Plasma Glucagon and Insulin in Rat Pregnancy

ROLES IN GLUCOSE HOMEOSTASIS

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ABSTRACT To determine if pancreatic glucoregulatory hormones can be implicated in the glucose fall of pregnancy, we have measured plasma immunoreactive insulin and glucagon (IRI and IRG) in rats. Fed rats in midgestation show a rise in IRI without a corresponding increase in IRG. In late gestation, IRG rises significantly, but only enough to keep pace with a further rise in IRI. On a molar basis, IRI remains the predominant hormone despite a marked fall in blood glucose. After a 48-h fast IRI falls to comparably low levels in pregnant and virgin rats. A small rise in IRG is seen in virgin but not in pregnant rats despite frank hypoglycemia in the latter. Thus, IRG secretion in pregnancy is diminished relative to IRI in the fed state and fails to increase in the fasted state despite the stimulus of a lower glucose in both instances.

To evaluate IRG secretory reserve, the IRG response to i.v. alanine was assessed in late gestation. In fed rats a greater IRG increase is seen in pregnancy; after fasting no difference is seen between pregnant and virgin rats. These results preclude an absolute deficiency in glucagon secretion. Pancreas hormone stores were also measured in an effort to explain the altered secretory state. We find reciprocal changes in IRI and IRG content favoring IRG in midgestation and IRI in late gestation. Thus, pancreas hormone storage is altered in pregnancy but does not account for the changes in hormone secretion. Rather, pregnancy exerts an effect on the islet secretory process itself. Release of IRI is enhanced relative to IRG regardless of the blood sugar level.

These observations suggest that in the pregnant rat circulating levels of insulin and glucagon may act to limit hepatic glucose output. Available evidence from the literature supports the concept of restrained glucose production. It is proposed that a lower blood glucose in rat pregnancy may be a lesser liability teleologically than would be the obligate nitrogen wasting which accompanies gluconeogenesis.

INTRODUCTION

Blood glucose concentrations fall progressively in the second half of rat pregnancy (1-8). This fall is significant when the animal is fed (8), and reaches hypoglycemic levels during fasting (1, 2, 4, 5, 7, 9-11). Blood glucose also falls progressively in human pregnancy after fasting overnight (12-19) or longer (18-19).

Several mechanisms are believed to contribute to glucose lowering in pregnancy. Increased glucose removal from the maternal circulation is attributed to fetal utilization, since the glucose fall is reversed by hysterectomy (1) or fetectomy (9-11). Also, gluconeogenesis could be limited by the reduction in gluconeogenic amino acids (7, 19). But the latter point applies only to the 24-h-fasting rat, since all gluconeogenic precursors are present in abundance in the fed, pregnant animal (7). Thus, despite the availability of adequate gluconeogenic substrate, a significantly lowered blood glucose persists in the fed, pregnant rat.

One mechanism for this adaptation might be an altered secretion of pancreatic glucoregulatory hormones that would limit hepatic release of glucose in both the fed and fasted states. To investigate this possibility in rat pregnancy, we have compared plasma glucose, immunoreactive insulin (IRI), and immuno-
reactive glucagon (IRG) in mid and late gestation. IRG response to alanine stimulation and pancreatic hormone content have also been measured. The data support the view that increases in IRI greater than in IRG can perpetuate glucose reductions in pregnancy. Some of these results have been presented previously (20).

METHODS

Animals. Pregnant primiparous and age-matched virgin female rats were obtained from Charles River Breeding Laboratories, Wilmington, Mass. Animals were maintained as previously described (8) with continuous access to Purina Chow pellets except for "fasted" animals who had access only to water for 48 h before sacrifice. Animals were bred at 180-190 g and the age at mating ranged from 47 to 60 days. As mating occurs at night, the following day is considered gestational day 1. Anesthesia consisted of 10-15 mg sodium pentobarbital administered i.p. Studies were initiated after the induction of anesthesia with a maximum duration of 30 min. All experiments were done between 9:00 and 12:00 a.m.

