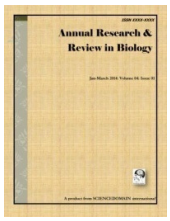

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Opioid System (β-endorphin) and Stress Hormones Profiling in Women with Polycystic Ovary Syndrome

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: In this study, we investigated serumβ-endorphin and its feedback system in women with polycystic ovary syndrome (PCO). Experimental and clinical studies show that there is an over activity of sympathetic nervous system in PCOS. It affects quality of life and can worsen anxiety and depression either due to the features of PCOS or due to the diagnosis of a chronic disease.
Study Area and Duration of Study: This study was carried out at Vali-e-Asr Clinic, a Reproductive Health Research Center, affiliated to Tehran University of Medical Sciences between February 2012 and April 2013.
Methodology: 77 women with PCO were studied with diagnose criteria and within the age range of 20-40 years without special disease. Stress neurohormones, beta-endorphine and melatonin serum levels were measured in study group and were compared with healthy women as control group. A questionnaire with items related to pieces of information about stress was used for data collection. Stress symptoms were assessed using the Understanding Yourself questionnaire. Statistical analyses were performed using SPSS Ver. 13.0 (SPSS Inc., Chicago, ILL, USA). The data are

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presented as mean ± SD or as frequency with percentages. A p-value less than 0.05 were considered as statistically significant.
Results: Data of serum levels of neurohormones showed, beta endorphin decreased (P<0.001) and adrenaline increased (P<0.001) in women with PCO. Significantly inhibited the opioid system may be one of the main reasons of the hyperactivity in two super systems: HPA and sympathetic nervous system.
Conclusion: Earlier reports stated that opioid system decreases sympathetic tone in the brain in normal condition, our results in this study confirm the over activity of sympathetic nervous system which was due to decreasing opioid system activity in women with polycystic ovary syndrom (PCO).

Keywords: Polycystic ovary syndrome (PCOS); stress; opioid system; β-endorphin; adrenaline; cortisol.

1. INTRODUCTION

1.1 Stress History

The concept of stress is as old as medical history itself, dating back at least to the time of Hippocrates who referred both to the suffering associated with disease (pathos) and to the toil (ponos) — the fight of the body to restore itself to normalcy [1]. In more recent history, both Walter Cannon [2] and Claude Bernard [3] described the ability of all organisms to maintain a constancy of their internal milieu or homeostasis, and 70 years ago Hans Selye, the pioneer of contemporary stress research, first described the General Adaptation Syndrome (GAS) as a chronological development of the response to stressors when their action is prolonged [4]. Early life events influence life-long patterns of emotionality and stress responsiveness and alter the rate of brain and body aging. The hippocampus, amygdala, and prefrontal cortex undergo stress-induced structural remodeling, which alters behavioral and physiological responses. As an adjunct to pharmaceutical therapy, social and behavioral interventions such as regular physical activity and social support reduce the chronic stress burden and benefit brain and body health and resilience [5].

1.2 HPA Axis: Historical Context

In 1936, Hans Selye reported a historic series of studies on sever stress in rats. Exposure to bacterial infection, toxic chemicals, and other life threatening insults consistently caused adrenal gland enlargement with high levels of corticosterone secretion, atrophy of the immune organs, and gastric ulcers. All three components of this nonspecific stress response are caused by prolonged activation of the hypothalamic–pituitary- adrenocortical axis (HPAC), resulting in secretion of stress levels of adrenocorticotropin (ACTH) and glucocorticoids. In spite of these harmful effects, glucocorticoids in normal levels are necessary for sustaining life. A challenge to homeostasis (a stressor) initiates the release of corticotrophin-releasing hormone (CRH) from the hypothalamus, which in turn results in release of adrenocortiotropin hormone (ACTH) into general circulation. ACTH then acts on the adrenal cortex resulting in release of a species-specific glucocorticoid into blood. Glucocorticoids act in a negative feedback fashion to terminate the release of CRH. The body strives to maintain glucocorticoid levels within certain boundaries and interference at any level of the axis will influence the other components via feedback loops.

