Direct effect of gonadal and contraceptive steroids on insulin release from mouse pancreatic islets in organ culture

Jens Høiriis Nielsen

Hagedorn Research Laboratory, DK-2820 Gentofte, Denmark

Abstract. Sex steroids are supposed to contribute to the normal glucose homeostasis and to the altered glucose and insulin metabolism in pregnancy and during contraception. In the present study isolated mouse pancreatic islets were maintained in tissue culture medium RPMI 1640 supplemented with 0.5% newborn calf serum and 100 ng/ml of one of the following steroids: oestradiol, progesterone, testosterone, megestrol acetate, medroxyprogesterone, chlormadinone acetate, norethindrone, norethindrone acetate, and ethynylestradiol. Release of insulin to the culture medium was measured during a 2 week culture period, and the islet content of insulin, glucagon, and DNA was measured at the end of the period. It was found that progesterone and its derivatives megestrol acetate, medroxyprogesterone, and chlormadinone caused a 2-fold increase in insulin release during the culture period. When islets cultured in the presence of oestradiol, progesterone, or testosterone were subjected to 30 min stimulation with 5.5, 11, 22 mmol/l glucose, only the progesterone-treated islets released more insulin in response to glucose than the control islets. It is concluded that progesterone and its derivatives have a direct effect on the glucose-stimulated insulin release probably by increasing the glucose sensitivity. The results suggest that the alterations in glucose and insulin metabolism in pregnancy and during treatment with certain oral contraceptives may in part be due to a direct effect of progestins on the β-cell.

Steroid sex hormones are supposed to contribute to the altered glucose and insulin metabolism in pregnancy (Costrini & Kalkhoff 1971) and during treatment with oral contraceptive agents (Hauschildt 1978). Since gonadal steroids are known to influence glucose metabolism (Hansen et al. 1980), it is still uncertain, however, whether the effects on the pancreatic islets are direct or indirect.

Studies on isolated pancreas or islets from pregnant rats have shown an increased glucose-induced insulin release (Malaise et al. 1969; Green & Taylor 1972; Sutter-Dub 1979). Similar effects were also observed after in vivo administration of oestrogen and/or progesterone (Sutter-Dub 1979; Howell et al. 1977; Ashby et al. 1978; Green et al. 1981). Direct exposure in vitro to the isolated perfused pancreas or islets has, however, given conflicting results (Costrini & Kalkhoff 1971; Sutter-Dub 1979; Howell et al. 1977). Since physiological effects of steroid hormones often are delayed in time, as previously demonstrated in our study of the effects of hydrocortisone on the islets (Brunstedt & Nielsen 1981), these discrepancies may be resolved in studies utilizing organ culture of isolated islets. The aim of the present study was to test whether various sex hormones affected the function of isolated mouse pancreatic islets in organ culture.

Materials and Methods

Isolation and culture of islets

Pancreatic islets were isolated from overnight fasted NMRI-mice of both sexes, weighing 20–22 g (Bomholtgaard, Ry, Denmark), by the collagenase method essentially as described previously (Brunstedt & Nielsen 1978). The excised pancreata were placed in 20 ml glass vials with 4 ml Hank’s balanced salt solution containing 20 mmol/l N-2-hydroxy-ethylpiperazine-N'-ethane sulfonic acid (HEPES) and 100 000 U/l penicillin and
100 mg/ml streptomycin and 1.5 mg/ml collagenase (type II Worthington, Freehold, N.J., USA).

