Acta Obstet Gynecol Scand 2000; 79: 180–188
Printed in Denmark · All rights reserved

Acta Obstetricia et Gynecologica Scandinavica
ISSN 0001-6349

ORIGINAL ARTICLE

Effects of electro-acupuncture on anovulation in women with polycystic ovary syndrome

ELISABET STENER-VICTORIN[1], URBAN WALDENSTRÖM[1], ULRIKA TÄGNFORS[1], THOMAS LUNDEBERG[3], GÖRAN LINDSTEDT[2] AND PER OLOF JANSON[1]

From the Departments of [1]Obstetrics and Gynecology and [2]Clinical Chemistry and Transfusion Medicine, Göteborg University, Göteborg, and the [3]Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden

Acta Obstet Gynecol Scand 2000; 79: 180–188. © Acta Obstet Gynecol Scand 2000

Background. The present study was designed to evaluate if electro-acupuncture (EA) could affect oligo-/anovulation and related endocrine and neuroendocrine parameters in women with polycystic ovary syndrome (PCOS).
Methods. Twenty-four women (between the ages of 24 and 40 years) with PCOS and oligo-/amenorrhea were included in this non-randomized, longitudinal, prospective study. The study period was defined as the period extending from 3 months before the first EA treatment, to 3 months after the last EA treatment (10–14 treatments), in total 8–9 months. The menstrual and ovulation patterns were confirmed by recording of vaginal bleedings and by daily registrations of the basal body temperature (BBT). Blood samples were collected within a week before the first EA, within a week after the last EA and 3 months after EA.
Results. Nine women (38%) experienced a good effect. They displayed a mean of 0.66 ovulations/woman and month in the period during and after the EA period compared to a mean of 0.15 before the EA period ($p=0.004$). Before EA, women with a good effect had a significantly lower body-mass index (BMI) ($p<0.001$), waist-to-hip circumference ratio (WHR) ($p=0.0058$), serum testosterone concentration ($p=0.0098$), serum testosterone/sex hormone binding globulin (SHBG) ratio ($p=0.011$) and serum basal insulin concentration (p=0.0054), and a significantly higher concentration of serum SHBG ($p=0.040$) than did those women with no effect.
Conclusion. Repeated EA treatments induce regular ovulations in more than one third of the women with PCOS. The group of women with good effect had a less androgenic hormonal profile before treatment and a less pronounced metabolic disturbance compared with the group with no effect. For this selected group EA offers an alternative to pharmacological ovulation induction.

Key words: androgens; electro-acupuncture; β-endorphin; ovulation induction; polycystic ovary syndrome

Submitted 23 October, 1998
Accepted 20 October, 1999

Polycystic ovary syndrome (PCOS) is a common endocrine and metabolic disorder, associated with anovulation, hyperandrogenism, obesity and insulin resistance (1, 2). Endocrine characteristics of PCOS are elevated serum concentrations of androgens and luteinizing hormone (LH) and decreased concentrations of sex hormone binding globulin (SHBG). The anovulation is associated with disturbances in the feedback from the ovarian steroid

Abbreviations:
ACTH: adrenocorticotropic hormone; BBT: basal body temperature; BMI: body-mass index; CGRP: calcitonin gene-related peptide; CI: confidence interval; EA: electro-acupuncture; FSH: follicle stimulating hormone; GnRH: gonadotropin releasing hormone; IRMA: immunoradiometric assay; LH: luteinizing hormone; NE: norepinephrine; NPY: neuropeptide Y; PCOS: polycystic ovary syndrome; RIA: radioimmunoassay; SHBG: sex-hormone binding globulin; TSH: thyrotropin; WHR: waist hip ratio.

hormones to the hypothalamus and pituitary, resulting in disturbances in the pulsatility of gonadotropin releasing hormone (GnRH) release (3).

It has been suggested that the elevated concentrations of LH are due to an abnormal feedback by estrogens (4–7) and that the high tonic concentrations of LH in PCOS are detrimental to follicular growth (5, 6). The low concentrations of SHBG are associated with a relative increase in unbound concentrations of androstenedione and testosterone concentrations, which may further increase clinical expressions of hyperandrogenism, such as hirsutism (4–7). Obesity is also seen and associated with insulin resistance and decreased SHBG as well as, in many cases, increased testosterone concentrations (8–10). Also psychological stress has been suggested to be more prevalent in women with PCOS (11, 12).

The etiology of PCOS is complex and probably multifactorial, and there is still disagreement whether the primary cause of the condition is located in the ovaries or in the central nervous system. Two hypotheses have emerged, involving the nervous system at two levels.

One of the hypotheses suggests that PCOS is due to an insufficient central β-endorphin inhibition of GnRH. This hypothesis is supported by studies showing that β-endorphin exerts a tonic inhibitory control on the GnRH pulse generator and on pituitary LH release (13–15). That β-endorphin plays a role in PCOS is also supported by the finding of elevated β-endorphin concentrations in plasma and the related hyperinsulinemic response. Interestingly, elevated β-endorphin concentrations are also seen following stress (11, 12, 15, 16). The second hypothesis is based on the finding that experimentally induced PCOS in rats is associated with hyperactivity in the peripheral sympathetic nervous system (17, 18). It has been demonstrated that rats with experimentally induced PCOS have increased concentrations of norepinephrine (NE) and a decreased number of β-adrenoreceptors in the ovaries. Also, transection of the superior ovarian nerve in the experimentally induced PCOS rats restored estrous cyclicity and ovulatory capacity (17, 18). Taken together, this would suggest that PCOS is associated with an elevated sympathetic tone in the ovaries resulting in steroidal hyperresponsiveness.