1.3 Stress System: Stress Syndrome Physiology

The central components of the stress system are located in the hypothalamus and the brain stem and include the parvocellular corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) neurons of the paraventricular nuclei (PVN) of the hypothalamus, the CRH neurons of the paragigantocellular and parabranchial nuclei of the medulla, and the locus ceruleus (LC) and other mostly noradrenergic (norepinephrine [NE]) cell groups of the medulla and the pons (the LC/NE-sympathetic system) [6,7]. The LC is densely innervated by processes exhibiting endogenous opioid peptides [8,9]. In vitro studies have shown that the LC contains a high concentration of μ-opioid receptors (μOR) [10,11]. Ultrastructural studies have demonstrated that μOrs are prominently distributed on somatodendritic processes in the LC and are specifically localized

to the plasma membrane of dendrites that are targeted by the enkephalin family of opioid peptides [11]. The interaction between CRH and endogenous opioids within the pontin nucleous, locus coeruleus (LC), a major noradrenaline nuclous (NA) in the brain, a region that has been implicated as a target in response to stress and opiates and distruption in CRH/opioid balance as a result of hyperactivity and hyperresponsivness of the LC-NA system and co-regulation of LC-NA system by CRH and opioids may be important in acute adaptation stress [10,12]. The intra-cerebroventricular (icv) administration of beta-endorphin (beta-End) to rats significantly increases plasma levels of ACTH and NA. Naloxone (NTX) could inhibit the latter increments. On the other hand intravenous (iv) adminstration of NTX attenuated the restraint stress-induced rise of plasma ACTH and NA levels. Iv administration of anti-rat CRH rabbit serum completely blocked the beta-endorphin-induced ACTH secretion without affecting the secretion of NA icv. The brain beta-endorphin can stimulate the secretion of ACTH and NA through opiate receptor and that brain CRH mediates the beta-endorphin-induced secretion of ACTH [9]. The endogenous opioids have a tonic inhibitory effect on sympathetic tone and have been implicated in the pathophysiology of vasodepressor syncope, because plasma beta-endorphin concentrations increase after vasodepressor syncope induced by stressor as exercise or fasting [13] and following the discovery of the cathecolamine tracts, it was proposed that activation of central alpha-receptors inhibits the hypothalamo-pituitary-adrenal axis, via α-1 adrenoceptors [14], whereas activation of α2- adrenoceptors supposed to inhibite ACTH secretion in stress condition (when the intrahypothalamic concentration of noadrenaline is very high), via α2-adrenoceptors [15] and further elucidated α-2 adreneceptorsubtyps involved in CRH/ACTH and beta-endorphin secretion [16]. The peripheral limbs of the stress system are the hypothalamic-pituitary-adrenal (HPA) axis along with the efferent sympatheticadreno-medullary system. Stressor-induced activation of the HPA axis and the SNS results in a series of neural and endocrine adaptations known as the "stress response" or "stress cascade" (Fig. 1.) [17,18].

