Insulin sensitizing drugs increase the endogenous dopaminergic tone in obese insulin-resistant women with polycystic ovary syndrome

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Abstract
To investigate whether the long-term administration of metformin or pioglitazone to women with polycystic ovary syndrome (PCOS) could induce changes in their hypothalamic dopaminergic (DA) tone and to analyze whether these changes correlated with modifications in insulin resistance, we originally studied 57 obese hyperinsulinemic, non-diabetic, insulin resistant women with PCOS, but only 34 completed the study. They were randomly divided into two groups: group one (n=17) received pioglitazone (30 mg/day) and group 2 (n=17) received metformin (850 mg, three times a day) over 24 weeks. All women were identically studied before (basal) and 6 months after (T6) drug administration, including clinical evaluations, a 2 h oral glucose tolerance test (75 g) (OGTT) for glucose and insulin measurements, followed a week later by a 2 h intravenous metoclopramide test (10 mg bolus) for prolactin (PRL) determinations. The areas under the insulin (AUC-insulin) and PRL (AUC-PRL) curves were calculated, along with the index of insulin resistance (HOMA-IR) and the indexes of insulin sensitivity (QUICKI and fasting glucose–insulin ratio). At baseline, women in both groups were of similar age, body weight, body mass index (BMI) and Ferriman-Gallwey hirsutism score (F-G score). At completion of the study, body weight and BMI remained unchanged but the F-G score significantly decreased. Fasting serum insulin concentrations and the AUC-insulin significantly decreased by the end of the trial in a similar fashion in both groups, while the AUC-PRL significantly increased at the end of the trial in both groups. At no time were significant correlations between AUC-PRL and AUC-insulin or the indexes HOMA-IR, QUICKI or fasting glucose–insulin ratio observed. The present results suggests that either pioglitazone or metformin administration was associated with a clear improvement in the endogenous hypothalamic DA tone, simultaneously with an amelioration of the insulin resistance status in these obese women with PCOS.

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Introduction
Polycystic ovary syndrome (PCOS) is the most frequent endocrine disorder in women of reproductive age, with an estimated frequency of between 4 and 7% (Lobo 2003), and is characterized by chronic anovulation and/or hyperandrogenism and/or polycystic ovaries by ultrasound in the absence of hypothyroidism, hyperprolactinemia, late-onset congenital virilizing adrenal hyperplasia and Cushing’s syndrome (The Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group 2003). The presence of insulin resistance (IR) and secondary hyperinsulinemia, mainly in obese women with PCOS (Burghen et al. 1980), is considered the key factor responsible for their hyperandrogenism (Dunaif 1997, Baillargeon et al. 2002). Previous studies have proposed that women with PCOS also have a disruption of the neuroendocrine mechanisms (mainly a deficiency of hypothalamic dopamine) regulating both gonadotropin-releasing hormone and prolactin (PRL) release (Quigley et al. 1981, Rosenfield, 1997, Taylor et al. 1997). Indeed, administration of dopamine (DA) or bromocriptine, a dopamine agonist, decreased the circulating levels of luteinizing hormone (LH) (Leblanc et al. 1976) and restored the cyclic ovarian function (Polson et al. 1987) in some of these patients. A hypothalamic deficiency of DA (Prelevic et al. 1988, Velardo et al. 1991) could also explain the mild hyperprolactinemia frequently present in women with PCOS (Luciano et al. 1984, Shoupe & Lobo 1984) which is further supported by the finding of a low DA
hypothalamic tone with increased PRL bioactivity in obese, hyperinsulinemic women with PCOS (Hernández et al. 2000). The influence of DA has also been implicated in the regulation of insulin secretion (Cincotta & Meier 1996, Uvnas-Moberg et al. 1996) and the presence of PRL receptors in islets of Langerhans (Sorenson & Stout 1995) along with a potent stimulatory effect of this hormone on both pancreatic islet ß cell division and insulin secretion have been documented (Sorenson & Parson 1985, Sorenson et al. 1987, Sinha & Sorenson 1993). Because IR is a cardinal feature of PCOS, the long-term administration of insulin sensitizing drugs, such as metformin and thiazolidinediones (TZDs) has become a basic therapeutic approach for women with this disorder. Up to now, there are no data on the possible influence of either drug on the hypothalamic DA tone in these women. Thus, the aims of this study were, first, to analyze whether the long-term administration of metformin and the TZDs compound, pioglitazone, to obese hyperinsulinemic women with PCOS could induce differential changes in the hypothalamic DA tone and, second, to analyze whether or not these changes were correlated with an improvement in insulin sensitivity.

