Role of 17β-estradiol and/or progesterone on insulin sensitivity in the rat: implications during pregnancy

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Abstract

The mechanism for the development of insulin resistance in normal pregnancy is complex and is associated with serum levels of both progesterone and 17β-estradiol. However, it remains unclear whether estrogens alone or progestins alone can cause insulin resistance, or whether it is a combination of both which produces this effect. We attempted to determine the role played by progesterone and/or 17β-estradiol on the phenomena of sensitivity to insulin action that take place during pregnancy in the rat. Ovariectomized rats were treated with different doses of progesterone and/or 17β-estradiol in order to simulate the plasma levels in normal pregnant rats. A euglycemic/hyperinsulenic clamp was used to measure insulin sensitivity. At days 6 and 11, vehicle (V)- and progesterone (P)-treated groups were more insulin resistant than 17β-estradiol (E)- and 17β-estradiol+progesterone (EP)-treated groups. Nevertheless, at day 16, the V, EP and E groups were more resistant to insulin action than the P group. On the other hand, the V, EP and E groups were more insulin resistant at day 16 than at day 6, whereas the P group was more insulin resistant at day 6 than at day 16. Our results seem to suggest that the absence of female steroid hormones gives rise to a decreased insulin sensitivity. The rise in insulin sensitivity during early pregnancy, when the plasma concentrations of 17β-estradiol and progesterone are low, could be due to 17β-estradiol. However, during late pregnancy when the plasma concentrations of 17β-estradiol and progesterone are high, the role of 17β-estradiol could be to antagonize the effect of progesterone, diminishing insulin sensitivity.


Introduction

Gestation is a highly important event in the life of mammals, and many metabolic and hormonal changes take place in this period (Herrera et al. 1991, Fernández et al. 1993, Metcalfe et al. 1994), including adaptations of carbohydrate metabolism characterized by a progressive state of insulin resistance that impedes maternal glucose utilization and consequently increases glucose fluxes to the developing fetus (Bliss et al. 1990, Kauffman et al. 1991, Parsons et al. 1992, Baaziz & Curry 1993). Pregnancy represents a severe stress test of carbohydrate tolerance. An important facet of these changes seems to be the development, in rats, of insulin resistance in the liver and extrahepatic tissues (Leturque et al. 1987). This could give a competitive advantage to the rat uterus during pregnancy, because uterine uptake and placental transport of glucose are relatively unaffected by maternal insulin (Hay et al. 1984, Rankin et al. 1986). The intense flux of maternal glucose to the fetus is partially compensated by maternal hyperphagia (Gutiérrez et al. 1991, Fernández et al. 1993), but the mother is also forced to develop mechanisms that reduce glucose consumption by her own tissues (Leturque et al. 1986). The mechanism(s) by which plasma insulin is elevated in pregnancy is still largely unknown. The process for development of insulin resistance in normal pregnancy is complex and is associated, at least in part, with increasing maternal serum levels of both progesterone and 17β-estradiol (Howell et al. 1977) or progesterone alone (Sutter-Dub & Dazey 1979).

Various clinical observations and experimental data from in vitro studies suggest that insulin and sex hormones interact on carbohydrate metabolism (Polderman et al. 1994). However, the effect of the menstrual cycle on this carbohydrate metabolism and insulin sensitivity remains to be determined. Clinical conditions such as pregnancy, where estrogen and progesterone concentrations are markedly raised, have a substantial effect on carbohydrate, lipid, and intermediary metabolism, as well as altering insulin sensitivity (Herrera et al. 1991, Baaziz & Curry 1993, Metcalfe et al. 1994, Saad et al. 1997). Artificially raised levels of the sex steroid hormones, estrogen and...
progesterone, in women taking the combined oral contraceptive pill were found to affect glucose tolerance and insulin sensitivity (Godsland et al. 1991, Watanabe et al. 1994). The influence of these hormones on the early steps of insulin action have not yet been investigated. However, the hypothesis that has gained most support is that the high insulin levels found in insulin-resistant states can stimulate the ovary, either through binding to classic insulin receptors or by inappropriate binding to the insulin-like growth factor-I receptor through a mechanism known as specificity spillover (Poretsky 1991). It remains unclear whether estrogens alone or progestins alone can cause insulin resistance, or whether it is only a combination of both which produces this effect.