Women with PCOS need some long-standing treatment to diminish their increased risk for endometrial-cancer, hypertension and type II diabetes (1). Traditional treatment in women with PCOS and anovulation is pharmacological induction of ovulation and the first choice is an antiestrogen – most commonly, clomiphene citrate (19). Antiestrogens are very effective, but side-effects such as nausea, multiple pregnancy and ovarian hyperstimulation syndrome are common (2). A need for alternative or complementary methods that allow the substitution or reduction of pharmocological interventions clearly exists. An alternative method to pharmacological induction of ovulation, known to influence both central β-endorphin systems and the sympathetic tone, is electro-acupuncture (EA) (20–22). Interestingly, Gerhard and Postneck (1992) have shown that, in infertile women with hormonal disturbances and anovulation, both auricular acupuncture and hormonal treatment resulted in equal pregnancy rates. In a previous study on 11 anovulatory women (eight with PCOS), EA was shown to induce ovulation in 5 of 13 menstrual cycles (23). This ovulation induction was associated with increased hand skin temperature and decreased plasma β-endorphin concentrations (23). The effect of EA on anovulation was attributed to an inhibition of a hyperactive sympathetic nervous system (24). Recently we have also reported that repeated EA treatments in infertile women due to a high uterine arterial blood flow impedance resulted in increased blood flow (25). The effects were attributed to a decreased activity in the sympathetic vasoconstrictor fibers innervating the uterus (25). The above findings and experimental observations support the possibility that EA could influence PCOS.

The aim of the present study was to elucidate the effect of EA in anovulatory women with PCOS. The outcome measures were basal body temperature and menstrual pattern. We included a large number of endocrine and neuroendocrine parameters to exclude major endocrine dysfunctions and also, in relation to ovulation and acupuncture, to allow for a more general investigation of the effects of EA in PCOS.

Materials and methods

Subjects

After approval by the Ethics Committee of Göteborg University and informed consent from the patients, 26 women with PCOS were included in this non-randomized, longitudinal, prospective study. They were between the ages of 24 and 40 years (mean 32 years). Two women were excluded because they were unable to attend the treatments.

Inclusion criteria were amenorrhea or oligomenorrhea with no more than four spontaneous bleedings per year and a typical ultrasonographic presentation of PCOS (multiple subcapsular follicles and thickened ovarian stroma) (26). Out of the 24 women 19 were clomiphene resistant, i.e. they had not ovulated on 150 mg clomiphene citrate for 5 days. All the ultrasound examinations were performed by transvaginal ultrasonography (Siemens Sonoline SL 200, serial number: SE00104, Germany) by one of the authors (UW). No hormonal

treatment had been given for 3 months prior to the start of the study or throughout the entire study period. Therefore, no hormonal treatment for 6 months before EA. The study started 3 months before EA.

Electro-acupuncture (EA)

EA was given twice a week for 2 weeks and then once a week, altogether 10–14 treatments. The needles were inserted intramuscularly to a depth of 15–40 mm in acupuncture points selected in somatic segments common to the innervation of the ovary and uterus (Th12-L2, S2-S4) (27). The location and stimulation of the needles were the same in all women (Table I). The needles (Hegu: Hegu AB Landsbro, Sweden) were made of stainless steel and were inserted and rotated to evoke 'needle sensation', often described as variable feelings of tension, numbness, tingling and soreness and reflecting activation of muscle-nerve afferents (A-delta fibers and possibly C-fibers). Four needles at the thoracolumbal and sacral level and four needles in the calf muscles were then attached to an electrical stimulator (WQ-6F: Wilkris & Co. AB, Stockholm, Sweden) and stimulated with low frequency (2 Hz) pulses of 0.5 ms duration for 30 minutes. The intensity was sufficient to cause non-painful local muscle contractions. Manually stimulated needles were rotated five times during each treatment session (Table I). All the acupuncture treatments were given by two of the authors (E S-V and UT).

Biochemical assays

Blood samples were drawn from an antecubital vein on three occasions;

1) within 1 week before the first EA treatment,
2) within 1 week after the last EA treatment and
3) 3 months later.

Serial sampling for LH and cortisol determinations were done with 30-minutes intervals during 4 hours (8 samples). LH and cortisol values are the mean of the serial samplings from each woman.

Serum LH and follicle stimulating hormone (FSH) were determined by immunoenzymometric assays (LH and FSH: AxSYM system; prolactin, IMx® system, Abbott Laboratories, Diagnostics Division, Abbott Park, IL, USA; upper reference limit for serum prolactin is 350 mIU/l). Serum testosterone was determined by a non-extraction competitive radioimmunoassay (RIA) using RSL 125I Testosterone (DA, ICN Pharmaceuticals, Inc., Costa Mesa, CA, USA; upper reference limit 3 nmol/l). Serum androstenedione was determined by a non-extraction competitive RIA (Coat-A-Count Direct Androstenedione, Diagnostic Products Corp, Los Angeles, CA, USA; reference intervals: age 20–29, 4.2–14.7 nmol/l; age 30–39, 21.7–7.7 nmol/l). Serum sex-hormone binding globulin (SHBG) was determined by using an immunoradiometric assay (IRMA) (Orion Diagnostica, Finland; reference interval 30–90 nmol/l). Serum cortisol was determined by RIA using Farmos Diagnostica RIA (Farmos Diagnostica, Espoo, Finland; reference interval: 200–800 nmol/l). Fasting serum insulin was determined by RIA using Pharmacia Insulin RIA 100 (Pharmacia, Sweden; reference interval <20 mU/l). Serum thyrotropin (TSH) were determined by immunoluminometric assay (Lumitest TSH, Brahms Diagnostica, Berlin, Germany; reference intervals: nonsmokers 0.4–4.0 mIU/l, smokers 0.1–4.0 mIU/l). Total T4 and T3 were determined by double antibody RIA:s (Diagnostic Products Corp, Los Angeles, CA, USA; reference intervals: T3, 1.5–3.0 nmol/l; T4, 60–160 nmol/l).

