Effects of cortisol and progesterone on insulin binding and lipogenesis in adipocytes from normal and diabetic rats

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ABSTRACT

Cortisol implants in normal and diabetic rats reduced body weight, adiposity, insulin receptor concentration and both basal and insulin-stimulated rates of lipogenesis in isolated adipocytes, whilst insulin sensitivity was unchanged. In normal but not diabetic rats these changes were accompanied by increased serum glucose and insulin concentrations.

In contrast, progesterone implants in normal and diabetic rats increased body weight gain, adiposity, insulin receptor concentration and both basal and insulin-stimulated rates of lipogenesis in adipose tissue, again without affecting insulin sensitivity. Progesterone did not affect serum insulin concentrations in normal or diabetic rats but accelerated the decline in serum glucose concentrations which occurred during an overnight fast in diabetic rats.

The results suggest that (1) cortisol inhibits lipogenesis in adipose tissue without affecting insulin sensitivity, (2) cortisol reduces insulin binding in adipose tissue without a requirement for hyperinsulinaemia, which might itself indirectly lead to down-regulation of the insulin receptor, and (3) in diabetic rats progesterone stimulates lipogenesis in adipose tissue without any increase in food intake or serum insulin concentrations suggesting that progesterone may have a direct anabolic role in adipose tissue.


INTRODUCTION

Insulin resistance, as manifested by glucose intolerance and hyperinsulinaemia, is a known consequence of glucocorticoid administration in vivo (Conn & Fajjan, 1956; Philips, Herrera & Renold, 1965; Feldman, 1977; Grunfeld, Baird, Van Oeverghen & Kahn, 1981; Yasuda, Hines & Kitabchi, 1982) and in vitro (Leboeuf, Renold & Cahill, 1962; Fain, 1964; Yorke, 1967; Olefsky, 1975; Cigolini & Smith, 1979). Part of this effect may be due to the reduced insulin binding which occurs in liver (Olefsky, Johnson, Liu et al., 1975; Kahn, Goldfine, Neve & De Meyts, 1978; Caro & Amatruda, 1982), adipose tissue (Olefsky et al., 1975; Kahn et al., 1978; Bezdrobnyi, Evdokimova, Eftimov & Yatsky, 1983), monocytes (De Pirro, Bertoli & Fusco, 1980) and erythrocytes (Yasuda et al., 1982) although moderate hypercortisolism apparently induces insulin resistance without affecting insulin binding (Shamoon, Soman & Sherwin, 1980; Rizza, Mandarino & Gerich, 1982). In-vitro insulin binding has been shown to be decreased (Olefsky et al., 1975; Cigolini & Smith, 1979; Grunfeld et al., 1981) or increased (Knopf, Torretti & Hoslet, 1978; Fantus, Saviolakis, Hedo & Gordon, 1982) in response to glucocorticoids and this lack of a consistent effect in vitro has led to the suggestion that insulin binding may be decreased by insulin itself due to the hyper-insulinaemia evident after glucocorticoid treatment (Fantus et al., 1982).

Insulin resistance is also a characteristic feature of pregnancy (Felig, 1975). Progesterone has been implicated in this effect as it increases basal and glucose-stimulated insulin secretion in vitro (Ashby, Shirling & Baird, 1978) and in vivo (Beck, 1969; Costrini & Kalkhoff, 1971; Howell, Tyhurst & Green, 1977; Ashby, Shirling & Baird, 1981) and reduces the hypoglycaemic effect of exogenous insulin (Sutter-Dub & Dazeys, 1981). Studies in vitro with adipose tissue have suggested that progesterone induces insulin resistance by inhibiting glucose oxidation via the pentose-phosphate pathway (Sutter-Dub & Dazeys, 1981). Paradoxically, numerous studies have shown that progesterone, like insulin, increases adiposity in the mouse and the rat (Wade & Gray, 1979; Shirling, Ashby & Baird, 1981). Whether this effect of progesterone is
direct is difficult to interpret because of its stimulatory effect on insulin secretion, although progesterone receptors are present in adipose tissue (Gray & Wade, 1979).

For these reasons we decided to investigate the effects of cortisol and progesterone on lipid deposition, insulin receptors and insulin sensitivity of isolated adipocytes from normal and diabetic rats in order to clarify the role of serum insulin concentrations in modulating the effects of these hormones on insulin binding and lipid metabolism in adipose tissue.

