Plasma β-endorphin Levels in Patients with Polycystic Ovarian Disease

Jung Gu Kim*, Yoon Seok Chang and Jin Yong Lee

Department of Obstetrics and Gynecology, Seoul National University College of Medicine, Seoul 110-744, Korea

Abstract—In order to determine endocrinologic changes by obesity in patients with polycystic ovarian disease (PCOD) and to assess the involvement of peripheral β-endorphin in the pathogenesis of PCOD, levels of plasma β-endorphin, gonadotropin, androgen and prolactin were measured by radioimmunoassay. Thirty-one PCOD patients (21 normal weight, 10 overweight) and 27 normally ovulating women, 20 normal weight, 7 overweight (normal controls) were studied. There were no significant differences in the plasma gonadotropin, androgen, and prolactin levels between the overweight and normal weight group in PCOD patients and normal controls. No significant difference in plasma β-endorphin levels was found between normal weight controls and normal weight patients. Plasma β-endorphin levels were similar in overweight controls and patients. These values were significantly higher than those in normal weight controls and patients respectively. There was a significant correlation between plasma β-endorphin levels and the percentage of ideal body weight in patients with PCOD and normal controls. These data suggest that peripheral opioid abnormality in PCOD may be a phenomenon associated with obesity and not related to the pathogenesis of disease.

Key words: Peripheral β-endorphin, Polycystic ovarian disease, Obesity.

INTRODUCTION

The most characteristic picture in women with polycystic ovarian disease (PCOD) is an abnormality of gonadotropin secretion, i.e., an increased serum luteinizing hormone (LH) and a normal or decreased serum follicle stimulating hormone (FSH) level, resulting in an increased LH/FSH ratio (Rebar et al. 1976, Bailey et al. 1977). Several studies have shown that the endorphins, a group of endogenous opioids, may play an important role in the release of gonadotropin (Quigley and Yen, 1980; Blankstein et al. 1981, Robert et al. 1981). Cumming et al. (1984) found the failure of LH respond to opiate antagonist, naloxone in PCOD patients, suggesting a lack of opioid inhibition of gonadotropin-releasing hormone (GnRH), and proposed that an abnormality of central opioid activity might be responsible for the increased gonadotropin secretion in PCOD patients. On the other hand, several investigators report that plasma β-endorphin levels are elevated in PCOD patients (Given et al. 1980, Aleem and McIntosh, 1984, Wortsman et al. 1984), but most of their patients were obese. Recently, an elevated levels of plasma β-endorphins also have been observed both in obese adults and

Therefore it is not clear whether this elevation in plasma β-endorphin levels in PCOD patients is associated with obesity or involved in the pathogenesis of disease. This study was undertaken to compare levels of various hormones including β-endorphin in the peripheral blood between overweight and normal weight PCOD patients and to assess the relationship of peripheral β-endorphin levels with body weight.

MATERIALS AND METHODS

1. Subjects

We studied 31 women, 20 to 30 years of age, with PCOD, 21 normal weight (mean weight ± S.E.: 98.91 ± 2.01% of ideal body weight) and 10 overweight (127.68 ± 2.65%). The criteria for diagnosing PCOD were amenorrhea or oligomenorrhea with or without hirsutism, anovulation determined by basal body temperature chart and/or endometrial biopsy, high LH basal levels with the LH/FSH ratio>2, and characteristic findings of polycystic pattern in transvaginal ultrasonography. Ideal body weights were determined from the standards of Korean women (Park, 1987). Overweight was defined as body weight greater than 120% of ideal body weight. Normal weight patients were within 20% of their ideal body weight. For controls, 27 normally ovulating women, 20 normal weight (98.70 ± 1.49%) and 7 overweight (126.58 ± 1.60%) were also included. All subjects were free of systemic and other endocrine diseases.

2. Collection of samples

All blood samples were collected between 9:00 AM and 11:00 AM randomly from amenorrheic patients and on menstrual cycle day 2 to 5 from menstruating women. Blood was collected in polypropylene tubes containing heparin, immediately centrifuged at 4°C for 15 minutes at 2,500 g, and the plasma stored at −20°C until assay.

