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Prolactin and growth hormone secretion after thyrotrophin-releasing hormone infusion and dopaminergic (DA2) blockade in infertile patients with minimal/mild endometriosis

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BACKGROUND: The origin of infertility in patients with endometriosis without tubal occlusion has not yet been clearly defined. Several reports show an abnormal pituitary–ovarian axis in this group of patients. Moreover, prolactin (PRL) and growth hormone (GH) secretion is closely related to reproductive status. This study aimed to evaluate PRL and GH secretion after metoclopramide and thyrotrophin-releasing hormone (TRH) infusion in infertile patients with minimal/mild endometriosis. METHODS: A total of 64 women participated in the study: 33 fertile patients without endometriosis; 10 fertile patients with minimal/mild endometriosis; and 21 infertile patients with minimal/mild endometriosis. TRH or metoclopramide was administered randomly in two sequential menstrual cycles (cycle days 3–5). Serum PRL and GH secretion before and after dopaminergic type 2 (DA2) receptor blockade and TRH were compared. RESULTS: Higher serum PRL levels were observed in patients with endometriosis at baseline and after 15 and 30 min of TRH administration. Also, infertile patients with endometriosis had lower serum estradiol levels than fertile patients. Moreover, the dopaminergic blockade did not result in abnormal PRL or GH secretion. CONCLUSIONS: Decreased serum estradiol levels and altered PRL secretion after TRH administration in infertile patients with minimal/mild endometriosis are related to ovulatory dysfunction and infertility in this group of patients without tubal occlusion.

Key words: endometriosis/GH secretion/infertility/prolactin secretion

Introduction

Endometriosis, a disease which has been associated with infertility, is estimated to affect 10–15% of the female population (Olive and Schwartz, 1993). Patients with moderate or severe endometriosis have an anatomical basis for their infertility, whereas the origin of infertility in patients with minimal or mild endometriosis has not been clearly defined (Olive and Haney, 1986; Candiani *et al*., 1991; Inoue *et al*., 1992).

Serum prolactin (PRL) and growth hormone (GH) levels and secretion are closely related to reproductive status. PRL has a pulsatile and circadian cycle, and the main regulatory mechanism for PRL secretion is the inhibitory action of dopamine (Kauppila *et al*., 1986; Schlaff, 1986; Veldhuis *et al*., 1989; Katz *et al*., 1993). The pattern of PRL secretion can be studied by inhibiting dopaminergic mechanisms, or by stimulating the release of PRL by means of a TRH stimulation test (Kauppila *et al*., 1986; Frohman, 1995).

A hormonal alteration of PRL secretion could be the origin of infertility in patients with endometriosis (Cunha-Filho *et al*., 2001), affecting their oocyte maturation and folliculogenesis (Cahill and Hull, 2000). Although some authors demonstrated that their infertile endometriotic patients had normal basal serum PRL levels, these levels were altered following a TRH stimulation test, suggesting an association between this altered secretion pattern and these patients' infertility (Muse *et al*., 1982; Gregoriou *et al*., 1999). However, this association is disputed by others (Panidis *et al*., 1992; Matalliotakis *et al*., 1996).

Patients who present luteal phase defects (Archer, 1987) or infertility (Asukai *et al*., 1993) but have normal serum levels of PRL have shown an exaggerated response to the thyrotrophin-releasing hormone (TRH) stimulation test or to metoclopramide administration (dopaminergic blockade). In such patients, this abnormal PRL secretion reveals an otherwise unknown state of hyperprolactinaemia, which could be the cause of infertility (Steinberger *et al*., 1990; Asukai *et al*., 1993; Kostal and Tosner, 1997).

The regulation of GH secretion is very controversial; it is

modulated by various mechanisms and neurosubstances (Devesa *et al*., 1992). In some conditions, such as acromegaly and polycystic ovarian syndrome (PCOS), GH secretion is impaired after TRH stimulation (Kaltsas *et al*., 1999).