**MATERIALS AND METHODS**

Nulliparous female Wistar rats weighing 180 g were used and were allowed free access to food and water. Ten normal and twelve diabetic (injected i.p. 5 days previously with 75 mg streptozotocin/kg; Sigma, Poole, Dorset) rats were each given a 50 mg pellet of pure cortisol (Sigma) s.c. in the nape of the neck, 12 normal and 12 diabetic rats were each given 2 × 100 mg discs of pure progesterone (Sigma) s.c. in the nape of the neck and 12 normal and 12 diabetic rats underwent sham-operations and served as controls.

Half of the animals from each group were killed 5 days after implantation, blood was collected from the trunk and allowed to clot at room temperature for 1 h. Serum was obtained by centrifugation at 1000 g for 10 min and stored at -20°C until used for the determination of serum glucose, insulin, progesterone and cortisol concentrations as described previously (Flint, Clegg & Vernon, 1981). Parametrical adipose tissue was removed and used for the preparation of isolated adipocytes by collagenase digestion (Flint et al. 1981); these cells were used to determine [U-14C]glucose incorporation into total lipid by the method of Moody, Stan, Stan & Gliemann (1974). Briefly, 10^4 adipocytes were incubated in 1 ml Krebs-Ringer phosphate buffer (pH 7.4) containing 1% bovine serum albumin, glucose (0.55 mmol/l) and 0.025 Ci [U-14C]glucose for 4 h at 37°C. Incubation was terminated by the addition of 4 ml toluene-based scintillation fluid which extracted the [14C]glucose converted to lipid.

Both the preparation of 125I-labelled insulin and the determination of the numbers of insulin receptors were as described by Flint, Clegg & Vernon (1980).

The remaining animals were fasted overnight on day 4 and blood samples were obtained from the tail at approximately 10.00 h on day 5 to determine fasting concentrations of glucose and insulin.

Statistical analyses were performed using Student's unpaired t-test.

**RESULTS**

Progesterone implants produced a 60% increase in body weight gain and associated with this was a similar increase in adipocyte volume indicating increased fat deposition (Table 1). [U-14C]glucose incorporation into total lipid in isolated adipocytes was also increased significantly in both basal and insulin-stimulated conditions (Table 1, Fig. 1) although sensi-

**TABLE 1. Adipocyte volume, insulin binding and glucose incorporation into isolated adipocytes, serum glucose, insulin, progesterone and cortisol concentrations in normal and diabetic virgin rats treated with either progesterone or cortisol. Values are means ± S.E.M.**

<table>
<thead>
<tr>
<th></th>
<th>Normal rats</th>
<th>Diabetic rats</th>
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<tbody>
<tr>
<td></td>
<td>Untreated</td>
<td>Progesterone</td>
</tr>
<tr>
<td>Number of animals</td>
<td>5-12</td>
<td>5-14</td>
</tr>
<tr>
<td>Body wt change (g/day)</td>
<td>2.5±0.2</td>
<td>4.0±0.4*</td>
</tr>
<tr>
<td><strong>Adipose tissue</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adipocyte volume (pl)</td>
<td>245±18</td>
<td>368±37**</td>
</tr>
<tr>
<td>Insulin bound (pg/10^6 cells)</td>
<td>13±2±1.4</td>
<td>21.8±2.1**</td>
</tr>
<tr>
<td>Insulin bound (fg/mm²)</td>
<td>6.7±0.5</td>
<td>9.3±0.6**</td>
</tr>
<tr>
<td>Glucose incorporation into total lipid (nmol/4 h per 10^6 cells)</td>
<td></td>
<td>23.9±3.8*</td>
</tr>
<tr>
<td>Basal</td>
<td>42±3±2.7</td>
<td>96±0±17.0**</td>
</tr>
<tr>
<td>Insulin (16 nmol/l) stimulated</td>
<td>77±6.7</td>
<td>174±132.9**</td>
</tr>
<tr>
<td><strong>Serum concentrations</strong></td>
<td></td>
<td>16±0±1.4</td>
</tr>
<tr>
<td>Progesterone (nmol/l)</td>
<td>68±14</td>
<td>251±25**</td>
</tr>
<tr>
<td>Cortisol (nmol/l)</td>
<td>32±9</td>
<td>17±3</td>
</tr>
<tr>
<td>Insulin (nmol/l) fed</td>
<td>0.3±0.0±8</td>
<td>0.27±0.05</td>
</tr>
<tr>
<td>Insulin (nmol/l) fasted</td>
<td>0.04±0.01</td>
<td>0.03±0.01</td>
</tr>
<tr>
<td>Glucose (nmol/l) fed</td>
<td>8.5±0.4</td>
<td>9.1±0.4</td>
</tr>
<tr>
<td>Glucose (nmol/l) fasted</td>
<td>3.8±0.4</td>
<td>4.1±0.4</td>
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*P<0.05, **P<0.01, ***P<0.001 compared with untreated rats (Student's unpaired t-test).
tivity to insulin appeared to be unchanged when expressed as a percentage of maximal response (Fig. 2). Insulin binding to the cells was increased significantly both per cell and per unit surface area (Table 1) and Scatchard analysis revealed that this was due to an increase in the number rather than the affinity of the receptors for insulin (Fig. 3).

