Relative Effects of Pregnancy, Estradiol, and Progesterone on Plasma Insulin and Pancreatic Islet Insulin Secretion

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Abstract Influences of estrogen and progesterone on the development of hyperinsulinemia and augmented pancreatic islet insulin secretion during pregnancy were assessed in this study. Groups of female rats were injected subcutaneously for 21 days with varying daily dosages of estradiol benzoate or progesterone in oil. On day 21, pancreatic islets were isolated by a collagenase method. Total insulin secretion was measured after 90-min incubations of 10 islets in buffered medium containing glucose. Higher physiologic dosages of estradiol or progesterone, singly or in combination, significantly increased islet secretion above values of untreated control rats and were comparable to augmented islet responses of term, 3-wk pregnant rats. Diameter and protein content of islets obtained from steroid-treated and pregnant rats exceeded control measurements in these instances. However, 2-hr preincubations of control islets with 1 or 10 μg/ml of either steroid did not influence subsequent glucose-stimulated insulin output.

In related studies, plasma insulin responses during 30 min intravenous glucose tolerance tests were significantly above control responses in term-pregnant rats and animals receiving comparable dosages of steroids for 3 wk. Unlike pregnancy or progesterone treatment, estradiol administration alone or with progesterone significantly lowered postchallenge plasma glucose concentrations.

These results indicate that estradiol and progesterone contribute to enhanced islet insulin secretion and plasma insulin responses to glucose administration during pregnancy. This change is not acutely produced but can be related to hypertrophy of islets following chronic hormonal administration. Although the data do not distinguish between direct and indirect beta-cytotrophic effects of these sex steroids, metabolic actions of estradiol and progesterone may differ, since estrogen treatment lowers plasma glucose curves following the induction of hyperinsulinemia.

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INTRODUCTION

During the course of human gestation augmented plasma insulin responses to a variety of insulinogenic stimuli occur. Carbohydrate tolerance is only minimally affected in normal pregnant women but often markedly deteriorates among gravid subjects with perexisting subclinical or overt diabetes, despite hyperinsulinemia (1–4). These changes very likely reflect a state of insulin antagonism. Since the intensification of this stress accompanies the growth and development of the fetal-placental unit and quickly disappears after parturition, it is logical to assume that the factors elaborated by this unit are primarily responsible for the adverse changes observed.

There is now good evidence that one placental hormone, human placental lactogen, contributes to the contra-insulin effects of human pregnancy (5–7). Nevertheless, one cannot ignore the possible modifying influence of the placental steroids, estrogens and progesterone, on the development of this condition, since the plasma concentrations of these hormones also increase markedly with advancing pregnancy (8) and parallel the rising titers of human placental lactogen (9). Moreover, sex steroids have been shown to influence carbohydrate

metabolism in many instances in both animals and man (10).

In the present study, the relative effects of pregnancy, estrogen and progesterone on carbohydrate tolerance, plasma insulin, and insulin secretion from isolated pancreatic islets, were assessed in the rat. The results indicate that both steroids significantly alter these parameters.

METHODS

Animals and diet. Sprague-Dawley virgin female rats, weighing 270-290 gm, and age-matched pregnant rats, in various stages of gestation, were employed in this study. Duration of pregnancy was estimated from the date of positive sperm tests and confirmed by allowing some animals that were excluded from the study to reach parturition. The average number of fetuses per pregnant animal was 10, and rats with less than 8 fetuses were not studied. The rats were housed in air-conditioned quarters at 72°F which provided light from 6 a.m. to 6 p.m. Liberal quantities of water and chow pellets with a caloric distribution of 58% carbohydrate, 11% fat, and 31% protein were provided.

Chemicals and reagents. Purified progesterone (pregn-4-ene-3-20-diol), estradiol (1, 3, 5, [10] estratrien-3, 17β-diol), estradiol benzoate, and bovine serum albumin (fraction V), were purchased from Sigma Chemical Co., St. Louis, Mo. Hanks balanced salt solution was obtained from Grand Island Biological Co., Grand Island, N. Y. Collagenase was supplied by either Nutritional Biochemicals Corp., Cleveland, Ohio, or Worthington Biochemical Corp., Freehold, N. J. Glucostat®, a commercially available kit for determining plasma glucose by a glucose oxidase method, was also obtained from Worthington Biochemical Corp.