Materials and Methods

The study protocol was approved by the Internal Review Board and the Human Ethical Committee of the Instituto Nacional de Perinatología, México City, México and written informed consent was obtained from all volunteers. The study was conducted according to the Declaration of Helsinki (as amended, October 2000).

Study population

Women with PCOS (n=57), aged 21 to 35 years, naive to any specific treatment, whose chief complaints were hirsutism (Ferriman–Gallwey [F-G] score >8) and/or sterility were recruited from the outpatient Endocrinology and Sterility Clinics of the Instituto Nacional de Perinatología, México City, México. The diagnosis of PCOS was based on at least two of the three following abnormalities: oligomenorrhea or amenorrhea, high serum testosterone (>2.9 ng/ml) and/or free testosterone (free T) (>3.075 pg/ml) concentrations, and/or polycystic ovaries detected by ultrasound (The Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group 2003). Additionally, all women had a body mass index (BMI) >25 kg/m², acanthosis nigricans, fasting hyperinsulinemia (>16 mU/ml) and a fasting glucose/insulin (G/I) ratio <4.5 (Parra et al. 1994). The presence of the following disorders was excluded by specific laboratory tests: type 2 diabetes mellitus (DM), hyperprolactinemia, thyroid disorders, late-onset congenital adrenal hyperplasia and Cushing’s syndrome. None of the women had been taking clomiphene citrate, oral contraceptives, antiandrogens, or drugs to control their appetite prior to the study. The presence of unsuspected pregnancy was excluded in all participant women prior to study entry. Criteria for exclusion during the study included: (a) diagnosis of pregnancy; (b) lost to follow-up; (c) non-compliance and (d) increased levels of serum transaminases.

Study design

Patients were randomly allocated to either one of two groups: group one (n=27) received pioglitazone (Zactos, Eli Lilly México, México) 30 mg/day oral single dose, during 24 weeks; group two (n=30) received metformin (Ficonax, Laboratorios Pisa, México City, México) orally administered at a dose of 850 mg, three times daily during 24 weeks. At no time during the study did any of the volunteer women receive instructions to modify their daily caloric intake or their physical exercise pattern. Randomization was by random number tables. The patients’ number treatment codes were held and kept until the end of the trial by a third party (not participating in the study) and patients’ names were disclosed after completion of the study. Either pioglitazone or metformin was started 2 weeks after written consent was obtained and the results of the basal (biochemical and hormonal) studies were available. All patients underwent clinical and hormonal evaluation at basal conditions (T0), and six months after initiation of treatment (T6). This included measurements of height, weight, BMI, waist/hip (W/H) ratio and hirsutism (F-G) score. After a 10–12 h overnight fast, an indwelling catheter was placed in an antecubital vein between 0800 and 0830 h, kept permeable by a slow infusion of 0.9% saline solution. After a 30 min rest a 2 h oral glucose tolerance test (OGTT) was performed with a 75 g oral glucose load and non-heparinized blood samples were obtained at 0, 30, 60, 90 and 120 min to measure serum glucose and insulin concentrations. A week later, and also after a 10–12 h overnight fast, between 0800 and 0830 h an indwelling peripheral catheter was placed following the same protocol as for the 2 h OGTT. After a 30 min rest, three basal non-heparinized blood samples were obtained at 15 min intervals (–30, –15, and zero min) and thereafter at 60, 90 and 120 min following a single 10 mg intravenous bolus of an antidopaminergic drug, metoclopramide (Pramotil, Laboratorios Pisa, México City, México). All patients were closely monitored for the possible occurrence of extrapyramidal symptoms. No sleeping, drinking of caffeinated beverages, or physical activity was allowed during the study. At each sampling time, the first 0.3 ml of blood was discarded to avoid a dilution error. All blood samples were centrifuged at 1000 g within the 30 min after being obtained and the serum was kept frozen at –20°C until assayed. After sixth months of treatment, the clinical evaluation along with the
2 h OGTT and the intravenous metoclopramide test were again performed in the same fashion.

