Acute cholinergic blockade with low dose pirenzepine reduces the insulin and glucose responses to a mixed meal in obese women with the polycystic ovary syndrome

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Summary

OBJECTIVES Pirenzepine, a selective muscarinic cholinergic antagonist, reduces plasma insulin and plasma glucose responses to a mixed meal in a dose dependent fashion in normals and in patients with non-insulin dependent diabetes. We have studied the effects of pirenzepine on plasma insulin, plasma glucose, growth hormone (GH), androstenedione, testosterone, insulin-like growth factor (IGF-I) and IGF binding protein 1 (IGFBP-1) responses to a mixed meal in obese clinically hyperandrogenic women with the polycystic ovary syndrome.

SUBJECTS AND METHODS Six obese women with polycystic ovary syndrome (BMI range 27.3–39.8 kg/m²) were studied in random sequence, and received either placebo or pirenzepine (single doses of 50, 100, or 200 mg) one hour before a standard test meal. Blood was sampled every 15 minutes for 2 hours after the meal and every 30 minutes thereafter for a total of 4 hours.

RESULTS Mean fasting plasma insulin concentrations were increased. Peak post-prandial plasma insulin concentrations were reduced significantly by all three doses used. Post-prandial integrated plasma insulin concentrations were reduced by the two higher doses. Peak post-prandial plasma glucose concentrations were also reduced. The late post-prandial GH surge was significantly suppressed by all three doses. However, plasma androstenedione, testosterone, IGF-I and IGFBP-1 concentrations were not significantly different when placebo was compared with pirenzepine 200 mg.

CONCLUSIONS Acute cholinergic muscarinic blockade with pirenzepine significantly reduces meal stimulated plasma insulin and plasma glucose concentrations in clinically hyperandrogenic women with polycystic ovary syndrome. The ability of pirenzepine to reduce plasma insulin without worsening glycaemia is a particular advantage and may be therapeutically relevant. Further studies are under way to assess the usefulness of pirenzepine in long-term suppression of plasma insulin in this group of patients.

Insulin resistance and hyperinsulinaemia are recognized features of both obese and non-obese women with the polycystic ovary syndrome (PCOS) (Burghen et al., 1980; Chang et al., 1983; Pasquali et al., 1983; Barbieri et al., 1988). There is increasing evidence linking insulin to hyperandrogenism in this disorder. Burghen et al. (1980) first showed a positive correlation between fasting insulin and serum androgen levels in women with PCOS. This may be the result of a ‘gonadotrophic’ effect of insulin on ovarian androgen production, mediated through either insulin or insulin-like growth factor (IGF-I) receptors (Poretsky & Kalin, 1987; Adashi, 1991). Insulin also affects transport and availability of androgens by its effect on sex hormone binding globulin (SHBG). In-vitro studies have demonstrated inhibition by insulin of SHBG secretion by cultured hepatoma cells (Singh et al., 1990), and indeed. SHBG levels bear an inverse relation to plasma insulin (PI) levels in hyperandrogenic women (Dunaif et al., 1987).

Selective reduction of insulin levels without worsening glucose tolerance would be of clinical and experimental benefit in such patients. Somatostatin reduces insulin but decreases glucose tolerance in patients with PCOS (Prelevic et al., 1990). In contrast, pirenzepine (PIR), a muscarinic cholinergic antagonist, reduces both insulin and glucose responses to food in normal individuals and patients with non-insulin dependent diabetes mellitus (Page et al., 1989; Bevan et al., 1991). We have therefore studied the dose related actions of PIR on insulin and glucose in response to food in obese clinically hyperandrogenic women with PCOS.

Subjects and methods

Six obese women aged between 21 and 42 years gave written informed consent to the studies which were approved by the
Ethical Committee of the University Hospital of Wales. They were all non-smokers and were on no medication. Their body mass index (BMI) ranged between 27.3 and 39.8 kg/m², and they initially presented with menstrual irregularity and hirsutism (Ferriman–Galwey scores of 16–21). High-resolution transvaginal ultrasound scanning revealed characteristic features of PCOS in all patients (Adams et al., 1986). Clinical, biochemical and ultrasound characteristics are shown in Table 1.