Study design. To determine plasma glucose, IRI and IRG concentration, pregnant and age-matched virgin animals were sacrificed in three separate sets: fed day 12, fed day 21, and fasted day 21. Since the plasma measurements for the virgin control rats did not differ in the two "fed" experiments, these results are pooled and are called day 0 (Table I). Another group of rats was used to evaluate the effect of alanine vs. saline on IRG secretion in 21-day pregnant and virgin animals. A final set of rats was studied to determine the effect of pregnancy on pancreatic hormone content.

Because of the limited availability of plasma, it was necessary to conduct two experiments to evaluate plasma hormone content: experiment I compared plasma glucose and IRI, experiment II compared plasma glucose and IRG. Comparison of mean glucose levels in each set of animals (Table I) reveals no significant differences between experiments I and II. This similarity supports the validity of a direct comparison of IRI and IRG as presented in Table I.

Plasma analyses. Plasma for glucose and hormone assays was obtained either from the cut tip of the tail (in the case of preinjection blood) or from the abdominal aorta (in the case of animals being sacrificed). Plasma glucose and IRI were measured in heparinized specimens by autoanalyzer techniques (Technicon Method N-2a, Technicon Instruments Corp., Tarrytown, N. Y.) as previously reported (8). Plasma IRG was measured on blood samples anticoagulated with 4 mg EDTA and treated with 0.1 ml (1,000 U) Trasylol per 3 ml whole blood (FBA Pharmaceuticals, New York). The assay system employed the highly pancreatic specific antiporcine glucagon antiserum 30-K of Rocha, Falona, and Unger (21). This antiserum measured plasma changes of 10 pg/ml with 95% reliability.

Alanine stimulation. Endogenous IRG secretion after i.v. saline was measured in anesthetized rats. An initial zero time blood sample of approximately 1 ml was taken for IRG from the cut tip of the tail. An alanine dose of 50 mg/kg in a solution of 25 mg alanine per ml of 0.154 M NaCl was then administered into a tail vein using a no. 27 gauge 1/2-inch needle. An identical quantity of saline (0.308 meq/kg) was administered to pregnant and virgin control rats. 15 min after injection the abdomen was opened and blood withdrawn from the aorta. In these experiments 50 µl of 1.0 M benzamidine was added to each ml of whole blood instead of Trasylol (22).

Pancreas analyses. Animals were sacrificed by a blow to the head. Pancreases were weighed and placed in chilled acid alcohol and immediately homogenized. Tissue extracts

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were neutralized with NaHCO₃ and phosphate buffer to a pH of 7.0 according to Malaisse, Malaisse-Lagae, and Wright (23). Because further attempts at purifying the extracts led to approximately 30% losses of labeled hormone as previously reported (24), the extracts were subjected directly to radioimmunoassay. IRI content was determined at a dilution of 1:100,000 using a rat insulin standard (courtesy Dr. J. Schlichtkrull, Novo Research Institute, Copenhagen) in the methods of Morgan and Lazarow (25). IGR was determined at a dilution of 1:100 using a porc glucagon standard. Alcohol was added to the glucagon standards to a final concentration of 0.1%, identical to that of the unknowns. IGR from these excised pancreas was determined not with the 30-K antiserum, but with a guinea pig antiporcine glucagon antiserum in an immunoassay conducted with minor modifications according to the method of Rocha et al. (21). This antiserum measured changes in pancreatic IGR content of 1 ng with 95% reliability, and is presumably not pancreas specific.

