a.m., 12:00 noon and 4:00 p.m., thereafter.

Statistical Methods

Friedman test was used to test the time effect over Estradiol 17β, LH, FSH Melatonin and Cortisol. Friedman test is a nonparametric version of repeated measure analysis of variance (ANOVA). This test was used to test the difference of outcomes at 6 time points. Post-hoc Wilcoxon rank sum T-tests was performed if significance was found. Bonferroni-corrected alpha was used to evaluate the pair-wise significance. The significance level was set at 0.05. Data analysis was performed with SAS 9.1.3 (Cary, NC, USA).

Results

17β-Estradiol, LH and FSH

Figure 1 shows the pattern of E_2, LH and FSH serum concentrations during the 8 hours which follow the administration of delestrogen at 8:00 a.m. A significant rise over time was observed for serum E_2 (p-value<0.001). The increase from 8:00 a.m. to 12:00 noon and 4:00 p.m. was significant. Overall, an almost linear increase was

observed for the rise in serum E_2 between 1 hour and 8 hours after treatment.

The decrease of level of LH over time was significant (p-value<0.001). A significant decrease in serum LH was not observed before 4 hours after treatment. Then, a linear decrease was seen up to 8 hours after treatment. The change from 8:00 a.m. to 4:00 p.m. and 10:00 a.m. to 4:00 p.m. was significant.

The level change of SFH over time was not significant at any time after treatment even if there was a trend for a very slow decline.

Melatonin and Cortisol

Figure 2 shows the pattern of E_2, Melatonin and Coristol serum concentrations during the 8 hours which follow the administration of delestrogen at 8:00 a.m.

Overall, there was a trend toward a slow constant decline for both hormones during the experimental period. However, the decrease in serum concentration was more pronounced for cortisol than for melatonin.
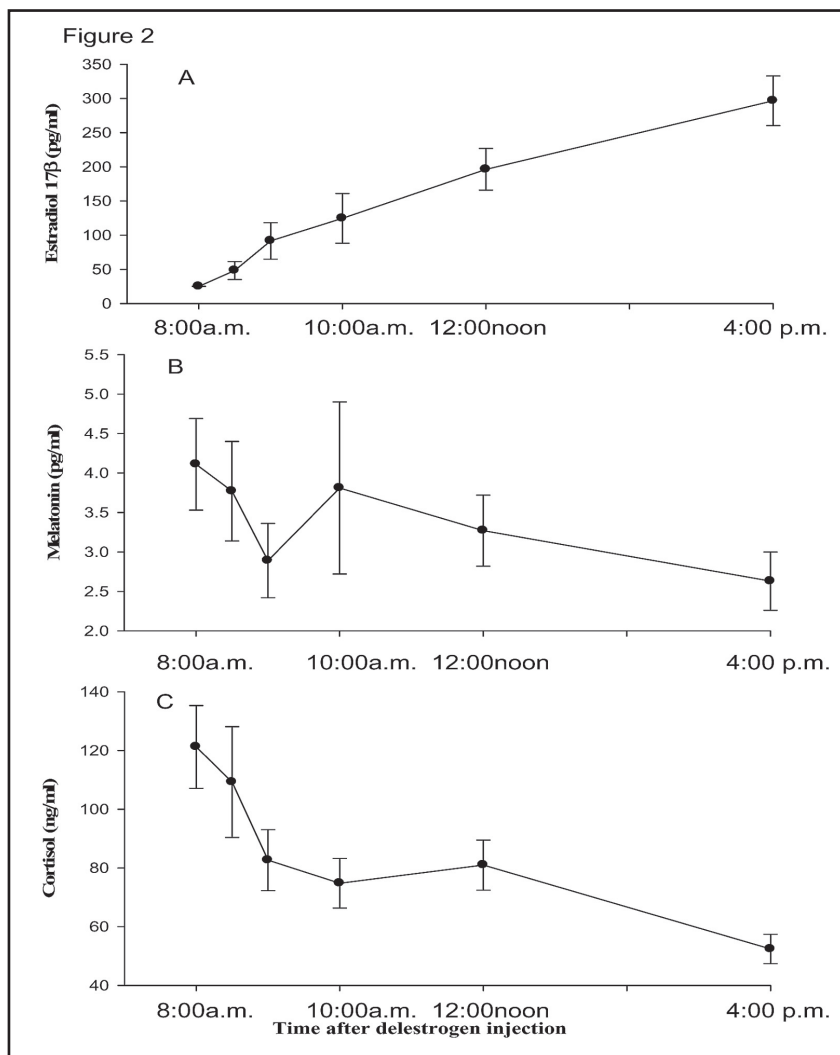
Figure 2. Pattern of mean of eight subjects (±SEM) estradiol-17β (part A), melatonin (part B) and cortisol (part C) in blood samples taken at 8:00 a.m. before the administration of 2 mg of delestrogen in 0.1 ml of oil and at 8:30 a.m., 9:00 a.m., 10:00 a.m., 12:00 noon and 4:00 p.m., thereafter.