Hormone treatment. Progesterone and estradiol benzoate were dissolved in sesame oil according to the method of Forbes (11) and stored at 45°C to prevent crystallization. Virgin normal rats were injected subcutaneously every 12 hr with either hormone separately or both hormones in combination for 21 days. Control rats received sesame oil alone. Total daily dosages for various groups of animals were 1.25, 2.5, and 5.0 mg of progesterone or 1.25, 2.5, and 5.0 μg of estradiol benzoate, respectively. Combined dosages were similar and always in a ratio of 1:1000 by weight of estradiol benzoate to progesterone (e.g., 1.25 μg of estrogen mixed with 1.25 mg of progesterone, etc.). Volume of each injection was 0.25 ml. Injection sites were rotated.

Intravenous glucose tolerance tests. After an overnight 12 hr fast all rats were wrapped gently in a towel with a small hole for nose breathing. A 50% solution of glucose was injected rapidly into a tail vein in a dosage of 1 mg/g body weight. Blood samples from tail tips were collected in heparinized tubes at 0, 10, 20, and 30 min and placed on ice. After centrifuging each tube at 4°C, we removed the plasma and froze it for subsequent determinations of glucose by a glucose oxidase method (Glucostat®) and of immunoreactive insulin with human insulin as standard (12). Hormone-treated rats received their last injection 2 hr before glucose tolerance tests.[1]

[1] In a preliminary study, injection of saline instead of glucose into tail veins of pregnant or hormone-treated rats similarly wrapped in towels was not associated with any changes of fasting plasma glucose or insulin concentrations during a 30 min period. Administration of anesthesia before the procedure was not required.

When glucose tolerance was studied in groups of pregnant or hormone-treated animals, appropriate control rats were included on a given test day. In addition, plasma samples obtained from both control and experimental rats on a specific day were analyzed for glucose and insulin concentrations in the same assays in duplicate.

Isolation of pancreatic islets. Control, pregnant, and hormone-treated rats that did not have assessments of intravenous glucose tolerance were decapitated and the abdomen was opened. Methods for retrograde perfusion of the pancreas with cold Hanks solution, removal of the organ, separation of individual islets of Langerhans from acinar tissue in collagenase incubates, and washing procedures have been described previously by Lacy and Kostianovsky (13). Collagenase incubations were monitored by removing portions at frequent intervals and examining the degree of separation under a stereomicroscope. The duration of appropriate incubation varied from one batch of enzyme to the next, but usually from 15 to 18 min were required. The final preparation of islets was contained in cold Hanks solution in a Petri dish that was placed in a larger Petri dish containing ice and water. Under a stereomicroscope, individual islets were identified and groups of 10 were transferred rapidly to incubation flasks with a 500 μl micropipette. Usually from three to four groups were transferred per rat pancreas, and only intact islets of uniform size were selected. Islets from groups of control and experimental animals were isolated and studied on the same day.

Pancreatic islet incubation procedures. Each group of 10 islets was placed in 5 ml Erlenmeyer flasks containing 2.0 ml of 2% albumin-Krebs-Henseleit bicarbonate buffer (14) with 5.5 mM concentrations of sodium fumarate, pyruvate, and glutamate at pH 7.4.[2] Two 0.025 ml portions of medium were removed at 0 time after which glucose in 0.5 ml volume was added in a final concentration of 3.0 mg/ml. After sealing, the flasks were agitated at 37°C in a Dubnoff metabolic shaker in an atmosphere of 95% O_2–5% CO_2 for 90 min. Two 0.025 ml portions were removed again at the end of incubation. Both 0 and 90 min samples were placed in suitable volumes of 5% albumin–0.075 M veronal buffer, pH 8.6, and frozen for subsequent insulin determinations. The difference between 0 and 90 min samples was recorded as total insulin secreted per 10 islets in 90 min. In all in vitro studies, insulin concentrations in media obtained from both control and experimental islet incubations on a given day were measured in the same insulin immunoassays.

Hormone-pancreatic islet incubation procedures. Quantitative dissolution of estradiol or progesterone into buffer was accomplished by the method of Lyngset and Velle (15). Portions of either hormone in 95% ethanol were added to small incubation flasks. Ethanol was evaporated at room temperature. The steroids were removed from the glass by introducing 1.0 ml of buffer and incubating them in a Dubnoff shaker for 30 min at 37°C. At this point, 10 pancreatic islets were transferred to the medium, and the total volume was brought to 2.0 ml with additional buffer and glucose in a final concentration of 0.5 mg/ml. The concentration of hormone was either 1 or 10 μg/ml. Preincubations were carried out for 2 hr at 37°C. Subsequently, the medium was carefully aspirated and the flasks and islets were repeatedly washed with warm buffer. The volume of buffer without steroids was brought to 2 ml. Samples of media were taken for insulin determinations at 0 min and again at 30 and 60 min after addition of glucose and incubations at 37°C.

