Insulin-related genes expressed in human placenta from normal and diabetic pregnancies

(RNA homology/fetal growth/somatotropins)

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ABSTRACT Rapid growth of human fetal tissues requires insulin or insulin-like growth factors. A high rate of human fetal growth occurs between implantation and about 14 weeks of gestation. Fetal pancreatic insulin secretion begins much later. Since maternal insulin does not cross the blood/placental barrier, other sources of insulin or insulin-like growth factors may be provided for fetal development. We report here that placental polyadenylated RNAs from the first and third trimester of normal pregnancy as well as from term pregnancies of diabetic mothers hybridize to a 32P-labeled cloned cDNA of an insulin-related sequence expressed in fetal pancreas. Moreover, placenta from diabetic women express much more of these sequences. These results suggest that insulin-related genes are expressed in placental tissue during fetal development and may be a source of growth-promoting hormones for the human fetus. Fetuses developing in diabetic women receive a large influx of glucose. This in turn may stimulate the expression of insulin-related sequences, which may result in higher utilization of glucose, thus bringing about the macrosomia and high incidence of malformation and stillbirths known to result from pregnancies in diabetics.

During the first 10 weeks of embryonic and fetal life, which is a critical period of rapid growth for the human conceptus, primary and secondary embryonic induction and organogenesis is taking place (1). Glucose is used as a major source of metabolic energy in the human fetus (2–4) and a glucose imbalance, in addition to affecting the growth of embryonic tissue, may well result in developmental abnormalities such as macrosomia, malformations, and increased rate of stillbirths, as is manifest in pregnancies of diabetic women. Insulin, a regulator of glucose metabolism, is an essential growth factor for fast-growing mammalian tissues, including human embryonic tissues (2–4); however maternal insulin does not cross the blood/placental barrier (5–8). Although it has not been established that insulin or an insulin-like growth factor is necessary for the early development of the human fetus in utero, such a requirement is not unlikely.

Explants of multiple tissues from fetal mouse synthesized insulin-like somatomedin (9). The production of the insulin-like growth factors IGF-I and IGF-II is developmentally regulated, and this pattern of expression is maintained in fibroblasts derived from fetal as well as older rats (10). Receptors for insulin-like growth factors (IGF-I, IGF-II, somatomedin A, and multiplication-stimulating activity) and insulin are present in human fetal tissues at different stages of development (11). This evidence suggests that fetal growth may be regulated by insulin and/or insulin-like growth factors.

The fetal pancreatic islets of Langerhans are capable of insulin synthesis only after 14 weeks of gestation, several weeks later than the critical growth period mentioned above. Further, the fetal pancreas normally exhibits only limited response to acute changes in cord blood glucose (12–14) and the factors that control the synthesis and release of insulin in the fetus are not known. Human maternal insulin levels can be elevated as much as 18-fold without any demonstrable transfer of this hormone across the blood/placental barrier to the fetus (15). Since the fetal pancreas has only a limited response to blood glucose levels and since maternal insulin does not cross the blood/placental barrier, these avenues are not likely to be the primary source of the insulin or insulin-like growth factors necessary for embryonic and fetal development. Therefore, the supply of hormones to the developing fetus in normal as well as pathological states must be synthesized and secreted by a tissue or tissues that communicate directly with the fetal circulation.

The placenta is the source of peptide hormones, such as chorionic gonadotropin and placental lactogen, and steroid hormones, such as estrogens and progestins, and therefore is a likely candidate for the synthesis of growth-promoting hormones, such as insulin and insulin-like growth factors, which may be essential for early embryonic development. Since there are numerous temporal changes in the production of hormones during normal pregnancy, we examined placenta for the temporal appearance, during fetal development, of RNA sequences related to the insulin gene family.

METHODS

Tissue. Placentas were obtained primarily by cesarean section at the Macdonald Hospital for Women, University Hospitals of Cleveland and were extracted within 30 min of delivery. Diabetics were classified according to White (16).

Total placental and pancreatic RNA were prepared as described by Chirgwin et al. (17). Poly(A)+ RNAs were isolated from total cellular RNAs by affinity chromatography on oligo(dT)-cellulose (18). The poly(A)+ RNAs were translated in the cell-free reticulocyte lysate system (New England Nuclear) under conditions suggested by the supplier. Translation products were resolved by NaDodSO4/12% PAGE (19). The gel was dried and exposed to Kodak XAR-5 autoradiographic film.

For hybridization of poly(A)+ RNA to cDNA probes, placental and pancreatic poly(A)+ RNAs were electrophoresed in an agarose minigel (15 ml gel volume) under denaturing conditions (20) and transferred to nitrocellulose filter in 3 M NaCl/0.3 M sodium citrate, pH 7.1–7.4, by the modified procedure of Thomas (20). Blotting of poly(A)+ RNA to nitrocellulose required 16–24 hr; the nitrocellulose filter then was removed from the assembly and baked in a vacuum for 2 hr at 60–80°C. Hybridization was carried out with nick-translated (21) cDNA insert under high-stringency conditions (50% formamide/0.9 M NaCl/0.09 M sodium citrate, pH 7.1–7.4, 42°C). The filter then was autoradiographed.

cDNA clones pUC81-1 and pHM-4 were provided by

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RESULTS

Poly(A)^+ RNA was extracted from placentas at various stages of normal development as well as from placentas of overt diabetic mothers. To examine the quality and integrity of the poly(A)^+ RNA, all samples were assayed in a cell-free reticulocyte lysate translation system. The in vitro translation products ranged in Mr from 3000 to well over 100,000, as determined by NaDodSO_4/PAGE (Fig. 1).