Methods

BMI was calculated using the equation: weight (kg)/height (m²) and the waist and hip circumferences were measured to the nearest centimeter with a soft tape in accordance with WHO criteria. Hirsutism was clinically evaluated using the F-G score, obtained by the same observer (COG); a score of >8 was considered as hirsutism. PRL concentrations were determined in duplicate using a commercially available immunoradiometric kit (Diagnostic Products Corporation, Los Angeles, CA, USA) and insulin levels were determined using a commercially available radioimmunoassay kit (Diagnostic Products Corporation). The intra- and interassay coefficients of variation were <6-0% and <7-8%, respectively. In each assay the samples were distributed equally relative to each group and to each time of the study. Plasma glucose was measured by a glucose oxidase method (GOD-PAP; Diagnostica Merck, México City, México) using an automatic enzymatic autoanalyzer (Vitalab Scientific, Dieren, The Netherlands). The intra- and interassay coefficients of variation were <2-5% and <3-9%, respectively. Based on serum glucose and insulin concentrations, both fasting and during the 2 h OGTT, the following parameters were calculated: (a) homeostasis model assessment for insulin-resistance (HOMA-IR)=(fasting serum insulin (µU/ml) × fasting serum glucose (mmol/l)/22·5 (Albareda et al., 2000); (b) insulin sensitivity index (QUICKI)=1/[log (I₀)+log (G₀)], where I₀=fasting serum insulin (µU/ml) concentration and G₀=fasting serum glucose (mg/dl) concentration (Katz et al. 2000); (c) fasting glucose–insulin (G/I) ratio=fasting serum glucose concentration (mg/dl)/fasting serum insulin concentration (µU/ml) (Parra et al. 1994, Dunai 1997); (d) area under the glucose curve (AUC-glucose) and area under the insulin curve (AUC-insulin) using a trapezoidal method (Tai 1994). Additionally, the area under the prolactin curve (AUC-PRL) during the 2 h intravenous metoclopramide test was calculated using a trapezoidal method (Tai 1994).

Statistical analysis

Statistics were done using the Statistical Package for Social Science, (SPSS) software, version 11·0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics and frequencies for all variables were performed. Within and between group differences among the variables studied were assessed by using one-way ANOVA and the paired Student’s t-test. Correlations between variables were analyzed using the Pearson’s correlation coefficient. Values in the text and figures represent mean ± S.E.M. unless otherwise indicated. A P value <0·05 was considered statistically significant.

Results

In group one, a total of ten women were eliminated: five were lost to follow-up (during the first seven weeks of the study) and 5 became pregnant (8, 11, 12, 18 and 21 weeks into the study). In group two, 13 women were eliminated: five were lost to follow-up during the first eight weeks of the study, five due to non-compliance for severe side effects during the first trimester of the study and 3 became pregnant (14, 15 and 20 weeks into the study). Thus, group one finally included 17 women and group two also consisted of 17 women (Fig. 1). The lost to follow-up cases, five in each study group, occurred within the first 7–8 weeks of the study but none of these ten women (17-5% of the original group of 57 women) differed in any clinical, biochemical or hormonal parameter at baseline evaluation from the remaining 34 women who completed the study. In all ten women, the loss to follow-up was due to socioeconomic reasons. At no time during the study were significant changes in serum transaminase levels detected and no extrapyramidal signs were recorded in any patient during the i.v. metoclopramide tests. At baseline, women in groups one and two were of similar age (28·8 ± 0·9 and 28·6 ± 0·7 years respectively), BMI, W/H ratio and F-G score. At completion of the trial, BMI and W/H ratio remained essentially unchanged but the F-G score diminished significantly (P=0·04) (Table 1). At no time were there significant differences between groups.

Serum glucose and insulin concentrations

At baseline, fasting serum glucose concentrations were normal in both groups, without significant intra- or intergroup differences by the end of the study. The AUC-glucose during the 2 h-OGTT showed a mild decrease in both groups by the end of the trial, with a borderline statistical significance (P=0·05) (data not shown). Fasting serum insulin concentrations were similarly above the normal levels before initiation of the trial in both groups, with a marked decrease thereafter. Also the AUC-insulin showed a significant decrease after six months of treatment with either drug (Fig. 2A). No significant differences were observed among the two groups. The insulin resistance index (HOMA-IR) at baseline was nearly identical in both groups with a subsequent decrease in both groups, although more pronounced in group one. On the contrary, the indexes of insulin sensitivity, QUICKI and G/I ratio showed a similar and marked increment above the pretreatment values. At no time were there significant differences between groups (Table 2).