Blood samples for analyses of regulatory peptides were collected in prechilled tubes containing EDTA and centrifuged at 2200 $\times$g for 15 min at +4°C within 1 hour. The plasma was separated and stored at −20°C. For determination of the various peptide concentrations using RIA, the samples were analyzed in serial dilutions optimized to linear part of the standard curve and corrected for nonspecific

Table I. Acupuncture points, their anatomical position and their innervation

Points	Stimulation	Segmental innervation	Muscle localization
BL 23 (bilateral)	EA	C6–8, Th9–12, L1–3	Fascia thoracolumbalis, mm. serratus posterior, erector spinae thoracolumbalis
BL 28 (bilateral)	EA	L4–5, S1–3	Fascia thoracolumbalis, m. erector spinae lumbosacralis
SP 6 (bilateral)	EA	L4–5, S1–2	Mm. flexor digitorum longus, tibialis posterior
SP 9 (bilateral)	EA	S1–2	M. gastrocnemius
PC 6 (unilateral)	manual	C8–Th1	M. flexor digitorum superficialis
TE 5 (unilateral)	manual	C7–8	M. extensor digiti minimi
GV 20	manual	Nn. trigeminus (V), occipitalis minor (C2) and major (C2–3)	Aponeurosis epicranii

BL=bladder channel. SP=spleen channel. PC=pericardium channel. TE=triple energizer channel. GV=governor vessel.

binding. Total interassay coefficient of variation, detection limit and normal reference intervals were: immunoreactive calcitonin gene-related peptide (CGRP) <12%, 10 pmol/l and <40 pmol/l, respectively; immunoreactive β-endorphin (negligible cross-reactivity against β-lipotropin i.e. <1.5%) <10%, 10 pmol/l and 30–45 pmol/l, respectively; immunoreactive neuro peptide Y (NPY), <7%, 12 pmol/l and <130 pmol/l, respectively; immunoreactive galanin, <10%, 10 pmol/l and 70–110 pmol/l, respectively; immunoreactive gastrin (antibody no. 7835, from Dr. J Rehfeld, Rikshospitalet, Copenhagen, Denmark), <8%, 5 pmol/l, <50 pmol/l, respectively. Adrenocorticotropic hormone (ACTH) was determined by immunoradiometric assay (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA), reference interval 2.0–11.5 pmol/l.

Skin temperature

The skin temperature was measured with a digital infrared thermometer (Microscanner D-series: Exergen, Watertown, Mass., USA). The accuracy of this self-calibrating equipment is ±0.3°C and the reliability is ±0.1°C. The recording sites were between the applied acupuncture needles in the sacrum and in the forehead, both in the midline. The measurements were made during the first, fifth and tenth EA treatments. The first measurements were made after 10 minutes' rest and just before EA. These were the 'baseline' values. Thereafter, further measurements were made every seventh minute during EA and one immediately after EA. The room temperature was constant during the three experimental sessions.

BMI and WHR

Measurements of body-mass index (the weight in kilograms divided by the square of the height in meters=BMI) and waist-to-hip circumference (waist hip ratio=WHR) measured with a soft tape, at the level of the umbilicus and the spina iliaca anterior superior with the women in the standing position, were made before the EA treatments and after the study period.

Study definitions

The study period was defined as the period extending from 3 months before the first EA treatment, to 3 months after the last EA treatment, in total 8–9 months.

The menstrual and ovulation patterns were confirmed by recording of vaginal bleedings and by daily registrations of the basal body temperature (BBT) throughout the entire study period.

The outcome in terms of ovulation pattern was measured as the difference between the time periods

1) 3 months before EA on the one hand, and

2) the treatment period plus the 3 months following on the other hand.

A woman was defined as having experienced a good effect if the BBT disclosed repeated ovulations (or pregnancy) during the treatment period and in the following 3 months. A woman was defined as having experienced no effect if the ovulation pattern did not differ before, during or after treatment.

Statistics

Fisher's permutation test was used for group comparison (good effect versus no effect) regarding BMI, WHR, hormones, steroids, neuropeptides and skin temperature. Fisher's test for paired comparison was used for analysis of difference before EA versus within a week after EA and 3 months after EA in

a) all women,

b) in women with good effect and

c) in women with no effect regarding ovulation, hormones, steroids and neuropeptides.

All tests were two-tailed and differences were considered to be statistically significant when $p<0.05$. The confidence interval (CI) was given when $p<0.05$.

Results

Ovulations

Of the twenty-four women, nine had experienced a good effect (38%) and fifteen no effect (62%). Of the nineteen women who were clomiphene resistant, seven had experienced a good effect (37%) and twelve no effect (63%).

