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ISLET CELL GROWTH AND FUNCTION

A REAPPRAISAL OF THE ROLE OF PROGESTERONE AND PREDNISOLONE

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Abstract—The mechanism of the inhibitory effect of steroid hormones, progesterone and prednisolone on the incorporation of [^{3}H]-thymidine into pancreatic islet cell DNA was investigated. Treatment with either hormone had no effect on the incorporation of ^{32}P-orthophosphate into islet cell DNA. Both prednisolone (10 μM) and progesterone (3 μM) markedly stimulated the activity of the enzyme thymidylate synthetase of islet cells possibly leading to increased synthesis of endogenous thymidine which resulted in dilution of the [^{3}H]-thymidine added to the islets in tissue culture. Prednisolone (10 μM) significantly increased both insulin biosynthesis and release, while at 5 μM it was effective in increasing only insulin release. In contrast, progesterone at the two concentrations employed did not affect insulin biosynthesis or release. The smaller doses of both hormones markedly stimulated the total protein biosynthesis.

The diversity of the responses of pancreatic islet cells to steroid hormones instigated controversy as to the actions of these hormones. Thus pregnancy, with its plethora of circulating progesterone and estradiol, was shown to be associated with increased mitotic frequency in islet cells [1, 2], islet hypertrophy [3], and increased synthesis of DNA and insulin, and of insulin secretion [4]. These effects could be abolished by restricting the food intake of pregnant animals to that of controls, which suggests that sex steroids may not be directly involved in these responses [5]. On the other hand, ovariectomy was found to result in increased islet cell DNA synthesis [6] which implies that they exert an inhibitory role on this process.

Several studies reported that glucocorticoids stimulate beta cell growth and replication *in vivo* [7, 8], and of insulin secretion in man [9]. In contrast, corticosterone was reported to directly inhibit insulin release both *in vitro* and *in vivo* [10] as did dexamethasone *in vitro* [11]. These responses were obtained with adult islets. In contrast, it was shown that fetal islet beta cell growth *in vitro* could be stimulated by low doses and inhibited by high doses of adrenal glucocorticoids [12, 13].

Recently, it was observed that steroid hormones *in vitro* decreased the rate of incorporation of tritiated thymidine into fetal islet DNA. In contrast, the incorporation of ^{32}P-orthophosphate into fetal islet DNA was not affected by this treatment [14]. This discrepancy was attributed to dilution of the radioactively-labeled thymidylate pool consequent upon stimulation of the activity of thymidylate synthetase by steroid hormones [14]. Since ovariectomy resulted in increased islet DNA synthesis [6], implying that ovarian steroids inhibit this process in adult rat islets, we decided to verify whether this inhibition could also be apparent due to dilution of radioactively-labeled thymidylate pool.

MATERIALS AND METHODS

Preparation and culture of islets. Pancreases were removed aseptically from adult male Sprague–Dawley rats weighing 200–250 g. Islets were isolated from the pancreatic tissue by collagenase digestion as described previously [15]. After washing with Hanks balanced salt solution, HBSS (Flow Laboratories, U.K.) batches of approximately 200 islets were transferred to 5 cm culture dishes that do not permit cell attachment (Sterilin, England) containing tissue culture medium RPMI 1640 (Flow) with 5.5 mM glucose and supplemented with 20 mM Hepes buffer (Flow), antibiotics (penicillin 100 U/ml, streptomycin 0.1 mg/ml; Flow) and 10% heat-inactivated fetal bovine serum (Flow). Cultures were incubated at 37° in a humidified atmosphere of 5% CO_2 in air for 48 hr. Islets were then transferred to "treatment medium" consisting of RPMI 1640 and supplemented as described above with or without steroid hormones added to the following final concentrations: prednisolone 1, 5, 10 or 100 μM (Merck, Sharp & Dohme International, West Point, PA) or progesterone 0.3 or 3.0 μM (Sigma Chemical Co. St. Louis, MO). Prednisolone was dissolved directly in culture medium while progesterone was initially dissolved in 70% ethanol and subsequently diluted 1:1000 with culture medium to attain the appropriate concentrations. Ethanol in concentrations similar to those introduced with progesterone was added to the control cultures. Islets were maintained free-floating for a further three-day period (treatment period) during which the treatment medium was changed daily.