Serum prolactin concentrations

Fasting serum PRL concentrations (mean value of three basal samples: −30, −15 and 0 min) in both groups were...
similar and well within normal values (Table 2). The AUC-PRL before initiation of the drug administration was subsequently increased in both groups after 6 months of treatment with either pioglitazone ($P=0.007$) or metformin ($P=0.003$). No significant differences between groups were observed (Fig. 2B). After 6 months of treatment with either drug the AUC-PRL and the AUC-insulin underwent opposite changes. At baseline, AUC-PRL had a weak but significant negative linear correlation with fasting insulin ($r=-0.470, P=0.055$) and HOMA-IR index ($r=-0.470, P=0.05$) and a positive linear correlation with QUICKI index ($r=0.470, P=0.05$). At the end of the study, AUC-PRL best correlated with the HOMA-IR index ($r=-0.360, P=0.034$).

*Ovulatory cycles and pregnancy outcomes*

Although the precise evaluation of ovulation rates was not a main goal of the study, 14 women in the pioglitazone group (82.3%) and 15 women in the metformin group (88.2%) had clinical signs of normal regular cycles during the 6 months of study. This was further confirmed by a baseline serum progesterone concentration of $1.8 \pm 0.4$ ng/ml and $4.6 \pm 0.9$ ng/ml at the 6 months evaluation in the pioglitazone group ($P=0.04$). Also, baseline serum progesterone concentration was $1.4 \pm 0.5$ ng/ml and $3.8 \pm 0.4$ ng/ml at 6 months in the metformin group ($P=0.05$). There were no significant differences among the groups. Of the eight pregnant women registered, four (pioglitazone $n=3$; metformin $n=1$) had a first trimester abortion; three developed gestational diabetes mellitus (pioglitazone $n=2$; metformin $n=1$) requiring diet management and insulin administration, and subsequently had an uneventful at-term vaginal delivery (>38 weeks of gestation). Only one woman (metformin group) had a full-term normal pregnancy. All four newborns (two in each study group) had a normal weight for gestational age, were clinically healthy and without perinatal complications.

**Discussion**

The present study disclosed that the administration of both insulin sensitizing drugs, pioglitazone and metformin, to obese, non-diabetic, PCOS women with severe IR was associated with a significant increase in AUC-PRL. Although a substantial amelioration in the degree of IR and hyperinsulinemia was also clearly documented in both groups of women at the end of the trial, there were no significant correlations between AUC-PRL and AUC-insulin or any of the indexes of IR or insulin sensitivity.
Table 1 Clinical characteristics in obese, non-diabetic, insulin-resistant women with PCOS receiving either pioglitazone or metformin during six months (mean ± S.E.M.)

<table>
<thead>
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<th>Group 1: pioglitazone</th>
<th>Group 2: metformin</th>
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<tr>
<td></td>
<td>Baseline</td>
<td>6 months</td>
</tr>
<tr>
<td>n</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>79·1 ± 2·6</td>
<td>82·3 ± 3·0</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>32·3 ± 1·1</td>
<td>34·0 ± 1·2</td>
</tr>
<tr>
<td>W:H ratio</td>
<td>0·88 ± 0·02</td>
<td>0·86 ± 0·02</td>
</tr>
<tr>
<td>F–G score</td>
<td>15·4 ± 0·87</td>
<td>10·4 ± 0·62*</td>
</tr>
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</table>

*P = 0·04 compared with corresponding baseline score value.

neither before nor at the end of the treatment period. All these changes occurred in the face of non-significant modifications in body weight, BMI or the W:H ratio. Under physiological conditions, basal production of pituitary PRL is mainly controlled by tonic inhibitory mechanisms mediated by DA (Ben-Jonathan 1985). Both basal serum PRL concentrations and its response to intravenous metoclopramide, a DA blocking agent, have been considered as an indirect way to evaluate the functional level of the hypothalamic DA tone in the clinical setting (Quigley et al. 1979, Parra et al. 1997, Birnbacher et al. 1998, Parra et al. 2001). In fact, a decreased PRL response to intravenous metoclopramide has been interpreted as evidence of a diminished endogenous DA tone and, thus, an increased production of pituitary PRL. On the contrary, an increased response to metoclopramide would represent an increase in endogenous DA tone and, consequently, diminished production of pituitary PRL.