Each subject was studied in random sequence on four occasions with an interval of at least one week between occasions with an interval of at least one week between

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (years)</th>
<th>BMI (kg/m²)</th>
<th>LH (U/l)</th>
<th>FSH (U/l)</th>
<th>PT (nmol/l)</th>
<th>SHBG (nmol/l)</th>
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<tr>
<td>1</td>
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<td>39.8</td>
<td>143</td>
<td>4.8</td>
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</tr>
<tr>
<td>2</td>
<td>28</td>
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<td>6.0</td>
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<td>26</td>
<td>38.8</td>
<td>20.1</td>
<td>3.4</td>
<td>1.3</td>
<td>31.3</td>
</tr>
</tbody>
</table>

Table 1 Clinical and biochemical features of six patients with polycystic ovary syndrome visualized on ultrasound scan

BMI, Body mass index; PT, plasma testosterone.

oration. Finland) with an intra-assay CV of <4.2%. Serum GH was measured using a commercial two-site immunodometric assay (Omnia GH IRMA kit. Immunodiagnostic Systems Ltd). Intra-assay CV was 5%, interassay CV was 10%; sensitivity limit of the assay was 0.13 ng/l. Serum IGF-1 was measured, after acid–ethanol extraction of its binding proteins (recovery 92.8 ± 4%), by RIA, using a polyclonal rabbit antiserum (R557A) raised against purified human IGF-1 (Taylor et al., 1990). Results are expressed in ng/l. The level of detection of this assay was 5.0 ng/l; the interassay CVs were 9.0, 4.5 and 6.2% at analyte levels of 65.4, 231 and 784 ng/l respectively, with an intra-assay CV of 4% at 231 ng/l. Serum concentrations of IGFBP-1 were measured by a specific RIA (Miell et al., 1993). Minimum detection limit of the assay was 6 ng/l. The interassay CV at 55 ng/l was 6.2%, and intra-assay CV at 35 ng/l was 4%.

Results are expressed as either peak values or area under curve (AUC). The areas under the curve for the responses of the assayed compounds to a mixed meal, were calculated by the trapezoidal rule. Summary measures for insulin were log transformed prior to statistical analysis and results are expressed as mean ± SEM for clarity. Comparisons of the summary measures were performed using the paired t-test (Matthews et al., 1990). Results are expressed as mean ± SEM.

Results

Mean fasting PI levels were raised as expected for obese subjects at 183.3 ± 12.6 pmol/l (normal range for laboratory derived from lean subjects was 28.6–179.3 pmol/l). Peak post-prandial PI was reduced by the administration of PIR 50, 100 and 200 mg when compared with PL (PL 1622 ± 369 pmol/l; PIR 50 mg, 894 ± 211 pmol/l, P < 0.01 vs PL; PIR 100 mg, 604 ± 152 pmol/l, P < 0.003 vs PL; PIR 200 mg, 685 ± 134 pmol/l, P < 0.005 vs PL) (Fig. 1). When insulin data are expressed as AUC, PIR doses of 100 and 200 mg produced a significant reduction (PL 156.8 ± 39.0 nmol min/l; PIR 50 mg, 116.2 ± 30.6 nmol min/l, P = 0.2; PIR 100 mg, 79.5 ± 19.1 nmol min/l, P < 0.009; PIR 200 mg, 91.1 ± 23.1 nmol min/l, P < 0.02).

Peak post-prandial PI was reduced significantly by PIR 50 and 200 mg and not by PIR 100 mg when compared to PL (PL 7.46 ± 0.13 mmol/l; PIR 50 mg, 6.54 ± 0.26 mmol/l, P < 0.02; PIR 100 mg, 6.95 ± 0.33 mmol/l, P = NS. PIR 200 mg, 6.55 ± 0.39 mmol/l, P < 0.04) (Fig. 2). Because of the wide scatter in basal PG values, the incremental change from basal to peak (Δpeak) was also calculated and suppression of Δpeak was statistically significant for all three doses of PIR used (PL 2.69 ± 0.18 mmol/l; PIR 50 mg, 1.36 ± 0.15 mmol/l.
Fig. 1 Plasma insulin levels (mean ± SEM) following a mixed meal in six obese females with PCOS pretreated with ○, placebo:
•, PIR 50 mg; ■, PIR 100 mg or
▲, PIR 200 mg.

Fig. 2 Plasma glucose levels (mean ± SEM) following a mixed meal in six obese females with PCOS pretreated with ○, placebo:
•, PIR 50 mg; ■, PIR 100 mg or
▲, PIR 200 mg.