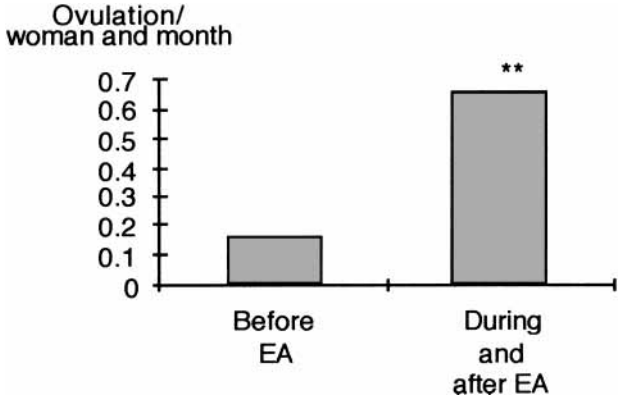

Fig. 1. Comparisons of the number of ovulations per woman and month in the period before EA (3 months) with the period during and after EA (5–6 months) in nine women with good effect. $^{**}p=0.004$ and CI=2.0, 4.0.

Table II. Mean and standard deviation (s.d.) for serum and plasma concentrations of gonadotropins, prolactin, steroids and neuropeptides from samples taken before, within a week after and 3 months after EA treatment in all women (n=24). * and ** indicate significance when comparing concentrations before EA with concentrations within a week after and 3 months after EA treatment

	Before EA		Within a week after EA		3 months after EA		Significance
	mean	(s.d.)	mean	(s.d.)	mean	(s.d.)	p (CI)
LH (IU/l)	9.7	(4.0)	9.7	(3.6)	8.2	(3.9)	n.s.
FSH (IU/l)	5.5	(1.3)	5.6	(1.3)	5.5	(1.6)	n.s.
LH/FSH ratio	1.7	(0.6)	1.9	(1.2)	1.47	(0.5)*	p=0.042 (-0.51, -0.01)
Prolactin (mIU/l)	154	(68.9)	166	(87.1)	181	(79.4)**	p=0.010 (9.6, 66.7)
SHBG (nmol/l)	29	(15.9)	27	(14.4)	25	(13.2)	n.s.
Testosterone (nmol/l)	1.88	(0.89)	1.83	(0.85)	1.78	(0.82)*	p=0.016 (−0.43, −0.05)
Testosterone/SHBG ratio	0.09	(0.07)	0.09	(0.07)	0.10	(0.07)	n.s.
Androstenedione (nmol/l)	9.1	(2.6)	9.3	(2.4)	9.3	(2.4)	n.s.
Cortisol (nmol/l)	272	(66.9)	265	(47.8)	241	(75.8)	n.s.
TSH (mIU/l)	1.6	(1.1)	2.0	(1.3)	1.9	(1.7)	n.s.
T3 (nmol/l)	1.4	(0.3)	1.4	(0.2)	1.3	(0.2)	n.s.
T4 (nmol/l)	91	(15.4)	90	(10.1)	89	(10.6)	n.s.
Basal insulin (mIU/l)§	16	(9.0)	19	(9.6)	28	(15.7)	n.s.
β-endorphin (pmol/l)	48	(18.1)	46	(17.8)	43	(17.4)*	p=0.013 (−11.6, −1.6)
ACTH (pmol/l)	3.9	(2.5)	4.3	(2.8)	1.7	(0.4)	n.s.
CGRP (pmol/l)	35	(46.9)	23	(30.7)	31	(35.1)	n.s.
Galanin (pmol/l)	109	(40.4)	114	(47.9)	118	(41.6)	n.s.
NPY (pmol/l)	146	(15.4)	147	(17.7)	144	(15.8)	n.s.
Gastrin (pmol/l)	39	(14.2)	38	(14.0)	38	(13.4)	n.s.

§ (n=10). * p<0.05. ** p≤0.01.

The group of nine women on whom EA had had a good effect displayed a total of 4 ovulations in the period of three months (in total 27 months) before EA treatments (0.15 ovulations/woman and month). This increased to 31 ovulations in the period during and after EA treatments (in total 47 months) (0.66 ovulations/woman and month) (Fig. 1). The difference was significant (p=0.004, CI=2.0, 4.0). The number of ovulations per woman and month did not change in the group with no effect.

BMI and WHR

Compared to the group of women with no effect, the group of women with good effect had a significantly lower BMI [32.02±5.37 versus 22.67±2.64 (M±s.d.) (p<0.001, CI=−13.3, −5.4)] and WHR [0.89±0.07 versus 0.81±0.06 (M±s.d.) (p=0.0058, CI=−0.14, −0.02)]. The BMI and WHR did not, however, change in either group during the study period.

Gonadotropins, prolactin, steroids and neuropeptides

When analyzing all included women together and comparing blood samples before the EA period with the samples 3 months after the EA period, a significant increase in mean prolactin concentrations (p=0.022, CI=9.6, 66.7) and a significant decrease in mean LH/FSH ratios (p=0.042, CI=−0.51, −0.01), in mean testosterone concentrations (p=0.016, CI=−0.43, −0.05) and in mean β-endorphin concentrations (p=0.013, CI=−11.6, −1.6) were found (Table II). No other significant changes could be detected when comparing all the women together.