Anovulation and infertility could be related to the altered secretion of GH, as proposed by Ovesen (Ovesen *et al*., 1994). GH may act as a co-gonadotrophin, and the literature supports the notion that the somatotrophic axis is associated with the reproductive process and with gonadal function (Mason *et al*., 1990; Katz *et al*., 1993; Rossato *et al*., 2000).

Therefore, the aim of the present study was to investigate a subtle alteration in PRL and GH levels after dopaminergic type 2 (DA2) blockade and TRH infusion in infertile patients with minimal or mild endometriosis, and to relate this physiological event to infertility.

Materials and methods

Design

A case–control study was designed to analyse the relationship between infertility in endometriosis and an altered PRL and GH secretion pattern. A total of 64 patients were studied between March 1997 and June 2000. They were selected among the patients seen at the Gynecological Clinic, Hospital de Clínicas de Porto Alegre (HCPA), and were divided into three groups, according to the presence of infertility and/or endometriosis.

Patients

Group 1 (control group) consisted of 33 patients without endometriosis who underwent laparoscopy for tubal ligation. Group 2 consisted of 10 patients with minimal/mild endometriosis who were submitted to laparoscopy for tubal ligation. Patients in groups 1 and 2 had proven spontaneous fertility within the previous 2 years. Group 3 was formed by 21 patients with minimal/mild endometriosis and infertility who were submitted to laparoscopy during infertility investigation. Endometriosis was diagnosed and categorized in all patients following the classification proposed by the American Society for Reproductive Medicine (American Fertility Society, 1985) during laparoscopy. All patients with endometriosis (groups 2 and 3) had their endometriotic foci cauterized during the laparoscopic procedure; the endoscopic procedure was done by the same investigator (J.S.C.F.).

Patients with previous endocrine disorders were excluded from the study, along with patients who were using drugs that could affect the parameters of the tests performed. Also excluded were patients with a history of allergic reaction to metoclopramide. Finally, we included only those patients whose minimal/mild endometriosis was the only abnormality associated with infertility. During their infertility evaluation they demonstrated regular menstrual cycles, day 3 FSH <8 IU/l, negative Chlamydia test, normal hysterosalpingography and tubal patency diagnosed by laparoscopy. In addition, their partners showed a normal sperm evaluation (World Health Organization, 1992).

All participants were informed of the procedures involved in the study and signed an informed consent form. The research project was approved by the Hospital's Ethics Committee, and followed the guidelines set by the Helsinki Declaration regarding human experimentation.

Hormonal assessment for analysis of PRL and GH secretion was performed in the early follicular phase (cycle days 3–5) on the first menstrual cycle following laparoscopy. Prior to dopaminergic (receptor type DA2) blockade with metoclopramide or TRH infusion, patients stayed at rest and were submitted to an 8 h fast. An i.v. catheter was placed in the antecubital vein 30 min before sample collection. After that, 10 mg of metoclopramide or 200 μg of TRH were administered i.v. Collections were made at 15 min intervals, for a total of six collections, at –15, 0, 15, 30, 45 and 60 min. TRH or metoclopramide was administered at random in two sequential menstrual cycles. The –15 and 0 min samples were used to measure estradiol, glucose, insulin, insulin-like growth factor (IGF)-1, thyroid-stimulating hormone (TSH), and basal serum PRL and GH levels.

All samples were centrifuged at 1270 *g* for separation of plasma, which was frozen at –20°C for later analysis. Hormones were analysed using chemiluminescence kits (Immulite Ltd, Los Angeles, CA, USA), IGF-1 was analysed with an immunoradiometric assay kit (Nichols Institute Diagnostics, San Juan Capistrano, USA), and glucose with the Glico-DH method (Merck Mega, Darmstadt, Germany). The largest inter- and intra-assay variations were 5.45 and 13.3% respectively for PRL; 15 and 16% for estradiol; 17.5 and 13.8% for TSH; 6.1 and 6.5% for GH; 7.6 and 4.3% for insulin; and 15.8 and 4.6% for IGF-1. The kits did not show a significant cross-reactivity between the hormones measured.

Statistical analysis

PRL and GH secretory patterns were compared within the same group by using a model for repeated measures (Friedman's test). Hormonal results for different groups were compared using the Kruskal–Wallis test. Dunn's post hoc test was performed to evaluate significant differences between the groups.