Effects of anesthesia and sampling methods. Because blood was sampled by three different techniques, data were analyzed to see whether systematic differences occurred when the technique was changed. (a) cut tip of the tail of conscious rats, (b) cut tip of the tail in pentobarbital anesthetized rats and (c) the aorta of pentobarbital anesthetized rats. In each instance, similar values for IGR were obtained in day 21, fed pregnant rats (mean±SEM of 8-14 observations): (a) 244±29, (b) 259±22, and (c) 221±18 pg/ml. Virgin controls (15-44 observations per group) were similarly reproducible: (a) 146±22, (b) 143±22, and (c) 160±7 pg/ml. For each experimental situation pregnant always exceeded virgin: (a) P < 0.02, (b) P < 0.01, and (c) P < 0.01 (see Results). We conclude that the techniques used have no detectable effect on plasma IGR in pregnant or virgin rats in contrast to the reported effect of pentobarbital in growth hormone and prolactin (26, 27).

Results were statistically analyzed using the Student's t test (28) performed directly or on the log10 transformed data when results were calculated as percentage increases (29).

RESULTS

In fed pregnant rats, little or no reduction in plasma glucose occurs by the 12th day of gestation (Table I). However, by the 21st day plasma glucose drops 39-44% below virgin controls. Compared to fed levels, a 48-h fast produces a further glucose decline. Virgin rats drop 25-27%, and 21-day pregnant rats drop 22-33%, the latter reaching hyperglycemic levels.8

Also in fed pregnant rats, plasma IRI rises progressively with a 60% increase on day 12 and a 2-fold increase on day 21. By contrast, no change is seen in IGR at midgestation and only at day 21 is there a significant increase of 40%. After a 48-h fast, plasma IRI declines precipitously in both pregnant and virgin rats to comparably low levels. Compared to fed levels, IGR in fasted virgin rats increases significantly by 17%. A small decline in IGR is noted in fasted pregnant rats but is not significant.

The peripheral IRI to IRG molar ratio has been used in man to estimate the integrated effects of these hormones on hepatic glucose balance (34). As seen in Table I, fed rats on the 12th and 21st days of gestation demonstrate a nearly twofold increase in the molar ratio. The primary factor in maintaining the molar ratio at an “anabolic” setting (34) is the increase in IRI. While IGR also rises in late gestation, presumably in response to the lower glucose, it merely keeps pace with the rise in IRI and is insufficient to alter the molar ratio.

In 48-h-fasted pregnant and virgin rats, IRI falls sharply and to comparably low levels (Table I). Only a small IGR rise is seen in virgin rats and none occurs in pregnant rats. The “catabolic” relationship of IRI and IRG is appropriate to the fasting state (34), but in view of the maternal hypoglycemia it is notable that further rise in IGR in lacking. Thus, the fasting hypoglycemia of pregnancy appears to result in part from the lack of an appropriate glucagon response.

Alanine-stimulated IGR secretion. In view of the predominance of IRI over IGR in fed pregnant rats and the lack of a rise in IGR in fasted pregnant rats, the possibility of an impaired secretion of IGR in pregnancy might be entertained. To evaluate this possibility 50 mg/kg i.v. alanine was administered to fed and 48-h-treated pregnant and virgin rats. Plasma IGR was measured before and 15 min after the injection. Results appear in Table II.

Alanine elicits a significant increase in plasma IGR in fed 21-day pregnant rats. A smaller increase in virgin rats is not statistically significant. Comparing pregnant and virgin rats, the IGR response to alanine is nearly sevenfold greater in pregnancy. Since alanine was administered on the per kilogram basis, it could be argued that the pregnant pancreas was exposed to a larger bolus of alanine before its equilibration through-out the body’s “alanine space.” This is unlikely, however, since the plasma volume increases as much as body weight in late gestation (30). In any case, these

8Levels of circulating glucose are herein considered to be hyperglycemic when below 50 mg/100 ml whole blood. Glucose concentrations were determined in plasma samples to eliminate the effect of a 10-15% drop in hematocrit at term in rat pregnancy (30, 31). Assuming a maternal hematocrit of 42.5, the results may be converted to whole blood by reducing the plasma value by 30% (32, 33) yielding values of 42.0 to 45.5 mg/100 ml in 48-h fasted 21-day pregnant rats (from Table I).