Before EA, the group of women having experienced a good effect had significantly higher mean SHBG concentrations (p=0.040, CI=9.5, 32.3) and significantly lower mean testosterone concentrations (p=0.0036, CI=−1.6, −0.2), testosterone/SHBG ratios (p=0.011, CI=−0.14, −0.02) and mean basal insulin concentrations (p=0.0054, CI=−24.0, −6.5) than did the group of women who had experienced no effect (Table III).

Comparisons of the blood samples taken before EA and 3 months after EA showed that only in the group of women with good effect did the mean prolactin concentrations increase significantly (p=0.047, CI=38.3, 167.1) (Table III).

Comparisons of the blood samples taken before EA and within 1 week after EA showed that, in the group of women with no effect, the mean testosterone concentrations decreased significantly (p=0.044, CI=−0.35, −0.01) and mean TSH concentrations increased significantly (p=0.011, CI=0.1, 0.9) (Table III).

No other significant changes were found when

Table III. Mean and standard deviation (s.d.) for serum and plasma concentrations of gonadotropins, prolactin, steroids and neuropeptides from samples taken before, within a week after and 3 months after EA treatment, according to response

		Before EA		After EA		3 months after EA		Significance and CI before EA *vs* within a week after EA and 3 months after EA	Significance and CI good effect *vs* no effect before EA
	Effect§	mean	(s.d.)	mean	(s.d.)	mean	(s.d.)	*p* (CI)	*p* (CI)
LH (IU/l)	*GE*	11.8	(4.5)	9.9	(2.7)	7.5	(3.8)	n.s.	n.s.
	NE	8.8	(3.4)	9.7	(4.1)	8.5	(4.1)	n.s.	
FSH (IU/l)	*GE*	5.7	(1.8)	5.8	(1.4)	5.1	(2.0)	n.s.	n.s.
	NE	5.4	(1.1)	5.9	(1.1)	5.7	(1.3)	n.s.	
LH/FSH ratio	*GE*	1.9	(0.8)	2.4	(1.8)	1.5	(0.6)	n.s	n.s.
	NE	1.6	(0.6)	1.7	(0.7)	1.4	(0.5)	n.s.	
Prolactin (mIU/l)	*GE*	123	(47.1)	179	(66.0)	212	(93.8)*	p=0.018 (38.3, 167.1)	n.s.
	NE	170	(74.6)	164	(99.2)	178	(85.2)	n.s.	n.s.
SHBG (nmol/l)	*GE*	43	(16.2)**	39	(15.8)	36	(15.3)	n.s.	p=0.0026 (9.5, 32.3)
	NE	22	(10.2)	21	(8.7)	20	(8.9)	n.s.	
Testosterone (nmol/l)	*GE*	1.3	(0.5)**	1.5	(0.9)	1.3	(0.8)	n.s.	p=0.0098
	NE	2.2	(0.9)	2.0	(0.8)*	2.0	(0.8)	p=0.044 (−0.35, 0.01)	(−1.6, −0.2)
Testosterone/SHBG	*GE*	0.04	(0.05)*	0.05	(0.06)	0.05	(0.05)	n.s.	p=0.011
ratio	*NE*	0.12	(0.06)	0.11	(0.06)	0.12	(0.07)	n.s.	(−0.14, −0.02)
Androstenedione	*GE*	7.9	(1.7)	8.5	(2.2)	8.3	(2.6)	n.s.	n.s.
(nmol/l)	*NE*	9.7	(2.8)	9.8	(2.4)	9.8	(2.3)	n.s.	
Cortisol (nmol/l)	*GE*	293	(88.0)	271	(51.2)	267	(86.5)	n.s.	n.s.
	NE	262	(54.6)	262	(47.8)	231	(71.7)	n.s.	
TSH (mIU/l)	*GE*	1.4	(1.0)	1.4	(0.5)	1.1	(0.3)	n.s.	n.s.
	NE	1.8	(1.2)	2.3	(1.5)*	2.2	(1.9)	p=0.011 (0.1, 0.9)	
T3 (nmol/l)	*GE*	1.4	(0.4)	1.2	(0.2)	1.2	(0.2)	n.s.	n.s.
	NE	1.4	(0.2)	1.4	(0.2)	1.4	(0.2)	n.s.	
T4 (nmol/l)	*GE*	93	(21.1)	88	(7.7)	86	(6.9)	n.s.	n.s.
	NE	90	(11.4)	91	(11.4)	91	(12.2)	n.s.	
Basal insulin	*GE*	6	(1.5)**	7	(1.6)	7	(1.1)	n.s.	p=0.0054
(mIU/l)§§	*NE*	21	(6.3)	23	(7.2)	34	(12.0)	n.s.	(−24.0, −6.5)
β-endorphin (pmol/l)	*GE*	39	(12.5)	34	(10.2)	30	(7.2)	n.s	n.s.
	NE	54	(18.8)	52	(18.4)	49	(17.5)	n.s.	
ACTH (pmol/l)	*GE*	3.5	(1.3)	3.5	(1.4)	3.53	(1.0)	n.s.	n.s.
	NE	4.1	(3.1)	4.7	(3.3)	3.5	(1.9)	n.s.	
Galanin (pmol/l)	*GE*	93	(19.9)	88	(17.2)	93	(19.7)	n.s.	n.s.
	NE	119	(46.5)	128	(55.1)	129	(45.3)	n.s.	
NPY (pmol/l)	*GE*	143	(13.9)	141	(17.4)	135	(14.7)	n.s.	n.s.
	NE	148	(16.4)	151	(16.8)	148	(14.6)	n.s.	
Gastrin (pmol/l)	*GE*	38	(12.7)	36	(11.3)	35	(11.9)	n.s.	n.s.
	NE	39	(15.5)	39	(15.4)	38	(14.5)	n.s.	