Table 2 Fasting serum glucose, insulin and prolactin concentrations and measurements of various indices of insulin resistance and sensitivity in obese, non-diabetic, insulin-resistant women with PCOS receiving either pioglitazone or metformin during six months (mean ± S.E.M.)

<table>
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<tr>
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<td>Baseline</td>
<td>6 months</td>
</tr>
<tr>
<td>n</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>92·5 ± 2·55</td>
<td>90·8 ± 2·3</td>
</tr>
<tr>
<td>Fasting insulin (µIU/ml)</td>
<td>31·1 ± 1·1</td>
<td>12·0 ± 1·8</td>
</tr>
<tr>
<td>Fasting G/I ratio</td>
<td>3·02 ± 0·14</td>
<td>9·84 ± 1·20</td>
</tr>
<tr>
<td>HOMA–IR index</td>
<td>7·03 ± 0·29</td>
<td>2·69 ± 0·41</td>
</tr>
<tr>
<td>QUICKI index</td>
<td>0·29 ± 0·001</td>
<td>0·34 ± 0·007</td>
</tr>
<tr>
<td>Fasting PRL (ng/ml)</td>
<td>9·35 ± 1·13</td>
<td>10·68 ± 1·16</td>
</tr>
</tbody>
</table>

*P values represent intragroup differences for 6 months compared to corresponding baseline.
of pituitary PRL (Quigley et al. 1979, Parra et al. 2001). Thus, a possible explanation for our PRL findings is that a diminished endogenous DA tone at baseline was present in women with PCOS, as previously described in both obese and lean women with PCOS (Hernández et al. 2000). This is in accordance with the proposal of the existence of a deficiency of hypothalamic DA in women with PCOS leading to disruption of the neuroendocrine mechanisms regulating PRL synthesis and/or release (Shoupe & Lobo 1984, Prelevic et al. 1988, Velardo et al. 1991) and the reason for a mild basal hyperprolactinemia seen in a subset of these women. This endogenous DA tone clearly improved after 6 months of treatment with either pioglitazone or metformin, thus contributing to the normalization of the endogenous secretion of PRL. This change could be due to either increased DA concentrations in the hypophyseal portal circulation or to an augmented sensitivity of the lactotrophs to DA. Although there are previous studies describing that both metformin (Ehrmann et al. 1997a) and troglitazone (Ehrmann et al. 1997b) failed to alter basal or stimulated LH and FSH levels when given for 12 weeks, there are no previous reports on the increased level of the endogenous DA tone associated with either pioglitazone or metformin administration (Fonseca 2003, Harborne et al. 2003). The increase of the AUC-PRL at the end of the trial can not be ascribed to an indirect effect of any other drug, since all women were naïve to any specific treatments. The possible influence of obesity in the low baseline AUC-PRL (Takemoto et al. 1994) could also be ruled out as body weight, BMI and W:H ratio remained essentially unchanged until the end of the study, yet the AUC-PRL significantly increased. Finally, the lack of significant differences in basal PRL concentrations between the first and second studies in both groups, in the face of significant changes in the endogenous DA tone, could have at least two explanations: firstly, the endogenous DA tone was diminished, but not abolished, at the initial study, and secondly, the mean of only three basal blood samples are not truly representative of the 24-h basal PRL secretion (Parra et al. 2001). Previous studies support the existence of an interplay between PRL and insulin (Sorenson & Parson 1985, Sorenson et al. 1987, Sinha & Sorenson 1993, Sorenson & Stout 1995) along with the influence of DA in the regulation of insulin secretion (Cincotta & Meier 1996, Uvnas-Moberg et al. 1996). In our study the correlations detected between AUC-PRL and AUC-insulin and the indexes of IR or insulin sensitivity, although weak, were statistically significant. This observation suggests that changes in the hypothalamic DA tone occurred in association with the improvement in insulin resistance seen in these women with PCOS. Thus, the increased endogenous DA tone (leading to a diminished pituitary PRL secretion), in association with the long term administration of pioglitazone or metformin, may participate in the amelioration of the IR status of these women. The study of the pioglitazone and/or metformin mechanisms of action involved in the improvement of the endogenous DA tone in these women with PCOS in the face of an unchanged body weight warrants further investigation. Furthermore, the study of the characteristics of the basal and pulsatile PRL secretion by frequent blood sampling may provide useful information.

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