[2] Hereafter, the word "buffer" refers to this specific type unless stated otherwise.

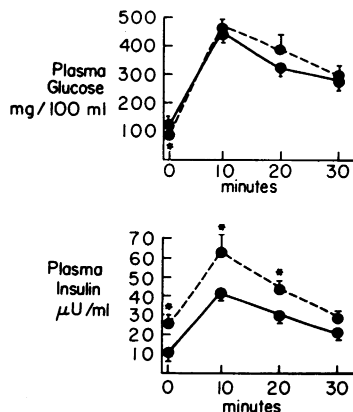

FIGURE 1 Intravenous glucose tolerance tests in 10 3-wk pregnant rats (broken lines) and in 10 control-animals (solid lines). Values are mean ±SE. Asterisk signifies the difference between mean values of pregnant and control groups, $P < 0.05$.

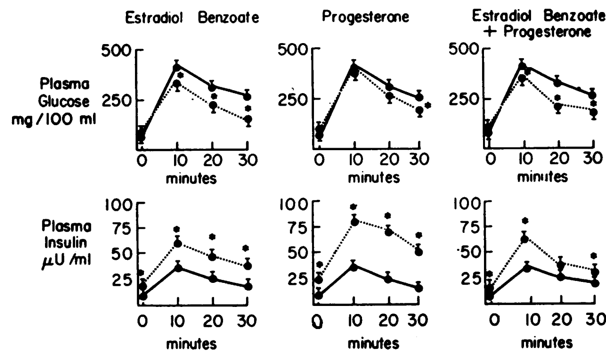

FIGURE 2 Intravenous glucose tolerance tests in 3 groups of 10 rats treated with steroids for 3 wk (broken lines) and in 10 control animals (solid lines). Daily dosages of estradiol benzoate or progesterone were 2.5 µg and 2.5 mg, respectively, administered separately or in combination. Values are mean ±SE. Asterisk signifies the difference between means of control and hormone-treated rats, $P < 0.05$.

Groups of control islets were treated in a similar fashion except that the original evaporation step was done with 95% ethanol that did not contain estrogen or progesterone.

In other studies the preincubation step with hormones was eliminated. Ten islets were transferred directly to 2.0 ml of buffer containing 1 or 10 µg/ml of estradiol or progesterone. Samples for insulin determinations were removed at 0 time and every 30 min for 90 min after additions of glucose and incubations at 37°C.

Measurements of pancreatic islet size and protein content. Pancreatic islets were isolated from organs of control, pregnant, and hormone-treated animals and transferred in a small volume of buffer evenly spread over a hemocytometer mounted on a stereomicroscope. The diameter of each islet was measured by a single determination.[3] Irregularly shaped or extremely large islets were purposely avoided. Other groups of 10 islets were homogenized in 10% trichloracetic acid with small ground glass tissue homogenizers, and samples were removed for protein determinations (17). In all instances, one individual selected islets for measurements of size and protein content and was not told from what group of animals the islets were removed.

Statistical analyses. Measured parameters of control and experimental groups were compared statistically by applying the Student's *t* test to unpaired data. In other instances, paired data analysis with the Student's *t* test was employed to compare changes within the same group (18).

RESULTS

Animal body weights. After 3 wk of sesame oil injections, body weight of control female animals was 309 ±3 g. Weights of rats treated for the same period with estradiol benzoate, progesterone, or the two hormones in combination, were within 10% of the control value. Weight of 1 wk pregnant rats (312 ±4 g) was similar to that of the control group, but rats pregnant for 2 wk (341 ±6 g) or 3 wk (381 ±9 g) were significantly heavier than control animals.[4]

Intravenous glucose tolerance tests. Late pregnancy in the rat was associated with decreased concentrations of fasting plasma glucose, but there was no change in glucose tolerance (Fig. 1). Plasma insulin levels were significantly greater than control values at 0, 10, and 20 min.