8The precise quantitative effect of the peripheral IRI/IRG molar ratio on hepatic glucose balance has not been established in vivo, although the opposing effects of insulin and glucagon on hepatic glucose balance in the perfused rat liver in vitro are well established (35-38). Insulin and glucagon are competitive at IRG concentrations below 2-4 X 10⁻⁸ M (35-39). Even allowing for higher levels in portal blood, IRG in the present report ranges substantially lower, between 4-6 X 10⁻⁸ M, in peripheral plasma.
The effects of handling and saline injection were also determined in fasted rats (Table II). No significant increases were seen.

Pancreatic content of IRI and IRG. We next investigated the possibility that an alteration in the quantity of stored pancreatic hormone might affect hormone secretion, by measuring pancreatic IRI and IRG content in pregnant and virgin rats. As shown in Table III, pancreas weights increased slightly at gestational days 12 and 21. A small (8.2%) but statistically significant reduction is seen in pancreatic IRI on gestational day 12 with a return to control levels at term. These changes parallel those reported by Malaisse, Malaisse-Lagae, Picard, and Flament-Durand, who found that pancreatic IRI was slightly reduced at gestational day 10 and increased at day 20 (3). Pancreatic IRG demonstrates a pattern reciprocal to IRI. A significant IRG increase is observed at day 12 and a significant decrease is seen at day 21 as compared to day 12.

After a 48-h fast, pancreas weight is slightly but not significantly reduced and no significant reduction from fed levels is seen in IRG. Results in virgin rats are in accord with the previous reports of pancreas IRG content in nonpregnant rats (39).

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DISCUSSION

Explanation of the lowering of blood glucose seen in late gestation requires consideration of glucose entry into and removal from the plasma compartment. We have referred to evidence that glucose consumption is in fact increased, due to metabolic demands of the fetus (9-11, 40). But why does maternal glucose production not increase enough to compensate for this glucose drain? Deficient gluconeogenic substrate has been offered as an explanation in the case of 24-h-fasting pregnant rats (7); but no such precursor deficiency exists in the 48-h-fasted pregnant, or the fed pregnant animals (7), although blood glucose is low. Evidence is presented herein to support the concept that the glucose-regulatory hormones insulin and glucagon may be important in the pathogenesis of gestational blood glucose lowering.

Midgestation. In midgestation we observed an increased plasma IRI confirming previous work by ourselves (8) and Sutter, Leclercq, Felix, Jacquot, and Sutter (11). The results are also in harmony with the increased secretion of insulin from pancreas pieces in vitro (3). Plasma IRG levels, on the other hand, were unchanged. With this midgestational setting of increased IRI and unchanged IRG, it is noteworthy that blood glucose is not diminished. Fetal utilization of glucose at this point is negligible, and food intake is increased (8). But a “resistance” to the hyperinsulinism may also be postulated, and evidence for such exists (1, 5, 29, 41). The effects of estradiol and progesterone are known to reduce the glucose production from pyruvate, alanine, and glycogen (42).

Late gestation. In late gestation, our data again confirm the well-known fall in blood glucose (1-8), coincident with a rise in IRI (4, 6, 8). We have, additionally, found that IRG rises in late gestation, although only to levels which, on a molar basis, remain well below the IRI rise. This persistent hyperinsulinism and relatively small glucagon increase is a distinctly unusual response to a glucose fall. Insulin is predominant and despite the late rise in IRG, an “anabolic” setting (34) is maintained irrespective of blood glucose reductions.

Fasting. In response to a 48-h fast, the blood glucose falls to hypoglycemic levels as seen in this and previous reports (1, 2, 4, 5, 7, 9, 11). The principal hormonal adaptation is a drop in plasma IRI in both virgin and pregnant rats. While IRG rises significantly in virgin fasted rats, pregnancy entirely eliminates a glucagon response to fasting hypoglycemia.