Total numbers of insulin receptors in progesterone-treated rats (423,000 ± 61,000 (s.e.m.), 7 observations) were significantly increased compared with untreated rats (251,000 ± 22,000, 6 observations) whilst they were significantly decreased in cortisol-treated rats (176,000 ± 27,000, 5 observations). Insulin degradation was unaffected by any of the treatments (mean % degradation per 100,000 cells was 7.5 ± 0.8, 28 observations). Progesterone implants significantly increased serum progesterone concentrations to values similar to those found in pregnant rats but did not significantly alter serum insulin concentrations in either the fed or fasted state in normal or diabetic rats.

Cortisol implants produced essentially the opposite effects to progesterone, leading to weight loss, decreased adiposity and decreased insulin binding to isolated adipocytes per cell although not per unit surface area (Table 1). Once again the sensitivity of adipose tissue to insulin appeared to be unchanged (Fig. 2). Cortisol also produced significant increases in serum glucose and insulin concentrations in both the fed and fasted state in normal rats. Serum cortisol concentrations reached values approximately two to three times the resting corticosterone concentration in the rat.

Diabetes led to increased blood glucose concentrations with low, barely detectable insulin concentrations (Table 1). Body weight gain was reduced by approximately 80% (Table 1). Progesterone and cortisol implants produced qualitatively identical effects in diabetic and non-diabetic rats with respect to body weight changes, adiposity, glucose incorporation into lipid and insulin binding to adipocytes. Cortisol did not, however, affect serum glucose or insulin concentrations in diabetic rats and the reduction in insulin binding produced by cortisol therefore took place without increasing serum insulin concentrations. In progesterone-treated diabetic rats there appeared to be an accelerated disappearance of glucose during fasting, since an 18-h fast led to significantly lower serum glucose concentrations in progesterone-implanted animals compared with untreated or cortisol-implanted diabetic rats, suggesting an insulin-independent effect of progesterone on glucose utilization (Table 1).

**FIGURE 1.** Glucose conversion to lipid in isolated adipocytes from untreated (○), progesterone-treated (□) and cortisol-treated (▲) rats with or without concurrent diabetes induced by streptozotocin (75 mg/kg). Values are means ± s.e.m. *P < 0.05, **P < 0.01, ***P < 0.001 compared with respective untreated rats (Student's unpaired t-test).
DISCUSSION

Numerous reports have described the development of insulin resistance in response to glucocorticoid treatment in a number of different species (Conn & Fajan, 1956; Leboeuf et al. 1962; Fain, 1964; Philips et al. 1965; Yorke, 1967; Olefsky, 1975; Feldman, 1977; Cigolini & Smith, 1979; Grunfeld et al. 1981; Yasuda et al. 1982). In-vivo studies in the rat have suggested that part of the effect may be due to impairment of insulin binding to both adipocytes and hepatocytes (Olefsky et al. 1975; Kahn et al. 1978; Caro & Amatruda, 1982; Bezdrobnyi et al. 1983). In contrast, physiological hypercortisolaemia induced in man produced insulin resistance in the absence of changes in insulin binding to circulating monocytes and erythrocytes (Shamoon et al. 1980; Rizza et al. 1982). In addition, in-vitro studies have suggested increased (Knopf et al. 1978; Fantus et al. 1982) as well as decreased (Olefsky et al. 1975; Cigolini & Smith, 1979; Grunfeld et al. 1981) insulin binding in response to glucocorticoids. The absence of clear-cut evidence for a decrease in insulin binding in vitro has led to the suggestion that the decrease in insulin binding after in-vivo glucocorticoid treatment may be produced indirectly by stimulating insulin secretion which then down-regulates its own receptors (Gavin, Roth, Neville et al. 1974). The results of the present study clearly illustrate that cortisol reduces insulin binding to adipocytes in diabetic rats where no increased insulin secretion occurred. This does not, of course, rule out the possibility that increased serum insulin concentrations may play some role in this process. Indeed cortisol implants in non-diabetic rats did significantly increase serum insulin concentrations in this study, in agreement with previous findings (Conn & Fajan, 1956; Philips et al. 1965; Feldman, 1977; Grunfeld et al. 1981; Yasuda et al. 1982). More surprising was the fact that, despite the decrease in insulin binding per cell, cortisol induced no
change in the sensitivity to insulin but reduced basal and insulin-stimulated rates of lipogenesis. This in turn suggests that the number of insulin receptors per unit surface area (which was unchanged by cortisol) may be the factor which determines sensitivity rather than the total number of receptors per cell. A post-receptor site of action for corticosteroids therefore seems most likely as has been previously proposed (Olefsky, 1975; Cigolini & Smith, 1979; Grunfeld et al. 1981; Rizza et al. 1982). Such a proposal is supported by the reduced V_max for lipogenesis in cortisol-treated rats (Fig. 1). The fact that cortisol did not affect the density of insulin receptors on the cell surface suggests that the decrease in insulin binding per cell could be due solely to the decreased adipocyte size.