Many findings (Simonson & Widom 1991, Valdes & Elkind-Hirsch 1991, Marsden et al. 1996) suggest that estrogen and progesterone can modulate insulin sensitivity in women. These investigations report a poor control of glucose homeostasis in diabetic women during the luteal phase while sensitivity to insulin action in normal women also decreases. It seems clear, therefore, that physiological variations in the concentrations of sex hormones during the menstrual cycle could have an effect on the receptor for insulin binding. All these considerations imply that progesterone is a modulating factor on receptor insulin binding, at least in adipose tissue (Simonson & Widom 1991, Valdes & Elkind-Hirsch 1991, Marsden et al. 1996). This interpretation would agree with the reduction in insulin sensitivity attributed to progesterone in women treated with the oral contraceptive pill (Spellacy 1982, Marsden et al. 1996).

The experimental findings of many authors (Leturque et al. 1987, Córdova et al. 1991, Lindheim et al. 1993, 1994) suggest that progesterone administration diminishes sensitivity to the action of insulin, whereas estrogen administration seems to maintain insulin sensitivity in female rats (Holmäng & Björntorp 1992). Ovariectomy causes a decrease in the glucose disappearance rate, which is restored by estrogen or estrogen+progesterone replacement but not by progesterone alone (Kumagai et al. 1993).

The present study was designed to determine the role played by progesterone and/or 17β-estradiol on the phenomena of sensitivity to insulin action that take place during pregnancy in the rat.

Materials and Methods

Animals

Twelve-week-old virgin female Wistar rats (from the Biotery of Faculty of Medicine, University of Oviedo) weighing 250–280 g, and kept under standard conditions of temperature (23 ± 3 °C) and humidity (65 ± 1%), and a regular lighting schedule of 12 h light:12 h darkness (0800–2000 h) were used. The animals were fed a standard diet (Panlab A04, PanLab s.l., Barcelona, Spain) and had free access to water. All experimental manipulations were performed between 0930 h and 1230 h. The experiments were carried out in accordance with the rules of laboratory animal care.

Experimental design

Three days before initiating the hormonal treatment (day −7), the rats were ovariectomized through a midline incision under light ether anesthesia. Ovariectomized rats were separated randomly into four groups: control (V), estradiol (E), progesterone (P) and estradiol+progesterone (EP) and housed individually throughout the experiment.

After surgery, ovariectomized rats were allowed 3 days to recover from surgery stress and for their hormone levels to decrease. From day −4 the rats were injected subcutaneously every 12 h (0900 h and 2100 h) for 20 days with 0.1 ml of a suspension in olive oil/ethanol (3:2, v/v) of 17β-estradiol (Sigma Chemical Co., St Louis, MO, USA), progesterone (Sigma Chemical Co.) or a combination of both hormones. A control group (V) injected with vehicle (olive oil/ethanol 3:2, v/v) was followed in parallel. Different doses of 17β-estradiol and/or progesterone were injected (Sutter-Dub et al. 1978, Aerts et al. 1980) in order to simulate the plasma levels that we observed in normal pregnant rats (González et al. 1997, 1998). Day 0 represents the start of hypothetic pregnancy.

The hormonal treatment was applied according to the temporal diagram shown in Fig. 1.

Groups (V, E, P and EP) were divided randomly into three subgroups (six animals/subgroup) and one subgroup was killed on days 6, 11 and 16. These days were selected as changes were found in sensitivity to the action of insulin during pregnancy in the Wistar rat (González et al. 1997).