Alanine stimulation. IRG secretion in response to alanine was assessed to rule out an absolute decrease in IRG secretion during pregnancy. The IRG response in pregnancy was merely sevenfold greater than control in fed rats and no different from control in fasted rats. This experiment precluded an absolute deficiency of IRG secretion in late gestation by demonstrating that, at least after pharmacologic alanine stimulation, pancreatic glucagon is released.

Pancreatic extracts. Finally, pancreatic extracts were made to determine whether the predominance of IRI over IRG could be explained by altered hormone stores in the pancreas. A reciprocal pattern between IRI and IRG was found. In midgestation IRG was increased and IRI slightly reduced; near term, IRI was reduced and IRI restored to normal. The reduction in IRG in late gestation is consistent with the degranulation of alpha cells seen in the pregnant rabbit (43). But changes in content do not explain the observed peripheral levels, since plasma IRI predominates over IRG in both mid and late gestation. Nonetheless, the measurements of hormone content in the pancreas illustrate another
unique effect of pregnancy on insulin and glucagon metabolism.

Several apparent inconsistencies with existing literature require mention. Recently, Girard et al. studied fed pregnant rats of 22-day gestation (21.5 complete days pregnant) (44). They found IRI only slightly elevated, and IRG definitely increased. Immunoassay techniques may account for some differences, particularly with regard to glucagon measurements, but their insulin values were considerably lower than ours. The difference is more likely due to the catabolic events immediately before parturition, when food intake declines (1, 8) and there is a drop in plasma progesterone (11, 45, 46). Whatever the mechanism, findings at term are not representative of the earlier course of pregnancy (8).

More fundamentally, our finding in fed rats of consistent IRI predominance over IRG might suggest that glucose production should be consistently suppressed. While in fasting pregnancy (when IRI is low) an increase in gluconeogenesis has been shown (4, 20), a body of evidence exists supporting the notion that hepatic glucose formation is reduced or at most unchanged in the fed, late gestational rat: despite lower blood glucose, glycogen stores are not diminished (4); total urea excretion in vivo is unchanged although urea produced by the fetus passes transplacentally to the mother at least in primate species (47), suggesting that the maternal contribution to urinary urea is diminished; urea release in vitro from liver slices is reduced (31), and a lesser rise in plasma urea is seen after intraperitoneal alanine or casein hydrolyzate (48); pyruvate conversion to glucose is unchanged in vivo (4) and is reduced in vitro (49) despite a 50% increase in liver size; and finally, a number of hepatic enzymes instrumental in converting amino acids into glucose share the trend toward reduced activity in pregnancy (9, 31, 47, 49-54).

Our data and the existing literature, then, are most consistent with the view that pregnancy alters the islet secretory process itself such that release of IRI is enhanced and IRG is minimized. The hormones of pregnancy may be responsible for this effect. Ample evidence is available that pregnancy stimulates insulin secretion in the rat (3, 6, 42, 55), and early data in human subjects suggests a reciprocal inhibitory effect on glucagon secretion (56).

The question may well be raised of how, in pregnancy, can an uncompensated glucose reduction benefit the growing fetus? We propose that the chief benefit of permitting the glucose fall would be to spare amino acids for incorporation into maternal and fetal protein. The reduction in circulating glucose appears to be a lesser liability than the potential waste of nitrogenous resources that would otherwise occur on restoring glucose to normal. This reduced circulating glucose, observed in both fed and fasted states, should not deprive the mother of an adequate energy supply, since increased fat mobilization provides an alternative energy fuel in late gestation (1, 4, 8, 27, 40). We therefore suggest that glucose lowering is a tolerable alternative to a greater amino nitrogen loss in pregnancy. This teleological "decision" appears to be implemented by the predominance of IRI over IRG in the fed state, and a lack of an IRG rise in the fasted state.

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