During a 3-wk period, dosages of estradiol benzoate (2.5 µg/day), progesterone (2.5 mg/day), separately or in combination, were administered to three groups of rats and resulted in significant elevations of most plasma insulin concentrations during glucose tolerance tests (Fig. 2). Exaggerated plasma insulin responses also were associated with a significant lowering of glucose concentrations at 10, 20, and 30 min in those animals given estrogen alone or in combination with progesterone. Progesterone administration by itself reduced only the 30 min glucose level despite insulin responses that were 2- to 3-fold greater than control values.

Pancreatic islet insulin secretion during pregnancy. Pregnant animals were studied at various intervals during a normal 3 wk gestation period. At 1 wk, mean islet insulin secretion did not differ from control values. After 2 wk of pregnancy, hormone secretion was distinctly increased, and after 3 wk, mean responses exceeded values of both 1- and 2-wk pregnant rats and control animals (Table I).

Effects of steroid treatment on islet insulin secretion. When estradiol benzoate (micrograms) or progesterone (milligrams) was administered separately to different

[3] One must assume that pancreatic islets are spherical in shape (16). Multiple determinations of a diameter of a single islet that was shifted from one place to another in the hemocytometer revealed no significant deviation from the initial measurement.

[4] The contribution of the fetal-placental unit to body weight in the rat is consistent with changes of weight observed in 2 and 3 wk pregnant animals in this study (19).

groups of animals, daily dosages of 1.25 or 2.5 for 3 wk had no effect on islet insulin secretion (Table II). However, at the 5.0 dosage level, hormonal secretion was elevated above control responses significantly with either steroid. Combination doses of 2.5 or 5.0 also elevated insulin secretion to levels that were higher than control responses. With the 5.0 combined dosage, islet secretion exceeded values of animals receiving 5.0 doses of estradiol or progesterone separately. The magnitude of islet insulin secretion of 3-wk pregnant rats was comparable to values of those animals treated with either 5 μg of estradiol or 5 mg of progesterone daily for 3 wk. However, term pregnancy responses were less than hormonal secretion of animals treated with the combined steroid regimen of 5.0 and more than the response of rats receiving the 2.5 dosage of both hormones together (Table II, Fig. 3).

Pancreatic islet diameter and protein content. In those pregnant and steroid-treated animals whose islet insulin secretion exceeded control hormonal responses (Fig. 3) there was also a significant increase in islet diameter and protein content (Table III). Although this was not a precise correlation from one group to the next, rats given a 5.0 combined dose of estradiol and progesterone, had the highest in vitro insulin output (Fig. 3) and the greatest changes in islet dimensions and protein content. Hormonal secretion of islets from rats treated with 2.5 μg of estrogen or 2.5 mg of progesterone failed to exceed control insulin output (Fig. 3), and there were also no differences among these groups with respect to islet diameter and protein.

TABLE I

Relationship between Duration of Pregnancy and Isolated Islet Insulin Secretion

Days of gestation		Number of determinations	Insulin secretion	*P* value*	*P* value‡	*P* value§
			μU/10 islets/ 90 min			
Control	(17)	49	572 ±25	—	NS	<0.05
6–7	(7)	20	560 ±25	NS	—	<0.001
14–16	(3)	11	689 ±22	<0.05	<0.001	—
18–21	(11)	31	865 ±51	<0.001	<0.001	<0.005

Values are mean ±SE. Numbers in parentheses indicate number of animals

* *P* value signifies differences between mean mean islet insulin secretion of control and pregnant animals.

‡ *P* value signifies differences between mean islet insulin secretion of 1 wk pregnant animals and all other groups.

§ *P* value signifies differences between mean islet insulin secretion of 2-wk pregnant rats and all other groups.

NS *P* value >0.05.

In vitro effects of estradiol and progesterone on pancreatic islets. Preincubation of pancreatic islets with estradiol or progesterone (10 μg/ml) for 2 hr did not influence subsequent islet secretory responses to glucose in concentrations of 0.6, 1.5, or 3.0 mg/ml (Fig. 4). Identical results were obtained when the concentration of either steroid in preincubations was reduced to 1 μg/ml. Direct incubation of islets with 10 μg/ml of estradiol or progesterone also did not change insulin secretion in response to glucose concentrations of 0.6 or

TABLE II

Relative Effects of Hormone Treatment and Pregnancy on Isolated Islet Insulin Secretion