The vials were incubated at 37°C for about 25 min in a solid thermostat (Grant UK) with shaking (150 r.p.m.). The islets were collected under a stereomicroscope either directly after washing or after centrifugation on a Percoll (Pharmacia, Uppsala, Sweden) gradient previously shown not to affect islet function (Brunstedt 1980). From a number of digests 500–1000 islets were pooled and about 50 islets were placed in each 60 mm bacteriological Petri dish (Nunc, Roskilde, Denmark) with 5 ml culture medium RPMI 1640 (Flow Laboratories, Irvine UK) containing 11 mmol/l glucose and supplemented with 100 000 U/l penicillin, 100 mg/ml streptomycin and 0.5% newborn calf serum (Gibco Europe, Paisley, UK). The pH of the medium was kept at 7.2 either by addition of 23.5 mmol/l bicarbonate and incubation in 5% CO2 (Figs. 1, 2 and Table 1) or by addition of 4.1 mmol/l bicarbonate with 20 mmol/l HEPES and incubation in air at 37°C (Table 2) previously shown to be equally effective in supporting islet function during culture (Brunstedt & Nielsen 1978). The medium was changed every 3–4 days. After the culture period the islets were subjected to 30 min stimulation with 5.5, 11, and 22 mmol/l glucose alone or 22 mmol/l glucose with 5 mmol/l theophylline in Hank's blanced salt solution with 20 mmol/l HEPES as described previously (Brunstedt & Nielsen 1981).

Insulin and glucagon determination
Insulin was measured in the culture media at each change, and insulin and glucagon were measured in sonicated homogenates of the islets after the culture period by radioimmunoassay with rat insulin and porcine glucagon (Novo, Copenhagen, Denmark) as standards and ethanol to separate antibody-bound and free hormone (Heding 1971). No significant decrease in the recovery of insulin added to the culture medium containing 0.5% newborn calf serum was found after incubation for up to 72 h at 37°C.

DNA determination
DNA was determined by a fluorometric method with diaminobenzoic acid (Fluka AG, Buchs, Switzerland) (Green & Taylor 1972).

Hormones
Progestrone, testosterone, ethynylestradiol (17-ethynylestradiol), norethynodrel (17α-ethynyl-17-hydroxy-5(10)-oestren-3-one), norethindrone acetate (19-nor-17α-ethynylestosterone acetate) and medroxyprogesterone (6α-methyl-17α-hydroxyprogesterone) were obtained from Sigma, St. Louis, Mo., USA; oestradiol (oestradiol-17β) and chloromadinone acetate (6-chloro-6-dehydro-17α-hydroxy-progesterone acetate) were from Merck, Darmstadt, FRG; and megestrol acetate (6-methyl-6-dehydro-17α-hydroxy-progesterone acetate) was kindly supplied by Novo A/S, Copenhagen, Denmark. All hormones were dissolved in 96% ethanol, and 5 µl hormone stock solutions were added to 5 ml of culture medium to give a final hormone concentration of 100 ng/ml in the medium. The same amount of ethanol was added to the control medium.

Statistical evaluation
Wilcoxon's test for paired observations was employed. In the figures and tables means ± SEM of the number (n) of dishes are indicated.

Results
Culture of islets in the presence of 100 ng/ml progesterone resulted in an enhanced release of insulin while oestradiol and testosterone had no effect on insulin release. The effect of progesterone (o), oestradiol (×), and testosterone (△) on insulin release from mouse islets maintained in organ culture in medium RPMI 1640 supplemented with 0.5% newborn calf serum (●) and 100 ng/ml of the hormones. The results are the accumulated values of insulin determination in the culture medium at each medium change. The bars indicate SEM (n = 10). Only the insulin release from the progesterone treated islets was significantly different from the controls on day 7, 10 and 14 (2α ≤ 0.01, Wilcoxon's paired test).

Fig. 1.
Insulin release in response to glucose 5.5, 11, 22, and 22 mmol/l plus 5 mmol/l theophylline (Th) from mouse islets cultured for 14 d in the absence (□), or presence of progesterone (■), oestradiol (▲), or testosterone (●), ng/ml. The ordinate indicates the insulin release in ng per islet during a 30 min incubation period. The bars indicate SEM (n = 8). Only the release from the progesterone treated islets was significantly different from the controls (2α < 0.01, Wilcoxon’s paired test).

Effect (Fig. 1). The glucose-induced release during short-term (30 min) serial incubations following the culture period was increased (2α < 0.01) in islets exposed to progesterone when compared with the islets cultured without hormones, while no significant differences were found in islets exposed to oestradiol or testosterone (Fig. 2). The difference was more pronounced when glucose was present alone than when both glucose and a phosphodiesterase inhibitor theophylline was added.