In order to exclude a confounding bias in the comparison of serum estradiol levels, we analysed the percentage of outliers and performed Levene's test for heterogeneity of variance.

The significance level was 0.05 (two-tailed).

Results

The relevant clinical and hormonal characteristics of the three groups are shown in Table I. Fertile patients with endometriosis were older than the patients in the other two groups. Infertile patients with endometriosis had lower serum estradiol levels than fertile patients with endometriosis. Fertile and infertile patients with endometriosis had higher baseline serum PRL levels than fertile patients without endometriosis. Body mass index (BMI), IGF-1, glucose, insulin and serum TSH levels were similar for the three groups (Table I).

The comparison of serum PRL levels after TRH infusion showed significant differences among the groups at 15 and 30 min ($P = 0.0001$ and $P = 0.036$ respectively). Fertile patients with endometriosis [median: 76.65 ng/ml; 95% confidence interval (CI): 26.60–142.70] and infertile patients with endometriosis (median: 72.30 ng/ml; 95% CI: 41.91–493.35) presented higher levels of PRL 15 min after TRH administration than fertile patients without endometriosis (median: 38.50 ng/ml; 95% CI: 13.54–120.50; $P < 0.05$, Dunn's post hoc procedure). This finding was confirmed after 30 min of TRH infusion: group 1 (median: 30.10 ng/ml; 95% CI: 14.71–119.00), group 2 (median: 66.25 ng/ml; 95% CI: 23.50–99.20) and group 3 (median: 52.70 ng/ml; 95% CI: 12.81–478.50; $P < 0.05$, Dunn's post hoc procedure) (Figure 1).

The analysis of PRL secretion after dopaminergic blockade showed no difference between the three groups. PRL secretion had a maximum response after 30 min, with a median (and

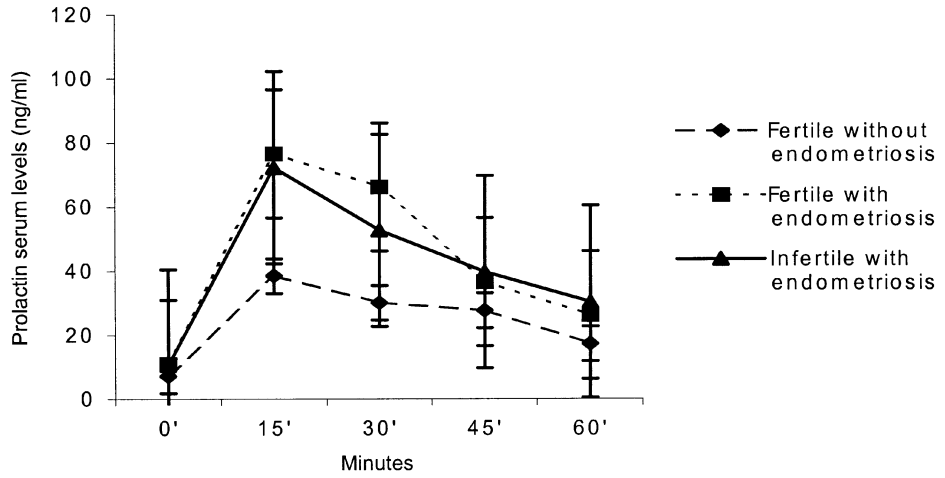

Figure 1. Serum prolactin levels after thyrotrophin-releasing hormone (TRH) administration (median ± SD). $P = 0.0001$ and $P = 0.036$ (Kruskal–Wallis's test), 15 and 30 min after TRH administration.