	1.25			2.5			5.0		
Hormone Treatment*	E	P	E + P	E	P	E + P	E	P	E + P
Number of animals	8	8	8	7	8	8	8	9	8
Number of determinations	26	21	26	21	19	20	23	32	31
Insulin secretion (μunits/10 islets/90 min)	570 ±24	529 ±21	630 ±20	645 ±36	637 ±21	679 ±20	816 ±48	896 ±39	1009‡ ±40
P value §:									
vs Control (572 ±25)	NS	NS	NS	NS	NS	<0.025	<0.001	<0.001	<0.001
vs 1 wk PG (560 ±25)	NS	NS	0.05	NS	<0.025	<0.001	<0.001	<0.001	<0.001
vs 2 wk PG (689 ±22)	<0.001	<0.001	NS	NS	NS	NS	<0.025	<0.005	<0.001
vs 3 wk PG (865 ±51)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.005	NS	NS	<0.05

* Female rats were injected daily for 3 wk with estradiol benzoate (E, micrograms), progesterone (P, in milligrams) or both hormones in combination ([E + P], in micrograms and milligrams, respectively). Values for islet insulin secretion are mean ±SE.

‡ Islet insulin secretion of the 5.0 E + P group was significantly greater than the values for the 5.0 E (*P* < 0.005) for 5.0 P (*P* < 0.05) groups.

§ Significance of the difference between mean islet insulin secretion of the hormone-treated group (above) and control or pregnant (PG) animals (below). Mean values for the control and pregnant rats are recorded in parentheses and number of animals and determinations for these groups are stated in Table I.

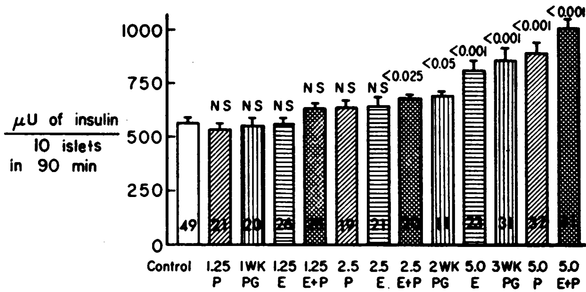

FIGURE 3 Isolated pancreatic islet insulin secretion in control, pregnant, and hormone-treated animals. Below the bar graphs, duration of pregnancy (PG) is indicated in weeks; daily dosages of estradiol benzoate (E) and progesterone (P) are in micrograms and milligrams, respectively, when given alone or in combination ($E + P$) for 21 days. Number of incubations are indicated within bar graphs. Values are mean ±SE. Significance of the differences between control responses and those of pregnant or hormone-treated animals are indicated above each bar. NS denotes $P > 0.05$.

3.0 mg/ml. Hormone concentrations of 1 μg/ml were without effect as well.

DISCUSSION

Pregnancy alters carbohydrate metabolism in the rat in a manner that resembles changes observed in women approaching parturition. Depression of fasting plasma glucose concentrations, in both instances, very likely reflects the preferential utilization of this substrate by the fetus (19). Elevated fasting plasma insulin levels (19) and exaggerated plasma insulin responses to injected glucose, which were uniformly observed in pregnant animals in the present study, are also characteristic of changes in hormonal response previously reported during similar investigations of women in late pregnancy (2–4).

TABLE III
Effects of Pregnancy and Hormone Treatment on Isolated Pancreatic Islet Diameter and Protein Content

Condition*	Islet diameter	P value‡	Islet protein	P value§
	μ		μg/islet	
Control	126 ±2 (170)	—	3.6 ±0.3 (12)	—
2 wk PG	150 ±3 (50)	<0.001	4.9 ±0.5 (12)	<0.025
3 wk PG	150 ±4 (50)	<0.001	5.0 ±0.4 (8)	<0.01
2.5 E	124 ±3 (50)	NS	3.9 ±0.3 (8)	NS
2.5 *P*	122 ±4 (50)	NS	4.1 ±0.4 (8)	NS
2.5 E + P	150 ±4 (50)	<0.001	4.8 ±0.4 (6)	<0.025
5.0 E	146 ±4 (50)	<0.001	5.0 ±0.2 (11)	<0.001
5.0 *P*	142 ±4 (50)	<0.001	5.8 ±0.5 (6)	<0.005
5.0 E + P	152 ±5 (30)	<0.001	6.8 ±0.6 (7)	<0.001

Results are expressed as mean ±SE. Numbers in parentheses indicate number of determinations done on pancreatic tissues from four to six animals. Each islet protein measurement was performed on 10 islets and the average value was used.