The level change of Melatonin over time was not significant (p-value=0.1135).

The decreasing trend of Cortisol level was tested as significant over time (p-value<0.001). The trend getting slow after 10:00 a.m. and the difference between 8:00 a.m. and 4:00 p.m. was significant (p-value=0.0008).

Values at eight hours post-treatment were 45% of initial values for cortisol, but only 65% of initial values for melatonin.

Discussion

This study confirms the estrogen negative feedback for LH but not for FSH. The negative feedback effect of estrogen on LH secretion has been already well-documented in the menopausal woman [9,10,12,20,21]. Also, the influence of estrogen on the circadian rhythm of serum cortisol has been documented [7,11].

Overall, our results show a decrease in the serum concentration of cortisol and melatonin after one single administration of conjugated estrogen to postmeno-pausal women. These results were observed during the morning and early afternoon, when the synthesis and secretion of melatonin and of cortisol are low after the nocturnal circadian peaks.

In humans, the effect of gonadal steroid on pineal secretion of melatonin is not fully understood. However, it seems that endogenous or exogenous ovarian hormones do not influence melatonin secretion during the time of estral or menstrual cyclicity.

Several groups of investigators [4,5,8,18] did not find changes in plasma melatonin levels during the menstrual cycle, which represents a situation of large variations of concentrations of 17β-Estradiol, as well as progesterone or a combination of concentrations or both steroids. However, changes in plasma melatonin levels were seen during the perimenopause. Very interestingly, a

significant correlation was noted between the serum E_2 concentration and the peak of serum melatonin concentration in premenopausal women close to the menopause (aged 40–50 years), but not in younger women (less than 40 years old) [18].

These results suggest that the sensitivity of the pineal gland to E_2 is developing with age being present close after the cessation of menstrual cyclicity. Also, an age-related decline in nocturnal melatonin secretion was found up to 15 years postmenopause, followed by an extremely gradual decline thereafter [18].

Also, after oral administration of conjugated estrogens for 8 weeks, there was a marked reduction of the nocturnal serum melatonin peak [18].

Our findings show that diurnal serum melatonin concentrations are also sensitive to estrogen administration. Similar observations were done throughout the estrous cycle in the female rat. In that species, the endogenous release of melatonin, followed by transpineal microdialysis, displayed no significant variation in cycling female rats. Similarly, neither 17β-estradiol, nor progesterone or a combination of both steroids were able to modify melatonin secretion in an in vitro perifusion system of the pineal gland. However, when the cycling female rats were exposed to a mammary carcinogen, the 7,12-dimethylbenz(a) anthracene (DMBA), E_2 treatment leads to a complete blunting of the isoproterenol-induced stimulation of melatonin secretion, far before the occurrence of mammary adenocarcinoma [3].

Our results also show that there is an almost parallel decline in serum cortisol concentration after administration of estradiol valerate. However, the decline in serum melatonin did not reach a significant level.

In summary, our results show that estradiol administered after the nocturnal serum melatonin surge is also able to significantly lower the diurnal cortisol and to a slight non significant extent melatonin secretion in the healthy postmenopausal woman.