Euglycemic insulin clamp

Clamp procedure was performed in anesthetized rats. After 12 h of fasting on days 6, 11 and 16, the animals were anesthetized with 3.3 ml/kg body weight i.p. ekytesin (0.96 g/ml sodium pentobarbital, 4.02 g/ml chloral hydrate, 2.12 g/ml magnesium sulfate, 40% propilenglicol, 10% ethanol). Body temperature was maintained at 37–38 °C with heating lamps. The left saphenous vein was catheterized for insulin and glucose infusion.

 Approximately 30 min after the end of surgery and as soon as anesthesia was assured by loss of pedal and corneal reflexes, in order to determine basal insulin concentration, a blood sample (1 ml) was collected from the jugular vein into heparinized tubes, centrifuged at 3000 r.p.m. for 20 min at 4 °C and plasma was immediately drawn off and stored frozen at −20 °C until assayed. A blood sample for the determination of basal blood glucose was collected from the tail. Plasma glucose was measured using an Accutrend System (Accutrend Alpha; Roche Diagnostic S.L., Barcelona, Spain).
The insulin was infused at a constant rate of 20 µl/min (0.4 IU/kg per h) into the left saphenous vein and the blood glucose level clamped at the level measured in the basal state by a variable infusion of glucose through the saphenous vein with a Precidor pump (Precidor Type 5003 Infusion Pump; INFORS AG, Switzerland). Insulin (biosynthetic human insulin Actrapid; Novo Nordisk, Bagsvaerd, Denmark) was dissolved in 0.9% NaCl containing 0.28% bovine serum albumin (Sigma Chemical Co.) and 0.125‰ heparin (sodium heparin 1%). The infusion of exogenous glucose (12–15% solution) dissolved in 0.9% NaCl, was initiated 5 min after insulin infusion. Blood (25 µl) was then sampled from the tail every 5 min and plasma glucose concentration was determined with an Accutrend System.

Adjustments in the exogenous glucose infusion rate were made to maintain euglycemia by altering the percent dial of the Precidor pump depending on the changes in blood glucose concentrations observed. These alterations were made empirically (Kraegen et al. 1983, Escrivá et al. 1992).

The duration of the experiment was about 1 h and the euglycemic clamp was easily reached 30–40 min after the beginning of insulin infusion. The total amount of blood volume taken was 5.5–6.5 ml from each animal. Plasma insulin was measured by RIA using a DGR Instruments GmbH (Marburg, Germany) kit for rat insulin. The sensitivity of the assay was 0.1 ng/ml, and the intra-assay coefficient of variation was 9.32%. The sample was assayed in duplicate. Plasma 17β-estradiol was measured by RIA using Immuchen kits of cover tubes (ICN Pharmaceuticals Inc., Costa Mesa, USA). The assay sensitivity was 10 pg/ml, and the intra-assay coefficient of variation was 12-26%. Plasma progesterone was measured by RIA using Immuchen kits of cover tubes (ICN Pharmaceuticals Inc.). The sensitivity of the assay was 0.15 ng/ml, and the intra-assay coefficient of variation was 11-48%. The sample was again assayed in duplicate. All samples were measured on the same day.

Finally, samples of different tissues were collected and immediately frozen in liquid nitrogen for future experiments and the animals were killed by bleeding.

Statistics

Data are expressed as means ± s.e.m. Intragroup comparisons for the period of hormonal treatment were made using an analysis of variance or Kruskal–Wallis one-way ANOVA test, and the Student–Newman–Keuls test or Mann–Whitney U–Wilcoxon Rank Sum W test. A \( P \leq 0.05 \) was considered as significant. Statistical analysis was performed using SPSS for Windows v.6-01.

Figure 1 Temporal diagram of hormonal treatment.