No difference was observed in islet DNA content in response to any of the hormones while the insulin content was reduced in islets cultured in the presence of progesterone (Table 1).

Islets were cultured in the presence of a number of synthetic steroids being constituents of oral contraceptive drugs and effects on insulin release compared during day 10 to 14 (Table 2). Only megestrol acetate, medroxyprogesterone and chlormadinone acetate caused a stimulation similar to progesterone while neither norethynodrel, nor-ethindrone acetate nor ethynylestradiol had any effect differing from the controls. Insulin, glucagon, and DNA content in the islets was measured

Table 1.
Effect of natural sex steroids on insulin and DNA content of cultured mouse islets. Hormone concentration 100 ng/ml. Insulin and DNA in ng/islet ± SEM (n = 10).

<table>
<thead>
<tr>
<th>Addition</th>
<th>Insulin content ng/islet</th>
<th>DNA content ng/islet</th>
<th>Insulin/DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>44.3 ± 8.5</td>
<td>17.0 ± 2.4</td>
<td>2.3 ± 0.3</td>
</tr>
<tr>
<td>Progesterone</td>
<td>28.8 ± 4.1*</td>
<td>17.7 ± 2.0</td>
<td>1.6 ± 1.0*</td>
</tr>
<tr>
<td>Oestradiol</td>
<td>47.7 ± 10.6</td>
<td>19.1 ± 2.4</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>Testosterone</td>
<td>39.3 ± 7.0</td>
<td>18.1 ± 3.0</td>
<td>2.2 ± 0.1</td>
</tr>
</tbody>
</table>

* 2α ≤ 0.01 (Wilcoxon’s paired test).
after the culture period (Table 2). There was a tendency to reduction in insulin and glucagon content of those islets showing elevated insulin release although it was most pronounced in the islets exposed to progesterone.

**Discussion**

The increased β-cell mass and the reduced susceptibility to diabetogenic agents found in female animals compared with males (Bonnevie-Nielsen 1980; Kromann et al. 1982), a difference which disappears after ovariectomy or by testosterone treatment (Houssay 1960) indicate an important role of the gonadal hormones in the regulation of glucose and insulin metabolism. The relative roles of the individual hormones as well as their mechanisms of action are poorly understood. Thus both the peripheral responsiveness to insulin and the responsiveness of the β-cell to glucose may be involved.

Although a similar effect of progesterone and oestradiol was found on insulin secretion in vivo (Bailey & Ahmed-Sorour 1980) the mechanism may be quite different. Thus oestrogens reduce food intake and body weight which may be secondary to metabolic changes as indicated by the reduced insulin-induced lipogenesis in isolated fat cells from ovariectomized rats treated with oestradiol (Hansen et al. 1980). A direct effect on parameters other than insulin secretion may be indicated by the demonstration of oestrogen receptor in the islets (El Seifi et al. 1981; Tesone et al. 1979) and the in vitro resistance of female islet cells to the cytotoxic action of streptozotocin (Kromann et al. 1982). Similarly, the deteriorating effect of testosterone on glucose tolerance (Bailey & Matty 1972) and experimental diabetes (Houssay 1960) may be indirect (cf. Fig. 1, Table 1).

There is general agreement on increased glucose-induced insulin release from islets of pregnant rats (Malaisse et al. 1969; Kalkhoff & Kim 1978; Green et al. 1978b), an effect which can be mimicked by progesterone treatment (Costrini & Kalkhoff 1971; Ashby et al. 1978) although the interpretations are conflicting. Thus, Sutter-Dub (1979) concluded that progesterone acts indirectly by causing peripheral insulin resistance. This conclusion disagrees with the present results and with the results of Howell et al. (1977) who found a small but significant increase in insulin secretion and biosynthesis in isolated islets after prolonged incubation with progesterone. Furthermore, progesterone receptors have been demonstrated in isolated islets (El Seifi et al. 1981; Green et al. 1978a).