^{32}P-orthophosphate incorporation into islet cell DNA. At the end of the treatment period, islets were incubated with ^{32}P-orthophosphate and processed as

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described earlier [14]. Following the final wash, islets in paired groups of 30 were transferred to plastic tubes, sonicated and treated for 1 hr at 37° either with 100 μl of 10 mg/ml pronase in 0.154 M NaCl (Sp. act. 6 U/mg; Boehringer Mannheim GmbH, W. Germany), or the equivalent volume of 0.154 M NaCl. This treatment hydrolyzes all proteins and phosphoproteins that may precipitate with the DNA in subsequent steps. At the end of the incubation period, each group of islets was treated with 0.5 ml of 0.3 M NaOH and incubated for 18 hr at 37° to saponify and solubilize membrane lipids, and to selectively hydrolyze RNA [16–18]. After duplicate 100 μl aliquots of the lysates were withdrawn for DNA estimation [19], the remaining lysates were cooled on ice, acidified with 5 M perchloric acid and filtered through glass microfiber filter discs (Whatman Ltd., England). The unbound ^{32}P, in the form of free nucleotides and saponification products were washed off the filter discs with cold 5% TCA until filtrates contained constant background radioactivity. The filters were further washed with 96% ethanol twice, dried overnight at room temperature, and the retained radioactivity representing DNA was determined by liquid scintillation spectrometry. The incorporation of ^{32}P-orthophosphate was expressed as dpm/μg DNA.

Incorporation of thymidine into islet cell DNA. After the treatment period, islets in the appropriate "treatment medium" were incubated for a further 24 hr with 1μCi/ml of [methyl-^3H]-thymidine (5 Ci/mmol, Amersham). The islets were then processed as described earlier [20].

Thymidylate synthetase activity. The tritium-release method of Dunlap [21] as modified in [14] was used and the results expressed as pmol/μg DNA/hr.

Insulin biosynthesis and release. Groups of 20 islets were used to study insulin biosynthesis and release as described in [20].

Statistical analyses. Data represented the mean ± SEM of the number of observations shown in parentheses (N). Significance of the differences from the control condition were tested by the two-tailed Student's *t*-test.

RESULTS

The incorporation of [^3H]-thymidine over a period of 24 hr into DNA of adult rat islets in culture is shown in Table 1. Progesterone at 0.3 μM concentration did not affect the rate of [^3H]-thymidine incorporation into DNA. Increasing the steroid hormone concentration 10-fold to 3 μM resulted in a statistically significant decrease of 26% in the incorporation of radioactively labeled thymidine into DNA during a 24 hr culture period. A more marked decrease was observed when the islets were treated with prednisolone at final concentrations greater than 1 μM in the culture medium. The decreases in [^3H]-thymidine incorporation into DNA in response to treatment with 5, 10 and 100 μM prednisolone were 48, 56 and 43% respectively. In contrast with the above findings, DNA synthesis determined as the incorporation of ^{32}P-orthophosphate into adult rat islet DNA during a 24 hr culture period was neither affected by 10 μM prednisolone nor by 3 μM progesterone which induced a significant reduction in the [^3H]-thymidine incorporation experiments.

The effect of treating the cultured islets with 3 μM progesterone or 10 μM prednisolone on the activity of the enzyme thymidylate synthetase which methylates deoxyuridylate to thymidylate is shown in Table 1. This reaction, in addition to the salvage pathway, provides the cell with the thymidylate required for DNA synthesis. Both hormones significantly increased the activity of the synthetase.

The effects of steroid hormones on the biosynthesis of (pro)insulin and of total protein in cultured islets are given in Table 2. Both concentrations of progesterone tested did not alter the synthesis of (pro)insulin by the islets but increased total protein biosynthesis especially at the low dose of progesterone ($P < 0.01$). In the presence of 10 μM prednisolone, the islets responded with a significant increase in the production of newly-formed (pro)insulin while the low dose was ineffective. Similar to progesterone at 0.3 μM, prednisolone at the low concentration increased the incorporation of [^3H]-phenylalanine into total TCA-precipitable islet protein ($P < 0.05$). the higher doses of the two steroids did not affect the total protein biosynthesis when compared with the control.