<table>
<thead>
<tr>
<th>DAYS</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>[EST+PROG]</td>
<td>E2+P5</td>
<td>E2+P5</td>
<td>E2+P5</td>
<td>E2+P5</td>
<td>E1+P6</td>
<td>E1+P6</td>
<td>E1+P7</td>
<td>E1+P7</td>
<td>E1+P7</td>
<td></td>
</tr>
<tr>
<td>[EST]</td>
<td>E2</td>
<td>E2</td>
<td>E2</td>
<td>E2</td>
<td>E1</td>
<td>E1</td>
<td>E1</td>
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</tr>
<tr>
<td>[PROG]</td>
<td>P5</td>
<td>P5</td>
<td>P5</td>
<td>P5</td>
<td>P6</td>
<td>P6</td>
<td>P7</td>
<td>P7</td>
<td>P7</td>
<td></td>
</tr>
</tbody>
</table>

P1=15.2mM; P2=22.9mM; P3=30.5mM; P4=68.7mM; P5=53.5mM; P6=76.4mM; P7=99.3mM;
E1=0.05mM; E2=0.025mM. * = day of killing.
Results

Plasma progesterone and 17β-estradiol values observed throughout the study are shown in Fig. 2. The plasma levels of progesterone in the P group were in concordance with normal pregnancy in the rat (González et al. 1997). However, in the EP group these were higher than in normal pregnancy. On the other hand, the plasma levels of 17β-estradiol were higher in the E and EP groups than in normal pregnancy in the rat (González et al. 1997).

Comparisons of body weight increase can be seen in Fig. 3. The increase in body weight was significantly higher on days 0 to 6 of the experimental period in the V, P and EP groups compared with days 11 to 16. No differences were noted in the E group. On days 0 to 6, the rise in body weight was significantly higher in the P than in the EP and E groups, in the V compared with the E group and in the EP compared with the E group. On days 6 to 11 the increase in body weight was significantly lower in the E than in the V, P and EP groups.

Table 1 shows fasting blood glucose, fasting serum insulin and serum insulin after clamp experiments. Fasting blood glucose levels were observed to be similar between groups and between days of the experimental period.

A significant decrease was found between days 11 and 16 in the fasting serum insulin levels of the EP group. Comparing this parameter for each day, we observed that on days 6 and 11 the levels of fasting serum insulin were significantly higher in the EP and E than in the V and P groups. However, on day 16 these differences only showed up between the P and EP and the P and E groups.

When comparing the groups each day, serum insulin levels after clamp experiments were similar in all groups at day 6. Although at days 11 and 16 this parameter was higher in the V than in the other groups, no significant differences were noted. A significant increase in the V and a significant decrease in the EP group were observed between days 6 and 16.

To investigate insulin resistance in rats at different days of the hormonal treatment, glucose clamp experiments were carried out under euglycemic and hyperinsulinemic conditions. Figure 4 shows the results of the clamp experiments. After insulin infusion, the blood glucose levels increased slightly from 0 to 10 min in some groups, but from 15–20 min to 60 min the blood glucose levels clamped around 110 mg/dl in all cases (Fig. 4A, B and C). On the other hand, on days 6 and 11 the glucose infusion rate of rats treated with vehicle and progesterone alone (V and P) were significantly lower than that of the groups.
Fasting blood glucose (mg/dl) between days 6 and 11, but significantly diminished between days 6–11 and 11–16. In the EP and E groups this rate was similar between days 6 and 11, but significantly diminished between days 6–11 and 11–16. However, a significant increase was found in the P group between days 6–11 and 11–16.

**Discussion**

Progesterone and estradiol have been implicated as etiological factors in the development of insulin resistance during pregnancy (Baaziz & Curry 1993, Kumagai et al. 1993, Sorenson et al. 1993, Nelson et al. 1994), but the effect of both hormones on glucose homeostasis remains relatively unclear. In this study, we have tried to clarify the separate roles of 17β-estradiol and progesterone on glucose homeostasis.