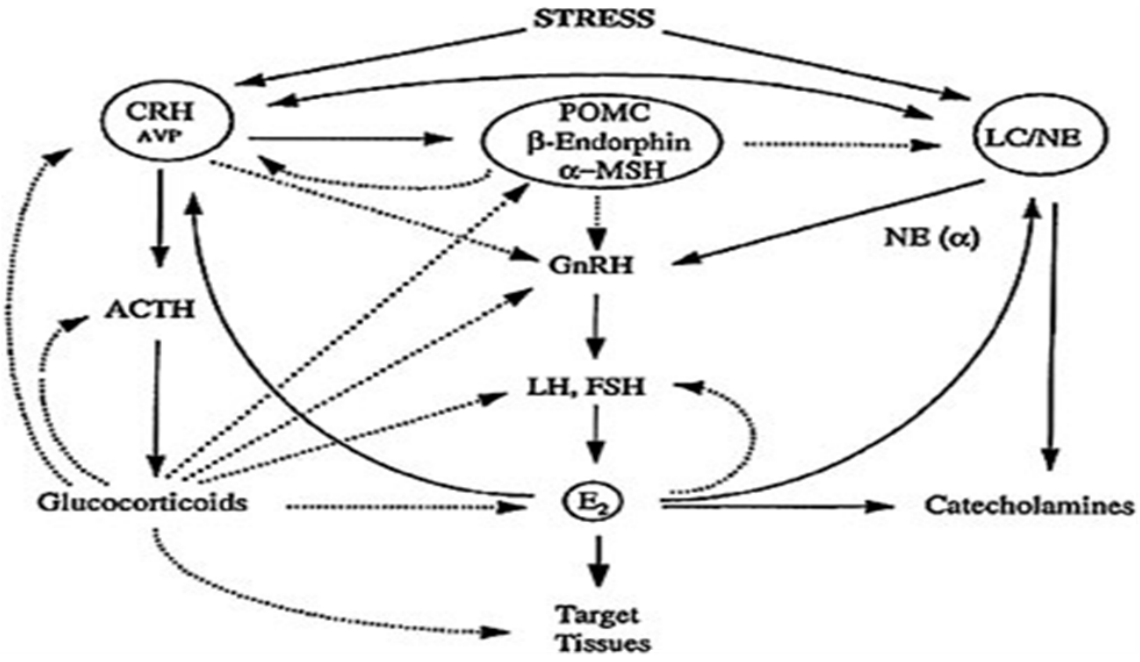

Fig. 1. Heuristic representation of the interplay among the hypothalamic-pituitary-adrenal axis, the locus ceruleus/norepinephrine (LC/NE) sympathetic system and the hypothalamic-pituitary-gonadal axis. The dotted lines represent inhibition while the solid lines represent stimulation [17]

1.4 Stress System & PCOS

Polycystic ovary syndrome (PCOS), the most common female endocrine disorder, is a complex and heterogenic disease with unknown etiology. PCOS is characterized by reproductive disturbances including chronic anovulation, hyperandrogenism and polycystic ovaries [19]. Its prevalence among infertile women is 15% - 20% [20]. The human ovary has a functional sympathetic innervation coupled to steroid secretion. The value for the concentration of norepinephrine in the ovary was similar to others previously reported and 10 times higher than rat ovarian tissue [21], this neurotransmitter is coupled to a steroidogenic response [22]. In this study we investigatedβ-endorphin and stress hormones (adrenaline, noradrenaline and cortisol) in women with PCOS. Previous studies have shown that PCOS may cause some psychological disorders. The relationships between the psychological health aspects and the clinical characteristics of PCOS are not yet clear. This study was conducted to determine and clarify the feedback systems of neurotransmitters between psychological statuses of women with PCOS.

2. MATERIALS AND METHODS

2.1 Participants

The sample included all women suffering from PCOS who visited Vali-e-Asr Clinic affiliated to Tehran University of Medical Sciences for the first time between February 2012 and April 2013. The diagnosis of PCOS was made according to the joint criteria of the European Society of Human Reproduction and Embryology and the American Society of Reproductive Medicine (ESHRE/ASRM) [23]. In this descriptive-analytical study, 77 patients aged 20–40 years participated who were not suffering from any illness except PCOS. This study was approved in ethical committee of Tehran University of Medical Sciences (publication No.116-15549, revised 2012).

2.2 Questionnaires

Data were collected from clinical and anthropometric variables, including hirsutism score, body mass index (BMI) and a demographic questionnaire inquiring about age, education, occupation, and duration of illness. BMI was calculated as weight (kg)/ height (m^2). Stress symptoms were assessed using the Understanding Yourself questionnaire. This questionnaire has been developed by psychologists to provide a comprehensive description of personality. It can be used to rate the personalities of children, adolescents, and adults of any age. Understanding Yourself and Others®: An Introduction to Interaction Styles reveals the four fundamental interaction style patterns for understanding oneself (and others). Within these patterns are clues to the "how" of our behaviors. Find out how you consistently seem to fall into certain roles in your interactions with others and how you can shift your energies to take on other roles when necessary. It includes 6 major questions and every question has several items with multiple choice answers as a self-report questionnaire that measures severity of stress. Understanding Yourself Questionnaire determines stress in four dimensions of anxiety, worries, hysteria and obsession. Stress score is calculated by adding scores of each question which range from 0 to 60, where a higher total score indicates more severe stress symptoms. Scores ≥26 were considered symptomatic stress. Scores below 26 are not indicative of stress 26–45 indicate neurotic stress, and stress scores higher than 46 indicate high levels of stress which need psychological intervention [24].

2.3 Clinical and Laboratory Investigation

Concentration of serum levels of Adrenaline, Nor-adrenaline (Oxidized LDL Elisa from Germany), Beta-endorphin (ELISA kit, Cousabio from USA), Cortisol (ELISA kit, Diametra from Italy) were measured at the Laboratory of Pathology of Vali-e-Asre.

2.4 Statistical Analysis

Data was presented as mean ± standard deviation also median if necessary. The t-test and Mann-Whitney test were used to compare hormones between study groups. Spearman correlation coefficient was used to evaluate relationship between hormones. All analysis was done in SPSS 18 (SPSS Inc, Chicago Ill) software. P-value less than 0.05 considered as significant level.