3. Radioimmunoassays

LH and FSH was assayed by a double antibody radioimmunoassay method with kits from Ameri-

sham. Pro lactin (PRL), testosterone (T), and dehydroepiandrosterone sulfate (DHEAS) assay were performed using radioimmunoassay kits purchased from Abbott Laboratories (North Chicago, IL), Diagnostic Products Corporation (Los Angeles, CA), and Serono Diagnostics (Braintree, MA), respectively.

Determinations of β-endorphin were performed after affinity gel extraction using radioimmunoassay kits from the Immuno Nuclear Corporation (Stillwater, MN). In brief, standards and unknown samples were extracted with anti β-endorphin sepharose complex in chromatography column and the absorbed β-endorphin was eluted from sepharose particle with 0.025 ml HCL. The mixture of sepharose-HCl extract (200 μl) and 1% bovine serum albumin borate buffer (100 μl) were incubated at 4°C for 18-hours with rabbit β-endorphin antisera (100 μl), followed by an 18 hour incubation with 125Iβ-endorphin (100 μl) and an additional 20 minutes with precipitating goat antrabbit serum (500 μl). The β-endorphin antisem used was 100% crossreactive, with human β-endorphin, below 5% with β-lipotropin, and did not crossreact (0.01%) with α-endorphin, leucine enkephalin, methionine enkephalin, adrenocorticotropic hormone (ACTH) or α-melanocyte stimulating hormone. Current β-endorphin assay sensitivity is 1 fmol/ml.

All samples were measured in duplicate in the same assay for each hormone. The intrassay variations were 7.1% for LH, 6.0% for FSH, 6.0% for T, 7.9% for DHEAS, 4.0% for PRL, and 6.5% for β-endorphin.

4. Statistical analysis

All results are expressed as the mean ± standard error (SE). Data analysis was performed using student t-test and correlation coefficients.

RESULTS

Various plasma hormone levels (Mean ± SE) in 20 normal weight and 7 overweight controls, and 10 overweight and 21 normal weight patients with PCOD are summarized in Table 1. When compared to the levels found in the normal weight controls, normal weight patients with PCOD had plasma concentrations that were significantly higher.
Table 1. Plasma LH, FSH, T, DHEAS, PRL levels and LH/FSH ratio in normal weight and overweight ovulatory women, and overweight and normal weight PCOD patients (Mean ± SE)

<table>
<thead>
<tr>
<th></th>
<th>Normal Weight (n=20)</th>
<th>Overweight (n=7)</th>
<th>Normal Weight (n=21)</th>
<th>Overweight (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH (mIU/ml)</td>
<td>12.01 ± 1.21a</td>
<td>12.87 ± 1.31a</td>
<td>33.92 ± 2.16b</td>
<td>35.43 ± 1.78c</td>
</tr>
<tr>
<td>FSH (mIU/ml)</td>
<td>12.27 ± 0.94</td>
<td>11.50 ± 1.01</td>
<td>11.99 ± 0.73</td>
<td>11.81 ± 0.86</td>
</tr>
<tr>
<td>LH/FSH ratio</td>
<td>0.98 ± 0.20b</td>
<td>1.12 ± 0.23c</td>
<td>2.93 ± 0.18d</td>
<td>3.09 ± 0.225e</td>
</tr>
<tr>
<td>T (ng/ml)</td>
<td>0.43 ± 0.04</td>
<td>0.44 ± 0.03</td>
<td>0.66 ± 0.07</td>
<td>0.69 ± 0.10f</td>
</tr>
<tr>
<td>DHEAS (ng/dl)</td>
<td>1342.00 ± 230.17b</td>
<td>1401.00 ± 221.27c</td>
<td>2083.08 ± 215.53d</td>
<td>2130.00 ± 232.98e</td>
</tr>
<tr>
<td>PRL (ng/ml)</td>
<td>10.27 ± 1.20</td>
<td>10.51 ± 1.05</td>
<td>11.00 ± 1.24</td>
<td>10.35 ± 1.18</td>
</tr>
</tbody>
</table>