Table I. Clinical characteristics and serum hormone levels. Values are expressed as medians and 95% confidence intervals

	Fertile without endometriosis ($n = 33$)	Fertile with endometriosis ($n = 10$)	Infertile with endometriosis ($n = 21$)	*P*-value (Kruskal–Wallis test)
Age (years)	33 (26–43)	38 (33–39)	31 (23–40)	0.003
BMI (kg/m^2)	23.80 (17.06–32.13)	22.85 (20.50–28.02)	22.00 (17.51–28.68)	NS
Estradiol (pg/ml)	52.10 (22.17–218.90)	122.00 (40.00–269.20)	48.40 (23.67–157.80)	0.021
TSH (μIU/ml)	1.47 (0.45–5.66)	1.68 (0.26–5.64)	1.50 (0.53–3.47)	NS
Prolactin (ng/ml)	7.20 (3.27–17.29)	10.96 (3.25–11.96)	10.55 (3.96–33.02)	0.006
Glucose (mg/dl)	85.00 (70.60–107.10)	89.50 (71.00–95.50)	77.00 (69.00–96.20)	NS
Insulin (μIU/ml)	9.60 (2.00–19.50)	7.05 (4.00–16.35)	6.00 (2.10–18.02)	NS
IGF-1 (ng/ml)	306.73 (80.53–505.46)	272.92 (146.45–365.54)	285.38 (140.06–416.81)	NS

BMI = body mass index; TSH = thyroid-stimulating hormone; IGF-1 = insulin-like growth factor-1; NS = not significant.

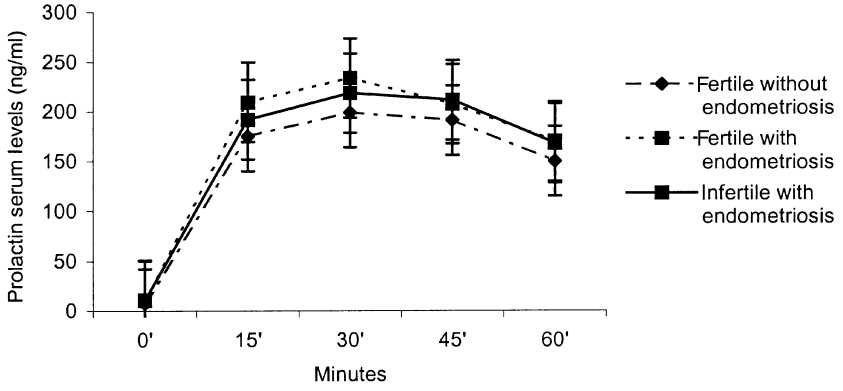

Figure 2. Serum prolactin levels after metoclopramide (DA2 blockade) infusion (median ± SD).

Table II. Growth hormone levels (ng/ml) after metoclopramide administration. Values are expressed as medians and 95% confidence intervals

	Fertile without endometriosis (n = 33)	Fertile with endometriosis (n = 10)	Infertile with endometriosis (n = 21)	P-value (Kruskal–Wallis test)
Basal	0.48 (0.13–2.60)	1.88 (1.20–2.10)	0.24 (0.21–2.60)	NS
15 min	0.50 (0.18–2.00)	1.80 (0.67–1.90)	0.52 (0.18–2.40)	NS
30 min	0.35 (0.17–1.30)	1.80 (0.20–2.00)	0.94 (0.21–2.00)	NS
45 min	0.60 (0.22–1.00)	1.95 (0.70–2.10)	0.74 (0.25–3.00)	NS
60 min	0.48 (0.18–1.40)	1.85 (0.40–2.40)	0.56 (0.29–3.90)	NS

NS = not significant.

Table III. GH levels (ng/ml) after thyrotrophin-releasing hormone infusion test. Values are expressed as medians and 95% confidence intervals

	Fertile without endometriosis (n = 33)	Fertile with endometriosis (n = 10)	Infertile with endometriosis (n = 21)	P-value (Kruskal–Wallis test)
Basal	0.43 (0.51–2.10)	1.65 (0.25–3.56)	1.20 (1.05–4.05)	NS
15 min	0.52[a] (0.44–1.24)	2.15[a] (0.88–3.03)	1.50 (0.99–3.84)	0.018
30 min	0.36[b] (0.34–0.78)	1.70[b] (0.73–2.11)	0.80 (0.57–2.02)	0.026
45 min	0.32 (0.24–0.71)	0.83 (0.38–1.73)	0.34 (0.27–1.05)	NS
60 min	0.27 (0.23–0.57)	0.47 (0.23–1.51)	0.28 (0.86–4.03)	NS

[a]NS (Dunn's post hoc procedure).
[b]$P < 0.05$ (Dunn's post hoc procedure).
NS = not significant.