3. RESULTS

Results showed that women in control groups had more education than PCO group (P = 0.003)

(Table 1). Employment rate was similar in both groups (P = 0.491). Delivery (P = 0.327) and abortion (P = 0.056) was same in both groups. Symptoms of PCO were higher in PCO group. Irregular menstrual (P < 0.001) and hirsutism (P < 0.001) significantly were higher in women with PCO. Overweight was same at both groups (P = 0.231). Mean of age in PCO group about 3 years was lower than control group (P < 0.001), but both groups had same marital duration (P = 0.935).

Analysis of neurohormones serum levels showed that according to Kolmogorov Smirniv cortisol and β endorphin had a normal distribution, but adrenaline and noradrenaline were not normally disturbed. Comparison of cortisol levels (P = 0.652) and noradrenaline (P = 0.737) did not show significant differences between the two groups. Adrenalin level was higher in PCO group (P < 0.001). Serum levels of Beta-endorphin (P = 0.001) was lower in women with PCO (Table 2). Spearman correlation shows that there was a significant correlation between cortisol and noradrenalin (r = 0.250, P = 0.001). This correlation is stronger in PCOS patients (r = 0.315) than control group (r = 0.198). Noradrenalin similarly had a significant correlation with β-endorphin (r = 0.177, P = 0.017) (Table 3).

4. DISCUSSION

Polycystic ovary syndrome (PCOS) is a common endocrine condition associated with long-term health risks, including type 2 diabetes and vascular dysfunction in addition to reproductive sequelae. Many of the common features of PCOS, such as central obesity, hyperinsulinaemia, obstructive sleep apnea (OSA) andmetabolic syndrome are associated with chronic sympathetic overactivity, suggesting that sympatho-excitation may be involved in the pathogenesis of this condition [25]. Emotional distress, depressive symptoms, hirsutism score, body mass index (BMI), waist-to-hip ratio (WHR), luteinizing hormone/follicle-stimulating hormone ratio, serum total testosterone, dehydroepiandrosterone sulphate levels and the insulin resistance index have been found to be significantly higher in women with PCOS than in healthy women [17,18,19].

Table 1. Comparison of demographic and history in study groups

Demographic factors	Description	Control	PCOs	P-value (Chi square)
Education	Lower diploma	30 (30.9%)	41 (53.2%)	0.003
	Diploma and more	67 (69.1%)	36 (46.8%)	
Occupation	Occupied	12 (12.4%)	7 (9.1%)	0.491
	House keeper	85 (87.6%)	70 (90.9%)	
Delivery	Yes	25 (25.8%)	15 (19.5%)	0.327
	No	72 (74.2%)	62 (80.5%)	
Abortion	Yes	11 (11.3%)	17 (22.1%)	0.056
	No	86 (88.7%)	60 (77.9%)	
Hirsutism	Yes	28 (28.9%)	53 (68.8%)	<0.001
	No	69 (71.1%)	24 (31.2%)	
Over weight	Yes	53 (54.6%)	49 (63.6%)	0.231
	No	44 (45.4%)	28 (36.4%)	

P value is significant at P≤ 0.05 and 0.001, Control n =97, PCOS n= 77, N=174, Chi-Square

Table 2. Mean ± SD Comparison of age and hormones in study groups

	Control	PCOs	P-value t-test
Age (year)	29.6 ± 5.2	26.6 ± 4.7	<0.001
Marital duration (year)	6.9 ± 4.4	7.0 ± 4.1	0.935
Hormones			
Cortisol (ng/ml)	197.08 ± 106.89	190.57 ± 75.07	0.652
β endorphin (pg/ml)	87.19 ± 9.39	81.58 ± 10.19	<0.001
Hormones			**Mann - Whitney**
Adrenalin (ng/ml)	3.61 ± 3.11 (3.10)	5.97 ± 4.49 (5.00)	<0.001
Noradrenalin (ng/ml)	0.74 ± 3.38 (0.00)	0.50 ± 1.35 (0.00)	0.737

P value is significant at P≤ 0.001, Control n =97, PCOs n= 77, N=174, t-test and Mann-Whitney (Median)