a,b,e,f,g: p<0.001; c: p<0.01; d,h: p<0.05

(p(0.001) for LH (33.92 ± 2.16 vs 12.01 ± 1.21 mIU/ml) but not for FSH (11.99 ± 0.73 vs 12.27 ± 0.94 mIU/ml). Plasma levels of PRL (11.0 ± 124 vs 10.27 ± 120 ng/ml) showed no differences between the normal weight controls and PCOD patients. The androgen levels revealed significantly elevated concentrations of T (0.66 ± 0.77 vs 0.43 ± 0.04 ng/ml, p(0.01), and DHEAS (2083.08 ± 215.53 vs 1342.00 ± 230.17 ng/dl, P<0.05). Significant differences in the plasma levels of LH (35.43 ± 1.78 vs 12.87 ± 1.31 mIU/ml, p(0.001), T (0.69 ± 0.10 vs 0.44 ± 0.03 ng/ml, p(0.001) and DHEAS (2130.00 ± 232.98 vs 1401.00 ± 221.27 ng/dl, p(0.05) between the overweight patients and controls. In both normal ovulatory controls and PCOD patients there were no significant differences in the plasma levels of LH, FSH, T, DHEAS, PRL and LH/FSH ratio between normal weight and overweight controls. In both normal ovulatory controls and PCOD patients there were no significant differences in the plasma levels of LH, FSH, T, DHEAS, PRL and LH/FSH ratio between normal weight and overweight controls.

Fig. 1. Plasma β-endorphin levels (Mean±SE) in normal weight and overweight controls, and normal weight and overweight PCOD patients.

Fig. 2. Correlation between plasma β-endorphin levels and the percentage of ideal body weight in normal controls (n=27).
controls, and overweight and normal weight patients with PCOD. The mean concentrations of plasma β-endorphin in overweight patients with PCOD (8.82 ± 1.21 fmol/ml) were similar to those found in overweight controls (8.14 ± 3.10 fmol/ml). There was no significant difference in the mean plasma β-endorphin levels between normal weight controls and patients (5.09 ± 0.58 vs 5.25 ± 0.50 fmol/ml). Plasma β-endorphin levels in overweight controls and patients were significantly higher than those in normal weight controls and patients respectively (p<0.02). As is shown in Fig. 2 and Fig. 3, a significant correlation was noted between percentage (x) of ideal body weight and plasma β-endorphin levels (y) in normal ovulatory controls (y = 0.093x – 3.961, r = 0.419, p<0.03, n = 27) and patients with PCOD (y = 0.084x – 2.708, r = 0.422, P<0.02, n = 31). There was no significant relationship of plasma β-endorphin levels with plasma LH, FSH, T, DHEAS, PRL levels and LH/FSH ratio.

**DISCUSSION**

Circulating gonadotropin concentrations are “inappropriate” with erratic and elevated LH and relatively constant and normal or low FSH levels in PCOD patients (Rebar et al., 1976; Baird et al., 1977). Obesity frequently observed in PCOD patients may cause or enhance hyperandrogenism, increasing the peripheral conversion of androstenedione to estrone in adipose tissue and decreasing sex hormone binding globulin levels, and affect inappropriate gonadotropin secretion. Indeed, Plymate et al. (1981) found that plasma LH levels are higher in the obese PCOD patients than in normal weight PCOD patients. In this study there was no difference in plasma gonadotropin levels between the overweight and normal weight patients. Our data is in agreement with findings of Petraglia et al. (1985).