Discussion

Our findings indicate that acute cholinergic muscarinic blockade with PIR significantly reduces the PI and PG responses to the physiological stimulus of a mixed meal in obese, clinically hyperandrogenic women with PCOS who were also hyperinsulinaemic. This inhibition was significant for all three doses used (PIR 200, 100 and 50 mg), when peak post-prandial PI levels were considered, although only the two higher doses significantly reduced the overall insulin response as measured by the area under the insulin curve.
insulin release from pancreatic β cells or a combination of both. In support of a direct pancreatic effect, it is well established that vagal activity and cholinergic agonists stimulate insulin release via muscarinic receptors on the β cells (Slcner & Freinkel, 1972; Grill & Ostenson, 1983).

Chronic hyperinsulinaemia has increasingly been linked to enhanced atherogenesis, hypertension, hyperlipidaemia and coronary artery disease (Stout, 1987; Reaven, 1988). The evidence for its role in ovarian hyperandrogenism is also increasing. Therefore any intervention which successfully reduces insulin levels without adverse effects on glycaemia would be potentially beneficial. We have shown that low doses of PIR can inhibit the meal stimulated PI and PG rise in obese, clinically hyperandrogenic, hyperinsulinaemic women with PCOS. Although we found no alterations in androgen, IGF-I and IGFBP-1 levels in this acute 4 hour study, the effects of more prolonged inhibition of insulin levels must now be investigated.

**References**


Table 2. Mean ± SEM of basal, peak post-prandial and AUC IGF-I and IGFBP-1 concentrations in six subjects with polycystic ovary syndrome after pretreatment with placebo (PL) and PIR 200 mg

<table>
<thead>
<tr>
<th></th>
<th>Basal (µg/l)</th>
<th>Peak (µg/l)</th>
<th>AUC (mg min/l)</th>
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<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>PIR 200 mg</td>
<td>Placebo</td>
</tr>
<tr>
<td>IGF-I</td>
<td>195.4 ± 35.1</td>
<td>232 ± 39</td>
<td>278 ± 22</td>
</tr>
<tr>
<td>IGFBP-1</td>
<td>132 ± 8.2</td>
<td>187.5 ± 11.3</td>
<td>221 ± 15</td>
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AUC. Area under curve; PIR, pirenzepine

Furthermore, this suppression of PI was achieved with a concomitant suppression of peak post-prandial PG and, more significantly, suppression of Δpeak glucose values.

Since the original description of Achard and Thiers (1921) of the ‘diabetes of bearded women’, the question of insulin resistance with hyperinsulinaemia and its relationship to ovarian hyperandrogenism has been studied extensively. In PCOS, the commonest variety of ovarian hyperandrogenism, hyperinsulinaemia with insulin resistance is present in 50% of patients (Singh et al., 1990), and is independent of body weight (Burghen et al., 1980). A large body of evidence suggests that insulin affects both production and transport of androgens (Poretsky & Kulin, 1987; Barbieri et al., 1988; Barbieri, 1991; Poretsky, 1991). Therefore, reduction of serum insulin levels in these patients might reduce androgen production. Previous studies have used several strategies for modulation of insulin levels. Weight loss over several weeks in obese patients with PCOS resulted in a significant reduction of hyperinsulinaemia and androgen levels (Kiddy et al., 1989; Pasquali et al., 1989). Acute opiate receptor blockade with naloxone has also been used to reduce hyperinsulinaemia with variable results (Givens et al., 1987; Laattkainen et al., 1990). Long-acting somatostatin analogue (Sandostatin) when used in PCOS reduced insulin levels but, predictably, worsened glucose tolerance (Prelevic et al., 1990). Acute cholinergic muscarinic receptor blockade using PIR 200 mg has been shown to reduce the insulin response to a mixed meal (Page et al., 1989; Bevan et al., 1991). We chose PIR in our study because of its proven efficacy, safety and its potential for long-term use in future studies.

PIR may affect the PG and PI responses to a mixed meal in several ways. The drug is thought to reduce upper gastrointestinal motility and impair glucose absorption, but significant reductions of insulin levels still occur following intravenous glucose in patients pre-treated with PIR (Coiro et al., 1986). This suggests additional mechanisms of either increased peripheral glucose utilization or inhibition of