95% CI) of 199.00 ng/ml (75.84–436.40) for group 1, 233.75 ng/ml (83.70–289.25) for group 2, and 218.50 ng/ml (18.20–476.40) for group 3 (Figure 2).

The secretion of GH after dopaminergic blockade was not different among the three groups (Table II). Significant differences in GH levels after TRH administration were observed among the three groups at 15 (P = 0.018) and 30 min (P = 0.026). However, post hoc testing at these two time points revealed significant differences ($P < 0.05$) between fertile patients with endometriosis and those without after 30 min only (Table III).

Levene's test, which was performed in order to exclude a possible confounding bias for serum estradiol comparisons, showed similar variances for all groups. According to this test, only one sample was considered to be an outlier.

Discussion

In the present study, we observed higher serum PRL levels in patients with endometriosis at baseline and after 15 and 30 min of TRH administration. Also, infertile patients with endometriosis had lower serum estradiol levels, and fertile women with endometriosis showed an altered GH secretion after TRH administration. Moreover, the dopaminergic blockade did not show an abnormal PRL or GH secretion. These results suggest the existence of a central PRL secretion dysfunction in patients with endometriosis, not involving the DA2 receptor system. As far as we know, no study has previously analysed the dopaminergic and TRH pathways in this particular group of infertile women as well as in fertile women with endometriosis.

We do not believe that the cauterization procedure for endometriosis treatment could have affected our results in terms of PRL changes, because even patients without endometriosis were submitted to cauterization for tubal ligation. Moreover, the dynamic function tests were carried out in the first menstrual cycle after the performance of cauterization.

Hirschowitz *et al*. were the first to describe a likely association between endometriosis and galactorrhoea (Hirschowitz *et al*., 1978). Other authors observed that infertile women with endometriosis had a basal PRL level twice as high as that of the control group, a difference that was not statistically significant. However, this difference became significant after the stimulus test with TRH, indicating the existence of a direct

relationship between endometriosis stage and the levels of PRL (Muse *et al*., 1982; Acièn *et al*., 1989). Many investigators have studied PRL secretion after TRH stimulation tests; however, only one group (Wallace *et al*., 1984) studied PRL secretion in relation to the dopaminergic pathway. Those investigators detected an increase in PRL secretion after metoclopramide infusion in patients with endometriosis, and also observed that it is possible to control the over-secretion of PRL in endometriotic patients by lowering the estrogenic stimulus. In other studies, the serum PRL levels of infertile patients with endometriosis were not statistically higher at baseline and after TRH stimulation. Our results differ from these mainly because the sample size (power calculation) employed in those studies is limited ($n = 20$) to investigate a subtle abnormality (Panidis *et al*., 1992; Matalliotakis *et al*., 1996).

A study of patients in all stages of endometriosis presenting with infertility demonstrated that hyperprolactinaemia might be the main cause of infertility in these patients (Gregoriou *et al*., 1999). We have previously described a higher prevalence of hyperprolactinaemia, luteal insufficiency and lower estradiol secretion in endometriotic women compared with fertile women (Cunha-Filho *et al*., 2001). This is in agreement with the present results, in that hyperprolactinaemia could affect follicular function and cause infertility in patients with minimal/mild endometriosis. Some investigators believe that the over-secretion of PRL after metoclopramide administration is caused by a stimulatory effect of estrogen (Wallace *et al*., 1984). In addition, other authors (Gregoriou *et al*., 1999) have shown that patients with endometriosis are hyper-responsive to TRH doses >200 μg; however, those authors did not control PRL secretion for estradiol, TSH or BMI as a possible confounding bias.

We demonstrated that in patients with endometriosis, PRL secretion is abnormal only after direct protein kinase C stimulation (TRH pathway). However, the DA2 blockade did not reveal any dysfunction in PRL secretion in those patients. Most likely, the mechanism involved in the abnormal PRL secretion does not include an inhibitory DA2 effect.

