Islet Cells Grow after Transplantation of Fetal Pancreas and Control of Diabetes

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SUMMARY

The capacity of a fetal rat pancreas to grow and function was assessed after its transplantation into adult diabetic rats. One month after induction of diabetes by streptozotocin (SZ) (72.5 mg/kg) in adult Lewis rats, one syngeneic 17–17½ day fetal pancreas was transplanted under the kidney capsule. Insulin, initially 3 U/day and decreasing to 0.5 U every other day, was given for 27.5 ± 1.7 ($\bar{x}$ ± SEM) days (total dose, 48 ± 4 U) until the diabetes reversed (45 ± 3 days). Plasma glucose, 481 ± 10 mg/dl after SZ, fell after transplantation and cessation of insulin injections during a 4-mo period to 129 ± 2 mg/dl. Urine volume fell from 82 ± 4 ml per day to normal (12 ± 2), and glucose excretion, which was 7.7 ± 0.4 g per day after SZ, completely disappeared from the urine. The disappearance rate of glucose injected into the circulation, which was 0.50 ± 0.07% per minute in untreated diabetes, reverted to normal, 2.78 ± 0.18% per minute, not different from that in the normal control, 2.55 ± 0.13% per minute. Plasma insulin in control diabetic rats was totally unresponsive to glucose injection, despite a rise in plasma glucose concentration from 471 ± 5 mg/dl to 621 ± 18 mg/dl. In transplanted rats, plasma insulin was moderately higher than normal before and at most time points after glucose injection. After removal of the transplanted pancreases, plasma glucose, urine volume, and glucose excretion returned to pretransplant levels and ketones appeared in the urine of all rats. Analysis of the insulin content of the pancreases removed 77 to 279 days after transplantation gave a value of 818 ± 43 mU, which is 22% of the insulin content of normal rats.

To achieve optimal growth and function of a transplanted fetal pancreas, careful control of the blood glucose is necessary during the period of growth and development. DIABETES *30*:9–13, January 1981.

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A fetal rat pancreas, when transplanted into a diabetic animal, has a remarkable ability to grow and to synthesize and release insulin.[1,2] Initial trials demonstrated that four or more syngeneic fetal rat pancreases transplanted under the kidney capsule of diabetic rats were necessary to reverse the diabetes completely.[3] Shunting of the venous drainage from the transplants into the liver, by anastomosing the end of the renal vein to the side of the portal vein, improved the response, so that two or three pancreases totally reversed the diabetic state.[4] An alternative method is to place the fetal organ in a normal, syngeneic rat for a period of growth and development before its transplantation to a diabetic host. Using this method, one fetal pancreas completely reverses the diabetic state, but the timing of transfer from the normal to the diabetic rat is critical.[5]

Although this approach is not applicable to diabetic man, the results suggest that normoglycemia, or at least control of the diabetic state, might improve the growth and function of the fetal pancreas. If a single fetal organ were able to completely reverse the diabetic state, matching of tissue types to alleviate rejection would become possible, and also the supply of donor organs would be enhanced. The ultimate goal is to apply this capability to diabetic man.

The results reported here reveal that, with prolonged insulin treatment, complete reversal of diabetes can be achieved by one fetal pancreas. We found that, when insulin content is used as the determining factor, the fetal organ reaches 20–25% of the function of an adult pancreas.

MATERIALS AND METHODS

Diabetes was induced in 20 adult Lewis rats (16 female and 4 male) by the intravenous injection of streptozotocin (courtesy of W. E. Dulin, Upjohn Co., Kalamazoo, Michigan). The dose of streptozotocin was 72.5 ± 5 mg/kg, and nine rats were injected with 90 mg/kg to produce severe diabetes. The animals were kept in metabolism cages to monitor the diabetic state. Plasma glucose levels of the tail vein were

measured weekly, and urine volume and glucose excretion were measured daily. After 2 wk of observation to establish the presence of diabetes, protamine zinc insulin (PZI) was injected daily for 14 days to ameliorate the severity of the disorder (mean total dose per rat, 39 ± 4 U). One month after the induction of diabetes by streptozotocin, one syngeneic fetal pancreas of 17–17½ days' gestation was transplanted beneath the kidney capsule of the diabetic animal.