§ *GE*=good effect, *NE*=no effect. §§ (*GE*; n=3 *NE*; n=7). * $p<0.05$.

values were compared within each of the effect groups.

Skin temperature

Zero time (0 minutes) was chosen to be after 10 minutes of rest before the start of an EA treatment, and the skin temperature at this time was referred to as the baseline temperature. During the EA treatments the skin temperature on the forehead increased significantly during all three experimental sessions, but there were no significant changes in the temperature of the skin covering the sacrum (Fig. 2).

Discussion

The main finding of the present study is that repeated EA treatments with low frequency (2 Hz)

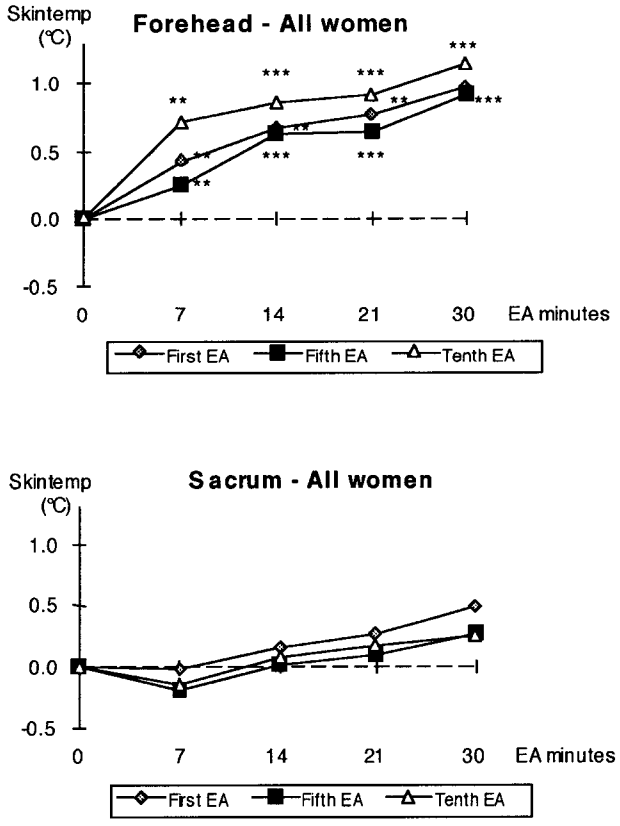

Fig. 2. The mean values of skin temperature (°C) changes in the forehead and sacral regions in all women during the first, fifth and tenth EA. *$p<0.05$, **$p<0.01$ and ***$p<0.001$ indicate the level of significance when compared with the baseline (dotted line).

induce regular ovulations in more than one third of the women with PCOS. In addition, it was possible to identify a distinct subgroup of PCOS women who responded well to EA. They were almost consistently characterized by comparatively low BMI, WHR, basal insulin and testosterone serum concentrations, but high SHBG serum concentration. Consequently, they were less androgenic and had a less pronounced metabolic disturbance. Also, these women had a significant increase in serum prolactin in response to EA. Except for one woman, all of the women with good effect had responded already during the EA period. Interestingly, in two of the women considered to have experienced a good effect, the ultrasonographic pictures taken of the ovaries 3 months after the EA period showed a disappearance of the multifollicular pattern.

In severe PCOS, the LH/FSH ratio is high, as are the concentrations of testosterone and β-endorphins. Interestingly, in the present study the LH/FSH ratio, the testosterone and β-endorphin concentrations decreased significantly following EA. Also, EA significantly increased the skin temperature on the foreheads of the PCOS women. It is thus possible that additional EA treatments would result in a higher overall success rate.

The results of the present study suggest that EA effects ovulation in women with PCOS. The exact mechanism behind this effect is unknown. Two hypotheses of the etiology of PCOS have emerged involving either an insufficient central β-endorphin inhibition of GnRH release or hyperactivity in the peripheral sympathetic neurons innervating the ovaries. Are there plausible explanations for the effects obtained with EA?

In acupuncture both physiological and psychological mechanisms may be involved. The EA stimuli excite mechanoreceptors with low and high thresholds in muscles and other tissues. Particular significance has been given to a group of receptors, denoted ergoreceptors, (28–30) found in muscles and physiologically excited during muscle contractions. It can be argued that EA and physical exercise with repetitive muscle contractions similarly activate these receptors and afferents. Both EA and muscle exercise result in the release of β-endorphin via two different systems (22). One system includes the hypothalamus and neuronal network that projects to the midbrain and brainstem nuclei; via this route it exerts an inhibitory effect on the vasomotor center resulting in a decreased sympathetic tone (20, 31–34). Via another system, β-endorphin is released in the blood in equimolar amounts to adrenocorticotrophic hormone (ACTH) (35). The two β-endorphinergic systems operate independently, (36) but both can be stimulated by afferent nerve activity. The described findings with regard to the central β-endorphinergic release and the decreased sympathetic tone support the idea that EA may be effective in PCOS (20, 22, 25, 37–43).