The plasma levels of 17β-estradiol and progesterone in the EP group were higher than expected with the administered doses (Fig. 2). In spite of these levels, the profiles of both hormones in this group were similar to pregnancy, that is to say, similar plasma concentrations between days 6 and 11 and a significant increase between days 11 and 16, in agreement with previous studies in rats treated with estradiol (EP and E), indicating the existence of insulin resistance in the V and P groups (Fig. 4D and E). However, on day 16, the infusion rate of rats treated with progesterone alone was significantly higher than that of the other groups (V, EP and E), revealing the existence of insulin resistance in the the V, EP and E groups (Fig. 4F).

Figure 5 shows the comparison of glucose infusion rates on days 6, 11 and 16 of the experiment. In the V group the glucose infusion rate significantly decreased between days 6 and 11. In the EP and E groups this rate was similar between days 6 and 11, but significantly diminished between days 6–11 and 11–16. However, a significant increase was found in the P group between days 6–11 and 11–16. In the present work we found that in groups treated with 17β-estradiol alone or 17β-estradiol and progesterone the fasting serum insulin concentration was significantly higher than in groups treated with progesterone alone or vehicle (Table 1). Our results therefore seem to indicate that 17β-estradiol is responsible for the increase in insulin secretion. Our findings might agree with several studies reporting that ovarian steroids affect β-cell function and

### Table 1: Fasting blood glucose, fasting serum insulin and serum insulin after clamp experiments of vehicle (V)-, progesterone (P)-, estradiol+progesterone (EP)- and estradiol (E)-treated rats on days 6, 11 and 16 of the experiment. Values are means ± SEM; there were six animals/group

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day 6</th>
<th>Day 11</th>
<th>Day 16</th>
<th>Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting blood glucose (mg/dl)</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>V</td>
<td>108.77 ± 4.00</td>
<td>110.00 ± 3.00</td>
<td>103.16 ± 2.38</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>112.71 ± 6.20</td>
<td>113.00 ± 5.61</td>
<td>110.00 ± 4.23</td>
<td></td>
</tr>
<tr>
<td>EP</td>
<td>110.40 ± 3.72</td>
<td>112.60 ± 6.83</td>
<td>99.60 ± 1.66</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>116.40 ± 4.82</td>
<td>120.80 ± 5.65</td>
<td>107.80 ± 2.59</td>
<td></td>
</tr>
<tr>
<td>Comparison</td>
<td>V vs EP; V vs E; P vs EP; P vs E</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Fasting serum insulin (ng/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>0.67 ± 0.19</td>
<td>0.72 ± 0.18</td>
<td>0.72 ± 0.29</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.59 ± 0.17</td>
<td>0.57 ± 0.17</td>
<td>0.32 ± 0.008</td>
<td></td>
</tr>
<tr>
<td>EP</td>
<td>2.59 ± 0.12</td>
<td>3.76 ± 0.81</td>
<td>1.72 ± 0.38</td>
<td>11 vs 16</td>
</tr>
<tr>
<td>E</td>
<td>2.17 ± 0.23</td>
<td>2.21 ± 0.29</td>
<td>1.74 ± 0.23</td>
<td></td>
</tr>
<tr>
<td>Comparison</td>
<td>V vs EP; V vs E;</td>
<td></td>
<td></td>
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<tr>
<td>Serum insulin after clamp experiments (ng/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>2.15 ± 0.55</td>
<td>7.45 ± 1.97</td>
<td>11.54 ± 3.78</td>
<td>6 vs 16</td>
</tr>
<tr>
<td>P</td>
<td>3.29 ± 0.97</td>
<td>4.53 ± 0.66</td>
<td>5.31 ± 1.63</td>
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<tr>
<td>EP</td>
<td>4.46 ± 0.81</td>
<td>2.35 ± 0.33</td>
<td>2.43 ± 0.21</td>
<td>6 vs 11; 6 vs 16</td>
</tr>
<tr>
<td>E</td>
<td>3.31 ± 0.20</td>
<td>5.19 ± 0.20</td>
<td>4.05 ± 2.40</td>
<td></td>
</tr>
</tbody>
</table>

Only significant differences are shown.
glucose homeostasis by increasing insulin production through the induction of β-cell hypertrophy (Yki-Jarvinen 1984, Magnaterra et al. 1997, Zhu et al. 1998, Nieuwenhuizen et al. 1999) and, furthermore, with those observing that chronic progesterone therapy has no effect on either basal glucose or insulin levels compared with control rats (Nelson et al. 1994).