* Pregnancy is abbreviated PG. Daily subcutaneous dosages of estradiol benzoate (E) are in micrograms, progesterone (P) in milligrams, the two steroids in combination (E + P) in micrograms and milligrams, respectively. Duration of administration was 3 wk.

‡,§ Significance of the difference between mean islet diameter (‡) and islet protein (§) of the control groups and pregnant or hormone-treated animals. NS: *P* value >0.05.

Parallelisms existed between the magnitude of plasma insulin responses and insulin secretion by isolated islets of pregnant rats. Enhanced insulin secretion, in turn, correlated with increased pancreatic islet size and protein content. These findings are in agreement with earlier reports that insulin secretion of pancreatic slices, measured by the indirect insulin antibody utilization technique, increases after 2 and 3 wk of gestation in this animal (20), and corroborate histologic evidence of islet hypertrophy in pregnant animals and human subjects (21, 22). Collectively, these data support the proposal that gestation induces a state of insulin antago-

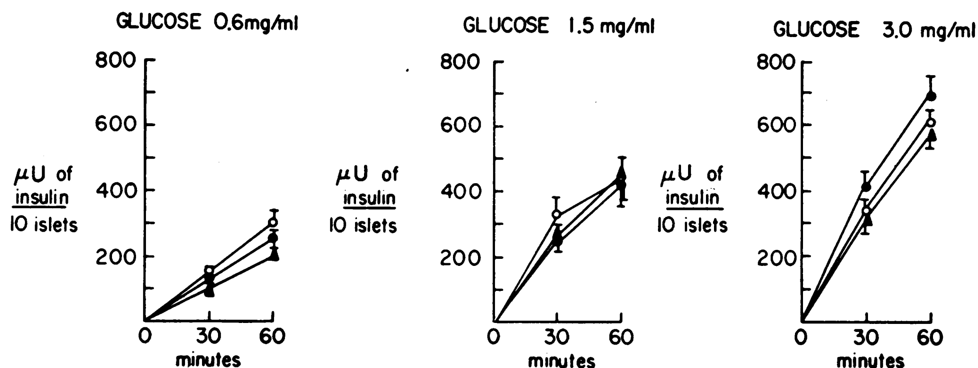

FIGURE 4 Islet insulin secretion during 1 hr incubations with different concentrations of glucose following 2 hr preincubations with estradiol (open circles), progesterone (solid triangles) or without steroid (closed circles). Preincubation steroid levels were 10 μg/ml. Each subsequent response curve is the mean ±SE of six incubations of islets from three normal animals. No significant differences between control and corresponding experimental means existed.

nism in the maternal host that requires compensatory hyperinsulinemia to maintain normal carbohydrate tolerance.

Human placental lactogen, also known as human chorionic somatomammotropin, is a placental peptide that has been strongly implicated in the development of gestational diabetogenic stress (5–7). Recently, peptides with immunochemical properties similar to this hormone have been isolated from placentae in a variety of animal species (23). Administration of placental lactogen to hypophysectomized rats greatly enhances islet insulin secretion (20), which is in accord with its marked effects on the plasma insulin response in association with impaired carbohydrate tolerance in hypophysectomized human subjects (6).

Findings in the present study, however, indicate that alterations of endocrine pancreatic function during pregnancy are not influenced by placental lactogen alone. Administrateion of estradiol benzoate and progesterone, singly and in combination, could reproduce characteristic changes in plasma insulin responses, isolated islet insulin secretion, islet size, and protein content that were observed in pregnant rats. Increased rat pancreatic islet size after estrogen or progesterone administration also has been reported previously (24, 25). Pancreatic islet effects of parenteral estradiol were approximately 1000-fold more potent than progesterone, but this difference is appropriate when one considers that daily amounts of progesterone secreted by the rat ovary during the estrus cycle or pregnancy as well as plasma levels normally may exceed estrogen secretion and plasma concentrations by this factor (26).[5] The combined dosage of estradiol and progesterone for 21 days, that was found to augment islet secretion to levels comparable to a 3 wk gestation, is between 2.5 and 5.0. It is of interest that these amounts are within the physiologic range recommended by other investigators to sustain metabolic effects of sex steroids that do not exceed those of pregnancy in the rat (27–29).