Several investigators have shown that β-endorphin levels in peripheral blood are elevated in patients with PCOD compared to normal controls (Givens et al., 1980; Aleen and McIntosh, 1984; Wortsman et al., 1984). In this study, elevated plasma β-endorphin levels were found not in normal weight PCOD patients but in the overweight controls and PCOD patients. We also confirmed the observations of others (Givens et al., 1980; Aleen and McIntosh, 1984) that plasma β-endorphin levels significantly correlate with body weight. On the other hand, Margules et al. (1978) found that both genetically obese mice and rats have elevated plasma and pituitary levels of β-endorphin. Similar findings have been reported in the peripheral blood of obese children and adults (Genazzani et al., 1986; Facchinetti et al., 1986, 1987; Giugliano et al., 1988). Our data in conjunction with those findings indicate that peripheral opioid abnormality in PCOD may be a concomitant phenomenon associated with obesity and not related to the pathogenesis of disease.

The physiological significance of elevated β-endorphin levels in obese patients with PCOD is conjectural at this time. It has been shown that β-endorphin stimulates the secretion and glucagon from the pancreas (Reid and Yen, 1981). Shoupe and Lobo (1984) demonstrated that hyperinsulinemia is present in obese women with PCOD. Thus the elevation of peripheral β-endorphin might be responsible for the obesity of PCOD by altering the insulin response to food and glucose.

Central opioid activity is probably unrelated...
to peripheral β-endorphin levels. Petraglia et al. (1985) reported that a naloxone-induced LH rise occurs in obese PCOD patients in a manner similar to that in normal healthy women; whereas naloxone does not elicit the increase in plasma LH levels in normal weight PCOD patients. Recent finding suggests that the reversal of peripheral ovarian responses induced by treatment for induction of ovulation may restore this impaired opioid activity in normal weight patients (Nappi et al. 1989).

The source of peripheral β-endorphin in obese PCOD patients is obscure. It is well-known that β-endorphin is released simultaneously with ACTH from the anterior pituitary (Guillemin et al. 1977). The demonstration of dexamethasone-resistant β-endorphin levels in obese children together with their cortisol being normally suppressed (Facchinetti et al. 1987) suggests that hyperandropinemia in these patients cannot be considered only as an overfunction of the anterior pituitary. Taking into consideration that human ovarian follicles contain β-endorphin levels 10 to 15 times higher than in the plasma (Petraglia et al. 1985), that immunoreactive β-endorphin is present in theca and stromal cells as well as luteinized granulosa cells (Aleem et al. 1986), and that the β-endorphin content in the follicular fluid of polycystic ovary is significantly higher than in normal ovaries (Aleem et al. 1987), it may be supposed that a possible release of β-endorphin from polycystic ovaries in part contributes to the peripheral pool of β-endorphin.

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Park SY. Studies on the physical growth and development, standard body weight and normal adapted
effect of this continued exposure upon melanocytes. In a previous study, we studied the effect of repeated UVR exposure for 2 weeks upon changes in the number of melanocytes by histochemical study (Kim and Youn 1988). The result was a significant increase in the population of melanocytes. In the present study, we decided to investigate the quantitative changes of melanocytes in C57BL mice upon repeated UVR exposure for 4 weeks by histochemical study with DOPA method, and we studied the ultrastructural changes to determine the subcellular events in melanocytes.

There are various skin reactions after exposure to UVR. There are immediate reactions such as erythema and pigmentation, and chronic reactions such as photocarcinogenesis and photocaging (Gange 1987). Topical sunscreens have been used to prevent the harmful effects of UVR (Pathak 1982).

In a previous study, we studied the effect of sunscreen upon erythema (Lee et al. 1984; Youn and Youn 1985). Sunscreens also could be used to prevent pigmentations which are an acute reaction caused by UVR. But the influence of sunscreens upon melanocytes after continued UVR exposure is not definite (Imokawa 1986; Chun 1984). The following study was undertaken to explore the effect of UVR on epidermal melanocytes and the influence of sunscreen by histochemical and ultrastructural study.

MATERIALS AND METHODS

Materials

1. Experimental animals
   The dorsal surface of ear skin of 20-25 gm, 8-10 week old C57BL male mice was used. A total of 30 mice were used in this study. They were divided into 6 groups according to the duration of irradiation.