Figure 1 reveals that progesterone at both the low and the high concentrations did not alter the rate of insulin secretion during the 2 hr incubation period. In contrast, prednisolone (5 μM) significantly stimulated the insulin output by the cultured islets ($P < 0.01$). Doubling the concentration of prednisolone in the culture medium resulted in more pronounced insulin secretion by islets ($P < 0.005$).

DISCUSSION

Early observations [1, 2] suggested that pregnancy could provide a stimulus for DNA synthesis in islet cells reflected as increased mitotic frequency. Alterations in beta cell mitotic activity could be correlated with changes in their functional activity expressed as the quantity of insulin they secrete [22]. With the widespread use of [^3H]-thymidine incorporation into DNA as an index of replicative activity, caution in the interpretation of the findings was called for since the [^3H]-thymidine labeling index could be explained in terms of altered uptake and or subsequent metabolism leading to changes in the specific activity of the DNA precursor pool [23]. This has since been confirmed as the mechanism underlying the apparent inhibition of DNA synthesis in rat fetal islet cells by steroid hormones [14]. In the present study with adult rat islets *in vitro*, there is a significant negative correlation between the degree of [^3H]-thymidine incorporation into DNA and the activity of thymidylate synthetase ($r^2 = 0.92$). This adds further support to the proposed dilution of the specific activity of the thymidylate pool as a cause of apparent reduction of incorporation of [^3H]-thymidine into DNA in islets treated with progesterone or prednisolone, and confirms the observations of Chick [11] and of Green *et al.* [6] but negates the contention that in pregnancy steroids are directly responsible for the associated islet cell hypertrophy. This

Table 1. DNA synthesis in cultured adult rat islets of Langerhans

Condition	Incorporation of (dpm/μg DNA)		Thymidylate synthetase (pmol/μg DNA/hr)
	^{3}H-Thymidine	^{32}P	
Control	1586 ± 109 (8)	2487 ± 148 (7)	0.53 ± 0.02 (6)
Progesterone [0.3 μmol/l]	1674 ± 166 (7)	—	
[3.0 μmol/l]	1171 ± 84 (7)†	2509 ± 322 (15)	0.65 ± 0.04 (3)*
Prednisolone [1 μmol/l]	1434 ± 128 (7)‡	—	
[5 μmol/l]	824 ± 109 (8)‡	—	
[10 μmol/l]	683 ± 112 (10)‡	2257 ± 158 (6)	1.14 ± 0.05 (3)‡
[100 μmol/l]	905 ± 86 (16)‡	—	

Data represent the mean ± SEM of the numbers of observations shown in parentheses. Statistically significant differences from the control values are denoted by *P < 0.05; †P < 0.01 and ‡P < 0.001.

Table 2. Steroid modulation of insulin biosynthesis in cultured adult rat islets of Langerhans

Condition	(N)	Insulin/(pro)insulin	TCA precipitate	%
Control	14	1.75 ± 0.16	7.02 ± 0.36	24.9 ± 2.14
Progesterone [0.3 μmol/l]	5	2.08 ± 0.19	9.04 ± 0.55†	23.0 ± 0.92
[3.0 μmol/l]	6	1.98 ± 0.10	7.70 ± 0.53	29.8 ± 1.19
Prednisolone [5.0 μmol/l]	8	2.05 ± 0.29	9.73 ± 1.23*	21.3 ± 2.07
[10.0 μmol/l]	8	2.28 ± 0.17*	8.16 ± 1.16	30.1 ± 2.60

Insulin (pro)insulin biosynthesis in cultured adult rat islets was estimated by measuring the incorporation of ^{3}H-phenylalanine into both the islet insulin/(pro)insulin and the TCA-precipitable protein fraction after incubation in RPMI containing 5.5 mmol/l glucose for 2 hr. Details of islet culture, incubation, and labeled protein analysis are given in Methods section. The figures are given as dpm × 10^{-5}/μg DNA for a 2-hr period. The values in column (%) refer to the percentage of the total incorporated radioactivity represented by the (pro)insulin/insulin fraction as calculated from each individual observation. Each value is the mean ± SEM of the number of observations in column (N). Significance of the difference between values obtained from control (untreated) adult islets: *P < 0.05; †P < 0.01.