Table 3. Spearman correlations coefficients between age and hormones

		Adrenaline			Noradrenaline			Cortisol			β-Endorphin		
		control	**PCOs**	**All**	**control**	**PCOs**	**All**	**control**	**PCOs**	**All**	**control**	**PCOs**	**All**
Adrenaline	r				0.073	**0.252**	**0.162**	-0.095	**0.355**	0.095	0.082	-0.050	-0.064
	P-value				0.478	**0.026**	**0.033**	0.356	**0.001**	0.209	0.426	0.661	0.399
Noradrenaline	r	0.073	**0.252**	**0.162**				0.198	**0.315**	**0.250**	0.185	0.209	**0.177**
	P-value	0.478	**0.026**	**0.033**				0.063	**0.005**	**0.001**	0.069	0.066	**0.019**
Cortisol	r	-0.095	**0.355**	0.095	0.198	**0.315**	**0.250**				-0.046	-0.108	-0.091
	P-value	0.356	**0.001**	0.209	0.063	**0.005**	**0.001**				0.654	0.347	0.233
β-Endorphin	r	0.082	-0.050	-0.064	0.185	0.209	**0.177**	-0.046	-0.108	-0.091			
	P-value	0.426	0.661	0.399	0.069	0.066	**0.019**	0.654	0.347	0.233			

r: correlation coefficients, P value is significant at P≤ 0.001, Control n =97, PCOs n= 77, N=174, Spearman correlation

Recent studies show that there is a complex condition with psychological, reproductive and metabolic manifestations in women with PCO [26,27,28]. Limited studies to date have reported that women who have PCOS are more prone to depression, anxiety, low self-esteem, negative body image, and psychosexual dysfunction [29,30]. The other critical aspect of psychosocial impact in PCOS is the negative impact of mood disturbance, poor self-esteem and reduced psychological well-being on motivation and on ability to implement and sustain successful lifestyle changes that are critical in this condition [31]. Surely, these issues all need to be explored and addressed as part of PCOS assessment and management. Management should focus on support, education, addressing psychological factors and strongly emphasizing healthy lifestyle with targeted medical therapy as required. Treatment for the large majority is lifestyle focused and an aggressive lifestyle-based multidisciplinary approach is optimal in most cases to manage the features of PCOS and prevent long-term complications. Disruptions in homeostasis (ie, stress) place demands on the body that are met by the activation of two super-systems, the HPA axis and the sympathetic nervous system (SNS). Stressor-induced activation of the HPA axis and the SNS results in a series of neural and endocrine adaptations known as the "stress response" or "stress cascade"[32]. The HPA axis together with the sympathetic system connects the brain with the periphery of the body [33]. The catecholamines play key roles in orchestrating the response to stress. While this is crucial to handle emergency situations, stress becomes maladaptive when prolonged or repeated, increasing allosteric load and susceptibility to a wide range of serious diseases. The cathecolaminergic influence can be dependent also on the nature of the stressor and on availability of adrenal steroids [34], then stress induced release of noradrenaline (NE) from postganglionic sympathetic neurons and adrenaline predominantly from the adrenal medulla. The noradrenergic input to the hypothalamic circuitry regulating LH secretion is itself under the influence of endogenous opioid peptides [35]. Thus opioid peptides suppress the release of hypothalamic noradrenaline [36] and the opioid antagonist naloxone can potentiate the rise in circulating LH concentrations that follow electrical stimulation of the ventral noradrenergic tract [37,38]. The orchestrated interplay of several neurotransmitter systems in the brain underlies the characteristic phenomenology of behavioral, endocrine, autonomic and immune responses to stress [33]. These transmitters include CRH, AVP, opioid peptides and cathecolamine. Increased knowledge on the mechanisms whereby CRH, β-endorphin and sympathetic tone mediate the effects of stress has helped us to understand the relationship between stress and disturbed reproductive function. The regulation of gonadal function in men and women is under the control of the hypothalamus and the pituitary gland by means of negative and positive feedback mechanisms by hypothalamus – pituitary- gonadal axis (HPG). This regulation is complex, and the details have only begun to be understood in recent years. Endocrine, paracrine, and autocrine mechanisms are involved, and the regulators include hypothalamic and pituitary hormones, neurotransmitters, and endogenous opioids in addition to steroidal and non-steroidal hormones from the gonads. There is a close functional interaction between the HPA-axis and the HPG-axis. Hypothalamic CRH neurons inhibit directly or indirectly through proopiomelanocortin neurons, the hypothalamic control of the gonadal axis (Dudás et al., 2002). These systems include neurons that contain many neurotransmitters and peptides from various brain regions that convey information to GnRH neurons, for example, norepinephrine, epinephrine, dopamine, serotonin, g-aminobutyric acid (GABA), glutamate, endogenous opiate peptides, NPY, and galanin. In general, catecholamines stimulate GnRH release, whereas endogenous opioid peptides and prolactin inhibit GnRH secretion (Marshall, 2001). Endogenous opioids have modulating role on cathecolamine secretion, and studies of these effect show that, opioids inhibit the release of cathecolamine during stress [39]. According to the interaction between the opioid and the sympathetic nervous system, our results show that serumβ-End was lower in women with PCOS, because their serum adrenaline was higher (overactivity of sympathetic, in peripheral) and the correlation coefficient shows that there is intraction with serum β-End and noradrenaline, noradrenaline with cortisol. In stress situation, inhibitory tone of opiates increases on sympathetic nervous system inhibiting the release of cathecolamines with decrease in an activity of sympathico-adrenal system and GnRH. The noradrenergic input to the hypothalamic circuitry regulating LH secretion is itself under the influence of endogenous opioid peptides [40]. About the role of opioid system, it was previously shown by Zubieta et al. in 2003 that induction of negative mood states is associated with a significant