Progestosterone has been implicated in the insulin resistance of pregnancy since it induces increased basal and glucose-stimulated insulin release in vivo and in vitro and reduces the hypoglycaemic effect of exogenous insulin (Beck, 1969; Costrini & Kalkhoff, 1971; Howell et al. 1977; Ashby et al. 1981; Sutter-Dub & Dazey, 1981), effects similar to those which occur during pregnancy (Felig, 1975). In-vivo and in-vitro studies using rat adipose tissue have suggested that progesterone induces insulin resistance by inhibiting oxidation of [1-14C]glucose, i.e. via the pentose phosphate pathway (Sutter-Dub & Dazey, 1981). A tentative link between progesterone and insulin binding has also been proposed, since they are negatively correlated during the menstrual cycle, although the insulin receptor measurements were made in circulating monocytes which do not possess any defined insulin response (Bertoli, De Pirro & Fusco, 1980). In addition, synthetic progestagens have been reported to decrease insulin binding to rat fat cells (Krauth & Schillinger, 1977).

In apparent conflict with these findings is the increased sensitivity to exogenous insulin reported for progesterone-treated ovariectomized mice (Ahmed-Sorour & Bailey, 1980), the improved glucose tolerance in progesterone-treated ovariectomized rats (Ahmed-Sorour, Bailey & Matty, 1978) and the positive correlation between high serum progesterone concentrations during pregnancy and insulin binding, which is also increased during pregnancy in rat adipocytes (Flint et al. 1980) and hepatocytes (Flint, 1980), porcine hepatocytes (Phillips, Bonde, Mason et al. 1980) and human erythrocytes (Moore, Kolterman, Weyant & Olefsky, 1981). The present study supports these latter findings and suggests that progesterone could be the causal factor for increased insulin binding during pregnancy. The fact that progesterone increased insulin binding to adipocytes without increasing the sensitivity of the tissue to insulin does perhaps suggest some post-receptor resistance to insulin, although it is difficult to appreciate why progesterone should increase the potential of such cells to respond to insulin and then induce some form of post-receptor resistance. Progesterone-induced insulin resistance is also difficult to reconcile with the fact that, in the present study, progesterone stimulated glucose conversion to lipid in the absence of insulin. These findings are in agreement with numerous reports of increased lipid deposition in rats treated with progesterone (Felig, 1975; Shirling et al. 1981) but are in apparent conflict with previous reports of decreased glucose oxidation via the pentose phosphate pathway with no effect on oxidation of glucose via other pathways (Sutter-Dub & Dazey, 1981). These latter findings involved high doses of progesterone which could conceivably cross-react with corticosteroid receptors and might explain the inhibition of glucose oxidation observed.

It is conceivable that progesterone may have stimulated the incorporation of glucose into lipid indirectly by increasing lipoprotein lipase activity (Kim & Kalkhoff, 1975) leading to increased lipid deposition and thereby cell size which could then result in increased glucose incorporation into lipid, since this is positively correlated with cell size (Hansen, Nielsen & Gliemann, 1974). Further evidence of a direct stimulatory effect of progesterone on glucose utilization was the increased rate of glucose disappearance from the blood when diabetic rats were fasted overnight. This effect was not apparent in non-diabetic rats where insulin obviously plays the major role in glucose homeostasis but it does suggest that progesterone may play an ancillary role in glucose utilization.

In summary, these results illustrate that cortisol decreases insulin binding by a mechanism independent of hyperinsulinaemia but that post-receptor mechanisms may be more important in corticosteroid-induced insulin resistance. They also illustrate that the effects of progesterone on blood glucose, insulin receptors and lipogenesis in adipose tissue are all consistent with an anabolic, insulin-enhancing role, rather than being involved in insulin-resistant states. This strengthens the proposal that placental lactogens, and not progesterone, may be the major hormones responsible for the insulin resistance of pregnancy (Tyson & Felig, 1971).

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REFERENCES