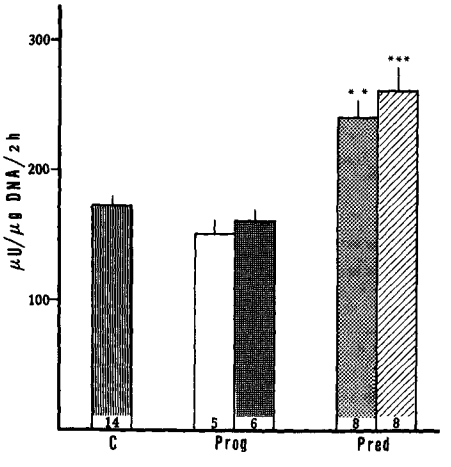

Fig. 1. Effect of progesterone (Prog) and prednisolone (Pred) on the glucose-stimulated insulin release from isolated adult rat pancreatic islets. Insulin release was measured in a 2 hr incubation period in the presence of the following steroid concentrations: progesterone 0.3 μM □ and 3.0 μM ▩; prednisolone 5 μM ▨ and 10 μM ▨ respectively. Bars represent the mean ± SEM of the numbers of observations shown at the bottom of each. Statistical significance of differences from control islets (c) ▥ is indicated by **, P < 0.01 and ***, P < 0.005.

hypertrophy could well be secondary to changes in the availability of nutrients since restriction of food intake abolishes it [5]. Since a similar decrease in [^{3}H]-thymidine incorporation was observed with fetal rat islets cultured in the presence of steroid hormones [14] it may be concluded that the effect is in part directly related to increased activity of thymidylate synthetase. Thus the observed inhibition of replication is only apparent due to dilution of the specific activity of the added [^{3}H]-thymidine consequent upon the synthesis of increasing amounts of endogenous thymidylate. This is further supported by the observation that the incorporation of ^{32}P-orthophosphate into islet cell DNA is unaffected by the presence of steroid hormones during the culture period since ^{32}P-orthophosphate labels the entire thymidine pool by the action of thymidylate kinase.

The present observation that high concentrations of prednisolone augmented the glucose-stimulated insulin release from adult islets in culture is in agreement with our previous observation with fetal islets [20]. However, it contrasts with other reports that several adrenal steroids directly inhibit insulin release *in vitro* [10–12]. It is possible that differences in the structure between the steroid used in the present study and those used in the other reports may account for the different responses. Thus it has

been proposed that 11β and 21 hydroxylations of the steroid nucleus are essential for differentiation of fetal and neonatal pancreas and for inhibition of insulin release *in vitro* [24]. It is also possible that differences in culture conditions could, in part, account for these divergent observations.

With regard to insulin biosynthesis, again prednisolone was effective in stimulating this process when present at high concentrations in the culture medium. The non-hydroxylated steroid progesterone did not increase (pro)insulin synthesis significantly. However, both progesterone and prednisolone stimulated total protein synthesis when present in low concentrations but were ineffective at high concentrations, probably due in part to non-specific toxic effects of high steroid concentrations, although the continuing stimulation of insulin secretion would argue against this. The similarity in response to both steroids disfavors the hydroxylation hypothesis of McEvoy and Hegre [24] and propounds that alterations in beta cell mitotic activity could be correlated with changes in their function as reflected by the quantity of insulin released [22] and synthesized [20].

In conclusion, steroid hormones do not appear to affect islet cell DNA synthesis *in vitro*. They affect, however, the rate of protein biosynthesis paradoxically, increasing it at low, and failing to affect it in the same way at high, concentrations. On the other hand, the trihydroxylated steroid hormone prednisolone tends to augment insulin release in a dose-dependent manner.

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