As pharmacological induction of ovulation in women with PCOS is associated with negative side effects, alternative or complementary methods are needed. Although the details are unknown, experimental and clinical evidence suggests that EA can reset the sympathetic system via β-endorphinergic mechanisms at the hypothalamic and brainstem levels. We propose that it is reasonable to believe that ovulation induction by EA does not cause serious side-effects or multiple pregnancies.

Interestingly, seven out of nine women who had experienced a good effect were clomiphene resistant, indicating a possibility that EA might serve as an alternative to second line therapies (ovarian drilling and gonadotropin treatment) in ovulation induction.

It is obvious to the authors that randomized, comparative studies are needed to verify the results of this study in conjunction with ultrasound to determine precisely what is happening at the

ovarian level and to exclude nonspecific effects. However, even if all of the effects were to be attributed to nonspecific effects, the results are impressive.

Considering all of these facts together, we suggest that EA may be an alternative or a complement to pharmacological induction of ovulation in women with PCOS who have a minor metabolic disturbance.

Acknowledgments

This study was supported by grants from the Hjalmar Svensson Foundation, Wilhelm och Martina Lundgrens Vetenskapsfond [Wilhelm and Martina Lundgren's Science Fund], Fonden för studerande av läkarvetenskapen vid Sahlgrenska sjukhuset [the Fund for Medical Science Students at Sahlgrenska University Hospital] and the Foundation for Acupuncture and Alternative Biological Treatment Methods, Swedish Medical Research Council (Project no. 4982). We would like to thank Professor Rolf Ekman, Department of Neurochemistry, Göteborg University, for constructive discussions and help with analyses of neuropeptides. Professor Sven Andersson, Department of Physiology, Göteborg University, is gratefully acknowledged for valuable suggestions. The authors would also like to thank Birgitta Josefsson, Elisabeth Bergqvist and Ingrid Jansson for invaluable help with the recruitment of patients and the collection of blood samples.

References

1. Dahlgren E, Janson PO. Polycystic ovary syndrome–long-term metabolic consequences. Int J Gynaecol Obstet 1994; 44 (1): 3–8.
2. Franks S. Polycystic ovary syndrome. N Engl J Med 1995; 333 (13): 853–61.
3. Knobil E, Hotchkis J. The physiology of reproduction. Knobil E, Neill JD, eds. The menstrual cycles and its neuroendocrine control. New York: Raven Press, 1988.
4. Rebar R, Judd HL, Yen SS, Rakoff J, Vandenberg G, Naftolin F. Characterization of the inappropriate gonadotropin secretion in polycystic ovary syndrome. J Clin Invest 1976; 57 (5): 1320–9.
5. Baird DT, Corker CS, Davidson DW, Hunter WM, Michie EA, Van Look PF. Pituitary-ovarian relationships in polycystic ovary syndrome. J Clin Endocrinol Metab 1977; 45 (4): 798–801.
6. Yen SS. The polycystic ovary syndrome. Clin Endocrinol (Oxf) 1980; 12 (2): 177–207.
7. McKenna TJ. Pathogenesis and treatment of polycystic ovary syndrome. N Engl J Med 1988; 318 (9): 558–62.
8. Dahlgren E, Janson PO, Johansson S, Lapidus L, Lindstedt G, Tengborn L. Hemostatic and metabolic variables in women with polycystic ovary syndrome [see comments]. Fertil Steril 1994; 61 (3): 455–60.
9. Holte J, Bergh T, Gennarelli G, Wide L. The independent effects of polycystic ovary syndrome and obesity on serum concentrations of gonadotrophins and sex steroids in premenopausal women. Clin Endocrinol (Oxf) 1994; 41 (4): 473–81.
10. Pasquali R, Casimirri F, Venturoli S et al. Body fat distribution has weight-independent effects on clinical, hormonal, and metabolic features of women with polycystic ovary syndrome. Metabolism 1994; 43 (6): 706–13.
11. Lobo RA, Granger LR, Paul WL, Goebelsmann U, Mishell DR, Jr. Psychological stress and increases in urinary norepinephrine metabolites, platelet serotonin, and adrenal androgens in women with polycystic ovary syndrome. Am J Obstet Gynecol 1983; 145 (4): 496–503.
12. Lobo RA. The role of neurotransmitters and opioids in polycystic ovarian syndrome. Endocrinol Metab Clin North Am 1988; 17 (4): 667–83.
13. Genazzani AR, Genazzani AD, Volpogni C et al. Opioid control of gonadotrophin secretion in humans. Hum Reprod 1993; 8 Suppl 2: 151–3.
14. Jenkins PJ, Grossman A. The control of the gonadotrophin releasing hormone pulse generator in relation to opioid and nutritional cues. Hum Reprod 1993; 8 Suppl 2: 154–61.
15. Wildt L, Petermann Sir T, Leyendecker G, Waibel Treber S, Rabenbauer B. Opiate antagonist treatment of ovarian failure. Hum Reprod 1993; 8 Suppl 2: 168–74.
16. Carmina E, Ditkoff EC, Malizia G, Vijod AG, Janni A, Lobo RA. Increased circulating levels of immunoreactive beta-endorphin in polycystic ovary syndrome is not caused by increased pituitary secretion. Am J Obstet Gynecol 1992; 167 (6): 1819–24.
17. Barria A, Leyton V, Ojeda SR, Lara HE. Ovarian steroidal response to gonadotropins and beta-adrenergic stimulation is enhanced in polycystic ovary syndrome: role of sympathetic innervation. Endocrinology 1993; 133 (6): 2696–703.
18. Lara HE, Ferruz JL, Luza S, Bustamante DA, Borges Y, Ojeda SR. Activation of ovarian sympathetic nerves in polycystic ovary syndrome. Endocrinology 1993; 133 (6): 2690–5.
19. Franks S, Adams J, Mason H, Polson D. Ovulatory disorders in women with polycystic ovary syndrome. Clin Obstet Gynecol 1985; 12 (3): 605–32.
20. Andersson S. The functional background in acupuncture effects. Scand J Rehabil Med Suppl 1993; 29: 31–60.
21. Debreceni L. Chemical release associated with acupuncture and electric stimulation. Critical Reviews in Physical and rehabilitation Medicine 1993; 5 (3): 247–75.
22. Andersson S, Lundeberg T. Acupuncture – from empiricism to science: functional background to acupuncture effects in pain and disease. Med Hypotheses 1995; 45 (3): 271–81.
23. Chen BY, Yu J. Relationship between blood radioimmunoreactive beta-endorphin and hand skin temperature during the electro-acupuncture induction of ovulation. Acupunct Electrother Res 1991; 16 (1–2): 1–5.
24. Chen BY. Acupuncture normalizes dysfunction of hypothalamic-pituitary-ovarian axis. Acupunct Electrother Res 1997; 22 (2): 97–108.
25. Stener Victorin E, Waldenstrom U, Andersson SA, Wikland M. Reduction of blood flow impedance in the uterine arteries of infertile women with electro-acupuncture. Hum Reprod 1996; 11 (6): 1314–17.
26. Adams J, Polson D, Franks S. Prevalence of polycystic ovaries in women with anovulation and idiopathic hirsutism. Br Med J 1986; 293: 355–9.
27. Bonica J. The Management of Pain. (2nd ed.) Philadelphia, London: Lea & Febiger, 1990. vol. 1.
28. Kniffki KD, Mense S, Schmidt RF. Muscle receptors with fine afferent fibers which may evoke circulatory reflexes. Circ Res 1981; 48 (suppl. I): 125–31.
29. Kaufman MP, Longhurst JC, Rybycki J, Wallach JH, Mitchell JH. Effects of static muscular contraction on impulse activity of groups III and IV muscle afferents. J Appl Physiol 1983; 55: 105–12.
30. Kaufman MP, Waldrop TG, Rybycki KJ, Ordway GA, Mitchell JH. Effects of static and rhythmic twitch contractions on the discharge of group III and IV muscle afferents. Cardiovasc Rec 1984; 18: 663–8.
31. Bloom FE. A growing family of pharmacologically perti-