deactivation in μ-opioid neurotransmission, and it expect a direct relationship between the level of euphoria and the opioid displacement [41] and this deactivation can result in overactivity of sympathetic nervous system. The brain beta-endorphin can stimulate the secretion of ACTH and NA through opiate receptor and brain CRH mediates the beta-endorphin-induced secretion of ACTH [18]. The interaction between CRH and endogenous opioids within the pontin nucleous in locus coeruleus (LC), as a target in response to stress and opiates, disruption in CRH/opioid balance as a result of hyperactivity, hyperresponsiveness of the LC-NA system and co-regulation of LC-NA system by CRH and opioids all may be important in acute adaptation stress [42,43]. The results of this study confirm that the reduced levels of beta-endorphin can be the main factor for reduced noradrenalin and increased levels of adrenalin. The intra-cerebroventricular (icv) administration of β-End to rats significantly increases plasma ACTH, NA levels and naloxone (NTX) could inhibit it. On the other hand intravenous (iv) administration of NTX attenuated the restraint stress-induced rise of plasma ACTH and NA levels. In women with PCOS, cortisol production rate is probably normal, although adrenal androgens can be overproduced in a subset of affected women [44]. Milutinovic's hypothesize in 2011 is that modulation of glucocorticoid receptor (GR) expression and function may underlie possible PCOS-related impairment of feedback inhibition of HPA axis activity and imply that PCOS is associated with increased GR protein concentration and HPA axis sensitivity to dexamethasone [45]. These findings suggest that the feedback system between the HPA axis and parvocellular nuclei (PVN) of the hypothalamus is disrupted in women with PCOS. Because β-End and noradrenaline both have low, whereas in normal β-End suppress the release of hypothalamic noradrenaline [36]. In view of animal data which show that acupuncture mediates sympatholytic and depressor effects in the central nervous system and reduces ovarian sympathetic tone in a rodent model of PCO [46], randomly assigned 20 moderately overweight patients with PCOS to low-frequency electroacupuncture (EA; n = 9), physical exercise (n = 5) or no treatment (n = 6) for 16 weeks [47]. BMI in our study was under 28. Early studies implied sympathetic activation in women with PCOS by measurement of catecholamines and their metabolites. Results of this study confirm that the serum adrenaline has increased but cortisol level in women with PCO is not changed because of a significant inhibition on opioid system resulting in aggravated sympathetic tone. Thus lowering the amount of beta-endorphins in women with PCO can increase sympathetic activity and therefore hyperactivity of the sympathetic system is a major factor in the change of feedback mechanisms in the etiology of this disease. Women with PCO should be aware that risk factors associated with sympatho-excitation i.e. hyperinsulinemia, blood pressure and central obesity are highly prevalent in this syndrome.

5. CONCLUSION

The results of this study confirmed the over activity of HPA axis and sympathetic nervous system that could be due to the reduction of opioid system.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Hippocrates. On airs, waters, and places. (Translated by WHS Jones, New York). New York: W. Heinmann; 1923.
2. Cannon WB. The Wisdom of the Body. New York: W.W. Nortyon and Co; 1939.
3. Bernard C. An introduction to the study of experimental medicine (1949).(2nd Ed.). New York: H.C. Greene; 1927).
4. Selye H. A syndrome produced by diverse nocuous agents. Nature. 1936;138:230–231.
5. McEwen BS, Wingfield JC. Allostasis and allostatic load. In: Fink G, editor. Encyclopedia of Stress. 2. Academic Press, New York. 2007;135–141.
6. Tsigos C, Chrousos GP. Physiology of the hypothalamic–pituitary–adrenal axis in health and dysregulation in psychiatric and autoimmune disorders. Endo Met. Clin North Am. 1994;23(3):451–466.
7. Charmandar E, Kino T, Souvatzoglou E, Chrouso GP. Pediatric stress: Hormonal