2. Radiation source
   High pressure mercury arc (Burdick Co., USA) provided radiation of 254, 263, 297, 303, and 366 nm wavelengths (peak output at UVB).

   Irradiance measurements were made with an IL 700A Research Radiometer (International Light Co., USA) fitted with a UVB sensor (SEE 240, International Light Co., USA).

3. Sunscreen
   Five % para-aminobenzoic acid (PABA) cream was used for sunscreen.

Methods

1. UV exposure
   The mice had both their ears irradiated daily for 5 days, from Monday to Friday, per week. One hundred mJ/cm² was exposed daily to the dorsal skin of the right ear (Subgroup A). Sunscreen was applied topically to the dorsal skin of the left ear 15 min before irradiation (Subgroup B).

   The mice were divided into 6 groups according to the duration of irradiation as follows: Group I (3 days), Group II (1 week), Group III (2 weeks), Group IV (3 weeks), Group V (4 weeks), and Group VI (unirradiated control). UV exposure was done in the afternoon to avoid diurnal variation.

2. Skin biopsy and tissue preparation
   The skin of both ears was biopsied after UV light irradiation for 3 days, 1 week, 2 weeks, 3 weeks, and 4 weeks.

   The biopsied specimen was cut into small pieces and processed for (1) histochemical study and (2) ultrastructural study.

1) Histochemical study
   The whole epidermal sheet was prepared by means of a split-DOPA technique (Erickson and Montagna 1975; Kim and Youn 1988). Epidermal unfixed tissues were split after incubation with 1N NaBr solution at 37°C for 2 hours. The split epidermis was incubated with buffered DOPA solution at 37°C for 2 hours followed by several hours of subsequent incubation at 4°C. The buffered DOPA solution was composed of 25 ml of 0.1% DOPA solution, 6 ml of 0.88% disodium hydrogen phosphate, and 2 ml of 0.9% potassium phosphate. The DOPA preparation was then fixed with 10% formalin solution and dehydrated by 70% and 99.5% alcohol solutions and xylol. The skin specimen was mounted on glass.slides with the dermal-epidermal junction facing up and examined with light microscopy.

   The number of melanocytes per square millimeter (No/mm²) was determined using a light microscope (Olympus, Japan) by counting the number of melanocytes in 10 independent fields in each specimen. The significance of the results bet-
ween Subgroup A and Subgroup B was examined by Wilcoxon's matched-pairs signed ranks test. The significance of the results of the number of melanocytes through the duration of irradiation was examined by the Friedman Two-way Anova test.

2) Ultrastructural study

The specimen for electron microscopy was cut into 1×1 mm pieces, fixed with 2.5% glutaraldehyde, postfixed with 1% osmium tetroxide, dehydrated with a graded ethanol series, and embedded in EPON 812. Thin sections were cut with a diamond knife, stained with uranyl acetate and lead nitrate, and examined under an electron microscope (JOEL JEM-100, Japan). The specimens for electron microscopy with DOPA staining were prepared by the same procedures as those for usual electron microscopy, except that the duration of fixation with glutaraldehyde was 1 hour (usual: 2 hours), and the pieces were incubated in the buffered DOPA solution at 37°C for 1 hour and in the changed fresh buffered DOPA solution at 4°C for several hours (overnight) (Hunter 1970; Jimbow 1971).

RESULTS

The Effect of UVR on Melanocytes

1. Histochemical findings

The irradiated skin contained strongly DOPA-positive melanocytes with stout dendrites as well as a clearly increased number of melanocytes compared to the nonirradiated skin. After UVR exposure for 3 days the number of DOPA-positive melanocytes increased from 29.3 ± 24.4 to 138.9 ± 116.3 cells/mm². The number of DOPA-positive melanocytes increased to 361.6 ± 68.6 cells/mm² after 1 week, 386.6 ± 73.2 cells/mm² after 2 weeks, 633.1 ± 90.0 cells/mm² after 3 weeks, and 919.7 ± 45.9 cells/mm² after 4 weeks. During the 4