The results from the acute glucose stimulation experiments (Fig. 2) indicate that the sensitivity to glucose rather than the maximal response in the presence of theophylline is increased. Whether this change is associated with a change in the cAMP-phosphorylation system remains to be determined (Lipson & Sharp 1978; Ashby et al. 1978). The

### Table 2.

<table>
<thead>
<tr>
<th>Addition</th>
<th>Insulin release</th>
<th>Insulin content</th>
<th>Glucagon content</th>
<th>DNA content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (16)</td>
<td>19.8 ± 2.5</td>
<td>30.2 ± 2.8</td>
<td>0.82 ± 0.07</td>
<td>14.5 ± 0.6</td>
</tr>
<tr>
<td>Progesterone (14)</td>
<td>74.3 ± 7.6**</td>
<td>23.8 ± 3.7**</td>
<td>0.66 ± 0.08**</td>
<td>14.5 ± 1.5</td>
</tr>
<tr>
<td>Megestrol acetate (8)</td>
<td>70.2 ± 13.7**</td>
<td>25.6 ± 2.6</td>
<td>0.55 ± 0.06*</td>
<td>13.5 ± 1.3</td>
</tr>
<tr>
<td>Medroxyprogesterone (8)</td>
<td>49.3 ± 7.5**</td>
<td>22.0 ± 2.4*</td>
<td>0.72 ± 0.13</td>
<td>13.0 ± 0.6</td>
</tr>
<tr>
<td>Chlormadione acetate (8)</td>
<td>45.0 ± 11.8**</td>
<td>28.9 ± 3.9</td>
<td>0.68 ± 0.12</td>
<td>14.7 ± 0.8</td>
</tr>
<tr>
<td>Norethynodrel (8)</td>
<td>22.7 ± 3.9</td>
<td>28.0 ± 3.9</td>
<td>0.70 ± 0.05</td>
<td>11.4 ± 1.6</td>
</tr>
<tr>
<td>Norethindrone acetate (8)</td>
<td>22.0 ± 2.4</td>
<td>33.5 ± 2.7</td>
<td>0.92 ± 0.07</td>
<td>13.5 ± 2.0</td>
</tr>
<tr>
<td>Ethynylestradiol (8)</td>
<td>21.1 ± 4.6</td>
<td>34.8 ± 3.7</td>
<td>0.89 ± 0.07</td>
<td>12.6 ± 1.7</td>
</tr>
</tbody>
</table>

* 2α ≤ 0.05. ** 2α ≤ 0.01. (Wilcoxon’s paired test).
reduced insulin content of the islets exposed to progesterone (Table 1) does not indicate an increased insulin biosynthesis as explanation for the increased insulin release. Alternatively, it may be due to an increased intracellular degradation of insulin induced by progesterone (Borg 1982).

The hyperplasia of β-cells found in islets isolated from pregnant rats (Green & Taylor 1972) was, however, not seen in the progesterone-treated islets as reflected in the unchanged DNA content (Table 1), and recent studies indicate that this phenomenon may rather be due to placental lactogen and/or prolactin, which were shown to have a direct stimulatory effect on the DNA synthesis in isolated islets in culture (Nielsen 1982).

The information from the numerous studies on the effects or oral contraceptive agents on glucose and insulin metabolism is conflicting partly because of the variety of hormone constituents. In a comprehensive review Hauschildt (1978) concluded that contraceptives containing nortesterone derivatives more often induced impaired glucose tolerance than those containing progesterone derivatives, and the progesterone-derived chlormadinone acetate was reported to improve glucose tolerance in subtotally pancreatectomized rats (Goberna et al. 1971).

The trends of these in vivo observations agree with and may in part be explained by the present in vitro studies. Thus the gestagens derived from progesterone (megestrol acetate, medroxyprogesterone and chlormadinone acetate) stimulated the insulin release from the islets (Table 2), while neither the 19-nortestosterone derivatives (nortydroxy and nordihydro acetate) nor the oestrogen (ethynyloestrodiol) had any effect on insulin release or the islets' content of insulin, glucagon and DNA (Table 2).

In conclusion, these results suggest that progesterone and its contraceptive synthetic analogues have a direct effect on the endocrine pancreas by increasing the glucose sensitivity of the β-cell. This mechanism may explain the hyperinsulinaemia seen in pregnancy and during treatment with certain oral contraceptive agents.

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References


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