Acknowledgments

REFERENCES

1 Atkinson HC and Waddell BJ. Circadian variation in basal plasma corticosterone and adrenocorticotropin in the rat: sexual dimorphism and changes across the estrous cycle. Endocrinol 1997; **138**:3842–3838.
2 Bartsch C, Seeger H Muck AO and Lippert TH. The effect of estradiol on production of melatonin in postmenopausal women. Int J Clin Pharmacol Ther 1995; **33**:401–403.
3 Jonage-Canonico, MB, Lenoir V, Martin A, Scholler R and Kerdelhue B. Long term inhibition by estradiol or progesterone of melatonin secretion after administration of a mammary carcinogen, the dimethyl benz(a)anthracene, in Sprague-Dawley female rat; inhibitory effect of melatonin on mammary carcinogenesis. Breast Cancer Res Treat 2003; **79**:365–377.
4 Berga SL and Yen SS. Circadian pattern of plasma melatonin concentrations during four phases of the human menstrual cycle. Neuroendocrinology 1990; **51**:606–612.
5 Brzezinski A, Lynch HJ, Seibel MM, Deng MH, Nader TM and Wurtman RJ. The circadian rhythm of plasma melatonin during the normal menstrual cycle and in amenorrheic women. J Clin Endocrinol Metab 1988; **66**:891–895.
6 Carey BP, Deterd CH, de Koning J, Helmerhorst F and de Kloet ER. The influence of ovarian steroids on hypothalamic-pituitary-adrenal regulation in the female rat. J Endocrinol 1995; **144**:311–321.
7 Cucinellii F, Soranna L, Barini A, Perri C, Leoni F, Mancuso S and Lanzone A. Estrogen treatment and body fat distribution are involved in corticotrophin and cortisol response to corticotrophin-releasing hormone in postmenopausal women. Metabolism 2002; **51**:137–143.
8 Delfs TM, Baars S, Fock C, Schumacher M, Olcese J and Zimmermann RC. Sex steroids do not alter melatonin secretion in the human. Hum Reprod 1994; **9**:49–54.
9 Gill S, Sharpless JL, Rado K and Hall JE. Evidence that GnRH decreases with gonadal steroid feedback but increases with age in postmenopausal women. J Clin Endocrinol Metab 2002; **87**:2290–2296.
10 Gill S, Lavoie HB, Bo-Abbas Y and Hall JE. Negative feedback effects of gonadal steroids are preserved with aging in postmenopausal women. J Clin Endocrinol Metab 2002; **87**:2297–2302.
11 Gudmundsson A, Goodman B, Lent S Barczi S, Grace A, Boyle L, Ershler WB and Carnes M.. Effects of estrogen replacement therapy on the circadian rhythms of serum cortisol and body temperature in postmenopausal women. Exp Gerontol 1999; **34**:809–818.
12 Hall JE and Gill S. Neuroendocrine aspects of aging in women. Endocrinol Metab Clin North Am 2001; **30**:631–646.
13 Hayashi KT and Moberg GP. Influence of the hypothalamic-pituitary-adrenal axis on the menstrual cycle and the pituitary responsiveness to estradiol in the female rhesus monkey (Macaca mulatta). Biol Reprod 1990; **42**:260–265.
14 Kerdelhue B, Jones GS, Gordon K, Seltman H, Lenoir V, Melik-Parsadaniantz S, Williams RF and Hodgen GD. Activation of the hypothalamo-Anterior pituitary Corticotrophin Releasing hormone, Adrenocorticotropin hormone and 17β-induced plasma LH surge in the ovariectomized monkey. J Neurosc Res 1995; **42**:228–235.
15 Kerdelhue B, Brown S, Lenoir V, Queenan Jr. JT, Jones GS, Scholler R and Jones Jr. HW. Timing of initiation of the preovulatory luteinizing hormone surge and its relationship with the circadian cortisol rhythm in the human. Neuroendocrinol 2002; **75**:158–163.
16 Kerdelhue B, Lenoir V, Scholler R, and Jones Jr HW. ACTH-cortisol activity during the 17β-estradiol and LH preovulatory surges of the menstrual cycle in the human. Neuro Endocrinol Lett 2006; **27**:101–107.
17 Kos-Kudla B, Ostrowska Z, Marek B, Kajdaniuk D, Ciesielska-Kopacz N, Kudla M, Mazur B, Glogowska-Szlag J and Nasiek M. Circadian rhythm of melatonin in postmenopausal asthmatic women with hormone replacement therapy. Neuro Endocrinol Lett 2002; **23**:243–248.
18 Okatani Y, Morioka N and Wakatsuki A. Changes in nocturnal melatonin secretion in perimenopausal women: correlation with endogenous estrogen concentrations. J Pineal Res 2000; **28**:111–118.

19 Skorupa AL, Garldou ML, Bothorel B, Saboureau M, Pevet P, Neto JC and Simonneaux V.. Pineal melatonin synthesis and release are not altered throughout the estrous cycle in female rats. J Pineal Res 2003; **34**:53–59.

20 Weiling Y and Gore AC. Neuroendocrine control of reproductive aging: roles of GnRH neurons. Reproduction 2006; **131**:403–414.

21 Weiss G, Skurnick JH, Goldsmith LT, Santoro NF and Park SJ. Menopause and hypothalamic-pituitary sensitivity to estrogen. JAMA 2004; **292**:2991–2996.