![Graph showing the effect of repeated UVR exposure on the number of DOPA-positive epidermal melanocytes.](image)

**Fig. 1.** The effect of repeated UVR exposure on the number of DOPA-positive epidermal melanocytes.

Table 1. The number of DOPA-positive melanocytes in repeated UV irradiation

<table>
<thead>
<tr>
<th>Group</th>
<th>Duration of Irradiation</th>
<th>No. of melanocytes/mm² (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Subgroup A</td>
</tr>
<tr>
<td>I</td>
<td>3 day</td>
<td>138.9 ± 116.3</td>
</tr>
<tr>
<td>II</td>
<td>1 wk</td>
<td>361.6 ± 68.6</td>
</tr>
<tr>
<td>III</td>
<td>2 wk</td>
<td>386.6 ± 73.2</td>
</tr>
<tr>
<td>IV</td>
<td>3 wk</td>
<td>633.1 ± 90.0</td>
</tr>
<tr>
<td>V</td>
<td>4 wk</td>
<td>919.7 ± 45.9</td>
</tr>
<tr>
<td>VI</td>
<td>Control</td>
<td>29.3 ± 24.4</td>
</tr>
</tbody>
</table>

* P<0.05, compared to Subgroup A
자외선조사에 의한 표피 멜라닌 세포의 변화 및 일반화된 대체
조직학적 및 조직학적 연구

서울대학교 의과대학 피부과학교실

주제일·이해영·이유신

자외선은 인체 피부에 석소반응을 유발하여 다양한 피부 반응을 일으킨다. 석소반응의
규명을 위하여 C57 BL mouse의 귀 피부에 고압수온등을 이용하여 매일 100 mJ/cm²의
자외선을 조사하여 3일 조사 후, 1주일 조사 후, 2주일 조사 후, 3주일 조사 후, 4주일
조사 후 멜라닌 세포의 수과 형태의 변화를 조직학적 및 전자미경적 검사로 연구
하였다. 또한 멜라닌 형성효소인 tyrosinase의 활성도를 알기 위해 DOPA-전치형성검사를
이용하여 시행하였다. 동시에 임상적적상, PABA의 효과를 알기 위하여 PABA
도포군에서의
변화를 연구하였으며 그 결과는 다음과 같다.

1. DOPA-양성 멜라닌 세포수는 비조사군에서는 표본 1병성 mm²당 29.3 ± 24.1였다.
   3일 조사 후는 138.9 ± 116.3으로, 1주 후는 361.6 ± 68.6으로, 2주 후는 386.6 ± 73.2로,
   3주 후는 633.1 ± 9.0으로 증가하였으며 4주 후는 919.7 ± 45.9로 조사기간이 길어짐에
   따라 점차 그 수가 증가되었다 (P<0.01).

2. 멜라닌 세포는 조사기간이 증가함에 따라 점차 세포가 커지고 수치증가가 증가
   되며, 길이가 길어졌다. 3주 후에 멜라닌 세포의 크기와 수치 증가가 가장
   빠르고 3주 후에는 세포수성비의 증가, 점착 멜라닌 석소 증가 및 시각물
   길어진 수지상모기 등을 나타내었다.

3. 전치형성검 및 DOPA-전치형성검사상 조사 3일 후에 멜라닌 소체 및 다수의 상
   발된 골기체가 관찰되었다. 또한 골기체 주위에 멜라닌 형성효소인 tyrosinase
   활성을 의미하는 DOPA 양성인 세포상 내에는 홍색의 구조가 관찰되었다. 이러한
   구조는 1주 후에 증가하였으며 2주 후는 약간의 변화가 발생하지 않으며, 3주
   후에 멜라닌 세포의 형태가 점차 두부형에서 소견을 나타내었다.

4. 일정단계 도포군에서는 4주 조사 후 DOPA-양성 멜라닌 세포의 수치가 일정단계에
   비도포군에 비해 유의하게 감소된 소견을 나타내었다 (P<0.05). 그러나 형질학적인
   변화는 관찰할 수 없었다.