nent peptides. Ann Rev Pharmacol Toxicol 1983; 23: 151–70.
32. Cuello AC. Central distribution of opioid peptides. Br Med Bull 1983; 39: 11–16.
33. Smyth DG. Beta-endorphin and related peptides in pituitary, brain, pancreas and antrum. Br Med Bull 1983; 39: 25–30.
34. Akil H, Watson SJ, Young E, Lewis ME, Khachaturian H, Walker JM. Endogenous opioids: biology and function. Ann Rev Neurosci 1984; 7: 223–55.
35. Crine P, Gianoulakis C, Seidah NG. Biosynthesis of beta-endorphin from beta-lipotropin and a larger molecular weight precursor in rat pars intermedia. Proc Natl Acad Sci USA 1978; 75 (10): 4719–23.
36. Rossier J, French ED, Rivier C, Ling N, Guillemin R, Bloom FE. Foot-shock induced stress increases beta-endorphin levels in blood but not brain. Nature 1977; 270: 618–20.
37. Yao T, Andersson S, Thoren P. Long-lasting cardiovascular depression induced by acupuncture-like stimulation of the sciatic nerve in unanaesthetized spontaneously hypertensive rats. Brain Res 1982; 240 (1): 77–85.
38. Kaada B. Vasodilatation induced by transcutaneous nerve stimulation in peripheral ischemia (Raynaud's phenomenon and diabetic polyneuropathy). Eur Heart J 1982; 3: 303–14.
39. Cao XD, Xu SF, Lu WX. Inhibition of sympathetic nervous system by acupuncture. Acupunct Electrother Res 1983; 8: 25–35.
40. Hoffman P, Thorén P. Long-lasting cardiovascular depression induced by acupuncture-like stimulation of the sciatic nerve in unanaesthetized rats. Effects of aurousal and type of hypertension. Acta Physiol Scand 1986; 127: 119–26.
41. Jansen G, Lundeberg T, Kjartansson J, Samuelsson UE. Acupuncture and sensory neuropeptides increase cutaneous blood flow in rats. Neuroscience letter 1989; 97: 305–9.
42. Hoffman P, Terenius L, Thorén P. Cerebrospinal fluid immunoreactive beta-endorphin concentrations is increased by long-lasting voluntary exercise in the spontaneously hypertensive rat. Regul Pept 1990b; 28: 233–9.
43. Sato A, Sato Y, Schmidt RF. The Impact of Somatosensory Input on Autonomic Functions. Heidelberg: Springer-Verlag, 1997. Physiology, Biochemistry and Pharmacology. pp. 73–95.

Address for correspondence:

Elisabet Stener-Victorin, R.P.T.
Department of Obstetrics and Gynecology
Kvinnokliniken
Sahlgrenska University Hospital
SE-413 45 Göteborg
Sweden