A cDNA clone (pUC8I-1) containing 240 bases encoding from amino acid 9 of the prepeptide to amino acid 9 of the A peptide of insulin was used to probe for authentic insulin sequences (22). The DNA sequence of the insulin-related probe (pBM-4) from fetal pancreas contained a 274-base-pair fragment with several regions of substantial homology to the insulin gene (22). Region one has 64% homology to the sequence encoding the NH_2-terminal and insulin B peptide and has an overall homology of 55% to the B-peptide-encoding sequence. Region two with 60% homology, begins at the end of sequences encoding the insulin B peptide and extends into sequences encoding the C peptide. A third region, corresponding to the COOH-terminal end of the C peptide, has 50% homology to this segment of the insulin sequence. The last region, with 52% homology, corresponds to part of the sequence encoding the leader peptide for insulin.

To determine whether these sequences were present in placental RNA, poly(A)^+ RNAs were separated by electrophoresis on agarose gels, transferred to nitrocellulose paper, and hybridized to the ^32P-labeled cDNA insert from the human insulin gene (Fig. 2A) or to the ^32P-labeled cDNA of the insulin-related sequence isolated from fetal pancreas (Fig. 2B). The DNA encoding adult insulin hybridized strongly to adult human pancreatic poly(A)^+ RNA (Fig. 2A, lane 1). However, placental poly(A)^+ RNAs showed no hybridization signals with this probe under the same hybridization conditions (Fig. 2A, lanes 2–4).

When the same RNA preparations were hybridized with the human fetal insulin-related probe under the same conditions, all placental poly(A)^+ RNA samples gave positive signals (Fig. 2B, lanes 2–4), whereas adult pancreatic poly(A)^+ RNA gave little or no hybridization signal (Fig. 2B, lane 1). With the human fetal insulin-related probe, a major band, corresponding to a molecular size of 6–10 S, appears with placental RNAs from first trimester and term normal pregnancies and from term pregnancies of diabetic mothers. However, larger species which hybridize more weakly are apparent with all placental poly(A)^+ RNA samples. It is also clear (Fig. 2B, lane 4) that poly(A)^+ RNA from term diabetic pregnancy contains quantitatively more of the sequences that hybridize strongly to the fetal insulin-related DNA probe. A human placental lactogen cDNA probe used as an internal control gave a similar hybridization signal with mRNAs from term placentas of both normal and diabetic pregnancies (22).

DISCUSSION

All of our placental poly(A)^+ RNA samples contained sequences that hybridized to the fetal pancreatic insulin-related DNA under conditions where only RNAs with substantial regions of ≥80% sequence homology would be expected to hybridize. Further, our experiments suggest that although these sequences are present both in first trimester and term placentas, the quantity of insulin-related sequences may vary with different pathophysiological conditions. From comparison with the hybridization results (not shown) of probing placental RNA preparations containing a known percentage of human placental lactogen, RNA, we estimate that the insulin-related sequences represent 0.03%–0.1% of the total poly(A)^+ RNA in placenta. Since this quantity of poly(A)^+ RNA could code for a substantial amount of protein, the protein product may well be a regulatory or growth-type hormone necessary for early embryonic development as well as other regulatory functions. Some of the known insulin-related growth factors, which have amino acid sequences...
similar to that of proinsulin, are the somatomedins (also known as insulin-like growth factors I and II, or IGF-I and IGF-II). Alternatively, these poly(A)\(^+\) RNAs may code for as yet unrecognized growth-promoting regulatory factors. Whether their possible effects are mediated through their own receptors or by their ability to bind to insulin receptors (24–27) awaits purification and identification of the sequences for further studies.

Insulin-related genes expressed in placenta may be analogous to those of the globin-gene cluster (28, 29), in which \(\gamma\)-globin is expressed in liver and \(\alpha\) and \(\beta\)-globins are synthesized in the bone marrow only after parturition. Thus, the first expression of the insulin multigene family may occur in placenta, and only later, after organogenesis, may the expression of other sequences from the insulin gene family occur in the fetal pancreas, brain, and liver.

Newborns of diabetic women are usually overweight due to macrosomia (30). Pedersen (31) proposed that this characteristic fetal overgrowth was a consequence of maternal hyperglycemia causing fetal hyperglycemia and hyperinsulinemia. Experiments utilizing fetal sheep preparations have shown that insulin increases fetal glucose uptake as well as fetal glucose utilization (13, 14, 32, 33). Studies of many animal tissues in culture, including human tissues, have demonstrated that insulin stimulates glucose uptake and protein synthesis in fetal skin, muscle, brain, adrenal, and kidney (2–4, 35–41). Since fetal pancreas exhibits none or only a limited response to acute changes in fetal blood glucose levels (12–14), the regulation of glucose metabolism in the fetus may be controlled by a hormone of extrapancreatic origin. Further, since glucose utilization by the fetuses of diabetic mothers is more rapid than normal, other sources of insulin-related factors may play an important role.

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