mediators and human development. Horm Res. 2003;59(4):161-79.
8. Drolet G, Van Bockstaele EJ, Aston-Jones G. Robust enkephalin innervation of the locus coeruleus from the rostral medulla., J Neurosci. 1992;12(8):3162–3174.
9. Reyes ASB, Glaser JD, Van Bockstaele EJ. Ultra structural Evidence for Co-localization of Corticotropin-Releasing Factor Receptor and μ-Opioid Receptor in the Rat Nucleus Locus Coeruleus. Neurosci Lett. 2007;413(3):216-221.
10. Mansour A, Fox CA, Akil H, Watson SJ. Opioid-receptor mRNA expression in the rat CNS: Anatomical and functional implications. Trends Neurosci. 1995;18(1):22-29.
11. Van Bockstaele EJ, Colago EEO, Valentino RJ. Corticotropin-releasing factor-containing axon terminals synapse onto catecholamine dendrites and may presynaptically modulate other afferents int he rostral pole of the nucleus locus coeruleus in the rat brain. J Comp Neurol. 1996;364(3):523-534.
12. Valentino RJ, Van Bockstaele EJ. Opposing regulation of the locus coeruleus by corticotropin releasing factor and opioids: potential for reciprocal interactions between stress and opioid sensitivity. J Psychopharm. 2001;158(4):331-342.
13. Szafarezyk A, Plotsky PM, Cunnigham ET, Widmayer EM. Cathecolam inergic madulation of corticotropin releasing factor and adrenocorticotropin secretion. Endocr Rev. 1989;10(4):437-458.
14. Damluji AI. Adrenergic mechanisms in the central of corticotropin secretion. J Endocrinol. 1988;119(1):5-14.
15. Zelena D, Kiem Dt, Barna I, Makara GB. α2-adrenoreceptor subtypes regulate ACTH and β-endorphin secretions during stress in the rat. J. Psychoneuroendocrin. 1999;24(3):333-343.
16. Ferin M. The menstrual cycle: An integrative view, Reproductive Endocrinology, Surg and Techno. 1996;1(183):103-121.
17. Miller DB, O'Callaghan JP. Neuroendocrine aspects of the response to stress. J. Metab. 2002;51(6 Suppl 1):5-10.
18. Wallbridge DR, MacIntyre HE, Gray CE, Denvir MA, Oldrgod KG, Rae AP, Cobbe SM. Increase in plasma beta-endorphins precedes vasopressor syncope. Br Heart J. 1994;71(5):446-8.
19. Norman RJ, Dewailly D, Legro RS, Hickey TE. Polycystic ovary syndrome. Lancet. 2007;370(9588):685-697.
20. Badawy A, Elnasha A. Treatment options for polycystic ovary syndrome. Int J Womens Health. 2011;3:25-35.
21. Yen SSC. In: Reproductive Endocrinology: Physiology, Pathophysiology and Clinical Management. Yen SSC, Jaffe RB, Barbieri RL, editor. W.B. Saunders Co., Philadelphia; The human menstrual cycle: Neuroendocrine regulation. 1999:191–217,
22. Baker FC, Driver HS. Circadian rhythms sleep and the menstrual cycle. Sleep Med. 2007;8(6):613-22.
23. Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod. 2004;19(1):41-7.
24. Berens LV. Understanding Yourself questionnaire. Rezakhani Z, translator. Place of publication: Signet Publications; 2001.
25. Lansdown A, Rees DA. The sympathetic nervous system in polycystic ovary syndrome: A novel therapeutic target? Clin Endocrinol (Oxf). 2012;77(6):791-801.
26. Adali EL, Yildizhan R, Kurdoglu M, Kolusari A, Edirne T, Sahin HG,Yildizhan B,Kamaci MJ. The relationship between clinico-biochemical characteristics and psychiatric distress in young women with polycystic ovary syndrome. J Int Med Res. 2008;36(6):1188-96.
27. Zangeneh FZ, Jafarabadi M, Naghizadeh MM, Abedinia N, Haghollahi F. Psychological distress in women with polycystic ovary syndrome from imam khomeini hospital, tehran. J ReprodInfertil. 2012;13(2):111-5.
28. Teede H, Deeks A, Moran L. Polycystic ovary syndrome: A complex condition with psychological, reproductive and metabolic manifestations that impacts on health across the lifespan.BMC Med. 2010;30:41.
29. Coffey S, Mason H. The effect of polycystic ovary syndrome on health-related quality of life. Gynecol Endocrinol. 2003;17(5):379-386.
30. Deeks A, Gibson-Helm M, Teede H. Anxiety and depression in polycystic ovary syndrome (PCOS): A comprehensive investigation. Fertil Steril. 2010;93(7):2421-2423.