We have previously demonstrated (González et al. 1997, 1998) that the fasting serum insulin levels decreased between days 5 and 10 and increased between days 10 and
In the light of the clamp experiments (Figs 4 and 5), it could be said that progesterone treatment increases the insulin sensitivity at the end of the experiment (the progesterone-treated group at day 16 (P16) being more sensitive than P6 and P11), but treatment with 17β-estradiol causes a decrease in the insulin sensitivity at the end of the experiment (E6 and E11 being more sensitive than E16). On the other hand, low concentrations of progesterone (days 6 and 11) produced a decrease in insulin sensitivity while 17β-estradiol or vehicle brought about an increase in insulin sensitivity (V, EP and E more sensitive than P at days 6 and 11). These results are in agreement with studies reporting that estrogens increase sensitivity to the actions of insulin (Kalkhoff 1975), others showing that progesterone administration decreases insulin sensitivity in rats (Ryan & Enns 1988, Leturque et al. 1989, Córdova et al. 1991) and other works suggesting that estradiol treatment seems to maintain insulin sensitivity in female rats (Holmäng & Björntorp 1992, Holmäng et al. 1992). However, we do not totally agree, because at day 16, when the progesterone plasma concentration of late pregnancy in the rat is reached, the P group is more sensitive than the EP and E groups. Therefore, we consider that the role of progesterone during early pregnancy, with low concentrations of 17β-estradiol, is to diminish insulin sensitivity while, in this period, the low concentration of 17β-estradiol favors insulin sensitivity. Nevertheless, during late pregnancy, the high concentrations of progesterone could be increasing insulin sensitivity but, in this period, the high concentrations of 17β-estradiol counteracted the effect of progesterone.

The present work seems to contradict the role that several studies attribute to progesterone (Leturque et al. 1987, Córdova et al. 1991, Lindheim et al. 1993, 1994, Kumagai et al. 1993). We consider that insulin sensitivity is influenced by sex hormone concentration, so progesterone per se increases insulin sensitivity at high concentrations and 17β-estradiol per se augments insulin sensitivity at low concentrations but diminishes insulin sensitivity at high concentrations.

It is likely that, during gestation, the roles of both hormones are complementary and are intimately related to the other hormones which are elevated during normal gestation in the rat (e.g. growth hormone, lactogenic hormones). In addition, it is feasible to think that the development of systemic insulin resistance to insulin action, related to pregnancy, is somewhat different if we study the role of both hormones on the three most important target tissues of insulin: liver, skeletal muscle and adipose tissue. In this way, during early pregnancy, progesterone could be favoring insulin resistance in some tissues (principally the liver) and could be increasing insulin sensitivity in others (mainly skeletal muscle and adipose tissue) while, during later pregnancy, the increase
in concentrations of this hormone could be favoring insulin sensitivity in the former (liver) and could be decreasing insulin sensitivity in the latter (skeletal muscle and adipose tissue), the action of 17β-estradiol antagonizing the action of progesterone in both periods of gestation.

In summary, our results seem to suggest that the absence of female steroid hormones results in a decreased insulin sensitivity. 17β-Estradiol could be responsible for the increase in insulin sensitivity during early pregnancy when the plasma concentrations of 17β-estradiol and progesterone are low. However, during late pregnancy, when the plasma concentrations of 17β-estradiol and progesterone are high, the role of 17β-estradiol could be to antagonize the effect of progesterone diminishing insulin sensitivity. The effect of both hormones as proposed in this paper could appear to be altered in the presence of high plasma concentrations of the lactogenic hormones and growth hormone, just as occurs during normal pregnancy.

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