Patients with latent hyperprolactinaemia have lower LH and serum estradiol levels in the mid-follicular phase (Kostal and Tosner, 1997). We believe that the finding of higher PRL levels after TRH could be associated with the follicular dysfunction and ovulatory abnormalities found in infertile patients with endometriosis. In fact, an altered steroidogenesis with decreased steroid release has already been described (Harlow *et al*., 1996). We observed lower serum estradiol levels in infertile patients with endometriosis, probably as a consequence of increased PRL secretion. Other authors have also demonstrated an impairment in oocyte quality and follicular development in infertile patients with endometriosis. These findings may be associated with an abnormal ovulatory mechanism (Cahill and Hull, 2000; Garrido *et al*., 2000).

The administration of bromocriptine to treat hyperprolactinaemia was proposed by Reinthaller (Reinthaller *et al*., 1988). As shown in those studies, high serum PRL levels interfere with follicular and oocyte development. The treatment of this dysfunction results in better oocyte quality and thus in increased fertilization rates. It is possible to speculate that infertile patients with endometriosis and transient hyperprolactinaemia may benefit from bromocriptine during assisted reproduction cycles to reduce the pulsatility of PRL and enhance follicular response. However, a large randomized trial is necessary to test this hypothesis.

We do not believe that the lower serum estradiol levels observed in our patients with infertility and endometriosis (group 3) was a random finding because: (i) all patients were evaluated on days 3–5 of the regular menstrual cycle; (ii) Levene's test showed that the groups had similar variances; (iii) the prevalence of outliers was extremely low. We therefore associate the lower estradiol levels in group 3 with a higher PRL pulsatility.

GH is associated with the reproductive function and prognosis of infertile patients, mainly due to its co-gonadotrophic modulation. Also, GH stimulates estradiol secretion; human granulosa cells have GH receptors, and an association between infertility and impaired GH secretion has been demonstrated (Mason *et al*., 1990; Ovesen *et al*., 1994).

GH secretion after metoclopramide infusion could be stimulated by an inhibitory effect on somatostatin secretion and on the adrenergic tonus (Cohen *et al*., 1979; Vance *et al*., 1987; Arce *et al*., 1991). However, this conclusion is disputed by others who did not find a difference in terms of GH secretion after dopaminergic blockade (Masala *et al*., 1978; Jordan *et al*., 1986). This disagreement could be explained by differences in the metoclopramide infusion protocols and in the control groups. Our results show that metoclopramide administration did not alter GH secretion in either fertile or infertile patients, probably because different pathways modulate GH secretion, and various neurohormones and transmitters are involved. The central role of dopamine in GH secretion depends mainly on its effect on adrenergic transmission to somatostatin neurons, so that it is possible to conclude that dopamine is a modulator, and that it does not have a major role in GH neuroregulation (Devesa *et al*., 1992).

The exact mechanism causing abnormal GH secretion after TRH stimulation is unknown. In some disorders (acromegaly, PCOS, anorexia nervosa, liver disease and depression), GH secretion after TRH administration is altered. Some investigators believe that the mechanism of TRH stimulation of pituitary cells is similar to that by which protein kinase C stimulates PRL secretion, or that TRH stimulates pituitary cells by acting on the hypothalamus (Kaltsas *et al*., 1999).

We reported an enhanced GH secretion in fertile patients with endometriosis, which is perhaps related to higher estradiol levels, since estradiol influences GH secretion.

In conclusion, patients with endometriosis had a dysfunction in PRL secretion after TRH infusion (protein kinase C stimulation); however, the central dopaminergic system was not affected. Moreover, infertile patients with endometriosis had an altered estradiol secretion, probably due to the increased serum PRL levels. Such an alteration in PRL secretion and the decrease in estradiol levels are strongly related to a dysfunction of the hypothalamic–hypophyseal–ovarian axis, and could be the cause of infertility in women with endome-

triosis without tubal occlusion. The importance of this finding should be investigated in a large, randomized clinical trial to evaluate the use of bromocriptine in infertile patients with minimal/mild endometriosis to improve oocyte and embryo quality.

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