Augmentation of pancreatic insulin secretion by estrogens and progesterone might occur as a result of direct beta-cytotrophic effects on the islet. In this instance the induction of beta cell hyperplasia would provide enough additional endocrine reserve to offset adverse factors, such as placental lactogen, which are known to oppose the action of endogenous insulin during pregnancy. The failure to observe these changes acutely in the present investigation after incubation of islets with these steroids does not exclude this possibility, since the duration of in vitro exposure may have been too brief.

An alternative explanation might be that estradiol and progesterone, like placental lactogen, are also insulin antagonists. The signal for pancreatic adaptation to occur would be a greater glycemic stimlus imposed upon the islet as a consequence of impaired peripheral tissue utilization of glucose. Ultimately, carbohydrate tolerance would be preserved to the extent that basal and postprandial insulin concentrations are increased relative to the degree of hormonal antagonism. Although certain amino acids are also stimulators of insulin release, their role in this specific process is doubtful, because total concentrations diminish during pregnancy (30). Progesterone is partly responsible for this change (31).

Evidence for a beta-cytotrophic effect of estradiol is mostly indirect but substantial. In the present study, postchallenge glucose concentrations during glucose tolerance tests in estradiol-treated rats were lowered significantly below control values in association with hyperinsulinemia. Similar results have been reported in normal rabbits and women after administration of this hormone, although plasma insulin measurements were not performed (32, 33). Estradiol also ameliorates diabetes in the partially pancreatectomized rat, and the beneficial outcome is attended by islet hypertrophy in the presence or absence of intact pituitary or adrenal glands. Islet enlargement can be demonstrated focally after implantation of estrogen pellets into rat pancreas which is more convincing evidence of a direct cytotrophic action (24).

In patients with diabetes mellitus, estrogen administration has been shown to reduce daily insulin requirements (34), although this has not been observed consistently (10, 33). Others also have reported that gonadectomized female rabbits have decreased insulin sensitivity (32). This suggests that the steroid, in addition to possible beta-cytotrophic effects, may enhance the glucose-lowering action of insulin by some unknown synergistic mechanism. Other effects of estradiol include the stimulation of hepatic glycogen deposition (35) and the accentuation of a specific mobile carrier transport system for glucose in rat uterus (36). Whether this steroid enhances glucose uptake and metabolism by other tissues remains to be determined, but such an effect could contribute, in part, to improved carbohydrate tolerance. All of these data do not support the view that naturally occurring estrogens are insulin antagonists. Similar arguments do not necessarily apply to synthetic estrogens commonly administered as oral contraceptive agents (10).

Positive effects of progesterone on plasma insulin also have been observed in both monkeys and man after

[5] The principle source of estrogen and progesterone during rat gestation is the ovary rather than the placenta (26, 27). Stimulation of increased ovarian secretion during a portion of gestation is presumably due to trophic hormone(s) released by the placenta. The subject has been reviewed briefly elsewhere (19).

glucose or tolbutamide administration (37, 38). In each investigation plasma glucose responses remained essentially undisturbed. In the human studies neither serum cortisol nor fasting plasma growth hormone concentrations were altered by this treatment, which suggests that indirect influences of these hormones were not required to produce these changes (38). From these results one might conclude that progesterone is an insulin antagonist, since this steroid, unlike estradiol, does not improve carbohydrate tolerance when similar degrees of hyperinsulinemia are induced. However, this hormone has little influence on the course of experimental diabetes mellitus (24), and, paradoxically, reverses glucocorticoid-induced impairment of carbohydrate tolerance in the monkey (37) and promotes deposition of adipose tissue in the rat (39). One must conclude that progesterone has a number of multicentric metabolic effects that escape precise definition at this time including the means by which it alters islet function.

On the basis of available evidence to date, it is reasonable to assume that placental lactogen is the principal generator of diabetogenic stress in the gravid state (5–7). Estradiol may serve to oppose this action, although its antidiabetogenic effect is not documented as well in human subjects as it is in other animal species. Progesterone, however, neither markedly aggravates diabetes, as placental lactogen does, nor improves carbohydrate tolerance in a fashion resembling estradiol despite its property of increasing islet insulin secretion and the plasma insulin response. Additional studies are necessary to define how these hormones influence carbohydrate metabolism in different tissues, and to what extent they directly modify pancreatic islet synthesis and release of insulin.

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