31. Moran LJ, Pasquali R, Teede HJ, Hoeger KM, Norman RJ. Treatment of obesity in polycystic ovary syndrome: A position statement of the Androgen Excess and Polycystic Ovary Syndrome Society. Fertil Steril. 2009;92(6):1966-1982.
32. Miller DB, O'Callaghan JP. Neuroendocrine aspects of the response to stress. Metabolism. 2002;51:5-10.
33. Chrousos GP. Stressors, stress, and neuroendocrine integration of the adaptive response. The 1997 Hans Selye Memorial Lecture. Ann NY Acad Sc.1998;851:311–335.
34. Feuvrier E, Auberi M, Maussel AL, Alonso G, Gaillet S, Malaval F, Szafarezyk A. Glucocorticoids provoke a shift from α2 to α1 adrenoceptor activities in cultured hypothalamic slides leading to opposite noradrenaline effect on corticotropin-releasing hormone release. J. Neurchem. 1998;70(3):1199-1209.
35. Bicknell RJ. Endogenous opioid peptides and hypothalamic neuroendocrine neurones. J Endocrinol. 1985;107(3):437-46.
36. Diez- Guerra FJ, Augood S, Emson PC, Dyer RG. Morphine inhibits electrically stimulated noradrenaline release from slices of rat medial preoptic area. Neuroendocrinology. 1986;43(1):89-91.
37. Crossman R,Diez-Guerra FJ, Mansfield S, Dyer RG. Neonatal testosterone modifies LH secretion in the adult female rat by altering the opioid noradrenergic interaction in the medial preoptic area. Brain Res. 1987;415(2):205-210.
38. Zangeneh FZ, Mohammadi A, Ejtemaeimehr SH, Naghizadeh MM, Aminee F. The role of opioid system and its interaction with sympathetic nervous system in the processing of polycystic ovary syndrome modeling in rat. Arch Gynecol Obstet. 2011;283(4):885-892.
39. Angelopoulos TS, Deny BS, Weikart C, Dasilvas SG, Mickael TJ, Robertson RJ. Endogenous opioids may modulate cathecolamine secretion during high intensity exercise. Eur J Appl Physiol Occup Physiol. 1995;70(3):195-9.
40. Kalra SP, Kalra PS. Opioid-adrenergic-steroid connection in regulation of luteinising hormone secretion in the rat. J Neuroendocrin. 1984;38(5):418-426.
41. Zubieta JK, Ketter TA, Bueller JA, Xu Y, Kilbourn MR, Young EA, Koeppe RA. Regulation of human affective responses by anterior cingulate and limbic mu-opioid neurotransmission. Arch Gen Psychiatry. 2003;60(11):1145-53.
42. Mansour A, Fox CA, Akil H, Watson SJ. Opioid-receptor mRNA expression in the rat CNS: Anatomical and functional implications. Trends Neurosci. 1995;18(1):22–29.
43. Valentino RJ, Van Bockstaele EJ. Opposing regulation of the locus coeruleus by corticotropin releasing factor and opioids: Potential for reciprocal interactions between stress and opioid sensitivity. J Psychopharm. 2001;158(4):331-342.
44. Pasquali R, Gambineri A. Cortisol and the Polycystic Ovary Syndrome. Expert Rev Endocrinol Metab. 2012;7(5):555-566.
45. Milutinović DV, Macut D, Božić I, Nestorov J, Damjanović S, Matić G. Hypothalamic-pituitary-adrenocortical axis hypersensitivity and glucocorticoid receptor expression and function in women with polycystic ovary syndrome. Exp Clin Endocrinol Diabetes. 2011;119(10):636-43.
46. Stener-Victorin E, Jedel E, Janson PO. Low-frequency electroacupuncture and physical exercise decrease high muscle sympathetic nerve activity in polycystic ovary syndrome. American Journal of Physiology: Regul Integ and Comp Physio. 2009;297(2):R387-R395.
47. Jedel E, Labrie F, Odén A. Impact of electro-acupuncture and physical exercise on hyperandrogenism and olig/amenorrhea in women with polycystic ovary syndrome: A randomized controlled trial. Americ J of Physio Endocrinol and Metab. 2011;300(1):E37–E45.

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