We injected 3 U of insulin subcutaneously every day, thereafter, until the urine volume became less than 40 ml/day, at which time we gradually decreased the dose. When the urine volume became normal and the plasma glucose was less than 250 mg/dl, we decreased the dose of insulin to 0.5 U every other day until plasma glucose became normal. The administration of insulin was then discontinued. The total duration of insulin treatment after transplantation was 27.5 ± 1.7 ($\bar{x}$ ± SEM) days, and the total dose per rat was 48 ± 4 U. The time from transplantation to the appearance of normal blood and urine values was 45 ± 3 days. All values for each rat were averaged for each phase of the study. Glucose tolerance tests were performed at 137 ± 12 days after transplantation, and at 173 days (range, 77–304) the grafts were measured with calipers, removed from the kidney surface, immediately frozen on dry ice, and stored at −70°C.

Assay procedures. For insulin assay, the frozen pancreases were homogenized in cold acid-alcohol and extracted overnight at 4°C. The insulin content was measured by radioimmunoassay against a rat insulin standard (Novo Industri, Copenhagen). The extracts were assayed in duplicate at three or more dilutions. Among 16 rats (12 females and 4 males) in whom diabetes completely reversed, 15 grafts were available for analysis and the tissue of one male rat that died was not usable. Grafts removed from two female rats in whom diabetes partially reversed and from two in whom there was no response were also assayed. To determine the insulin content of the normal rat pancreas, the total content of insulin in the pancreases removed from 13 adult Lewis rats, 4–24 mo of age, was measured. Under ether anesthesia, the pancreatic duct was distended with saline, the pancreas was removed and minced, and insulin was extracted and assayed by the same method.

Glucose tolerance tests. Rats were fasted overnight, weighed, restrained in a towel wrap, and given d-glucose at a dose of 0.5 g/kg of body weight by a rapid injection into the tail vein. Blood samples were collected from a tail vein in 250 μl heparinized tubes before injection and at 10, 20, 30, and 40 min after injection. The rats were not restrained at these times. This method does not affect the k value for glucose disappearance, but it reduces the peak insulin concentration modestly compared with values obtained using chronic, indwelling catheters.[4] After the plasma was separated from the blood, the glucose content was determined on a Beckman glucose analyzer and the remaining plasma was frozen for radioimmunoassay of insulin content using a rat insulin standard. The k value for the rate of glucose disappearance was determined using the 10, 20, and 30 min values. Normal control rats of the same age were subjected to the glucose tolerance study, and untreated, control diabetic rats were studied one month after the streptozotocin injection.

Pregnancy. After transplantation and complete normalization of their diabetes, five female rats were mated with fertile male Lewis rats. After pregnancy and parturition, the glucose tolerance study was repeated and the transplants were removed as already described. Glucose tolerance tests were first performed 15 to 25 days before pregnancy and 8 to 46 days after parturition; none of the mothers were nursing.

RESULTS

Plasma glucose levels, daily urine volume, and urine glucose excretion of 16 rats were measured for 2 wk after the injection of streptozotocin and before insulin injections, and the mean values were determined (Figure 1). After the transplantation and the insulin injections, plasma glucose levels and urine volume fell to normal, and glucose completely disappeared from the urine. Insulin injections were then discontinued, after which the values remained normal for approximately 4 mo. Mean values during this 4-mo period are given in Figure 1. After removal of the transplanted fetal pancreases, the hyperglycemia, polyuria, and glycosuria returned to pretransplant levels, and tests of the blood and urine of all rats with Acetest reagent tablets were positive for ketones. The weight of the rats between the time of transplantation and the removal of the graft increased from 221 ± 17 g($\bar{x}$ ± SEM) to 301 ± 9 g.

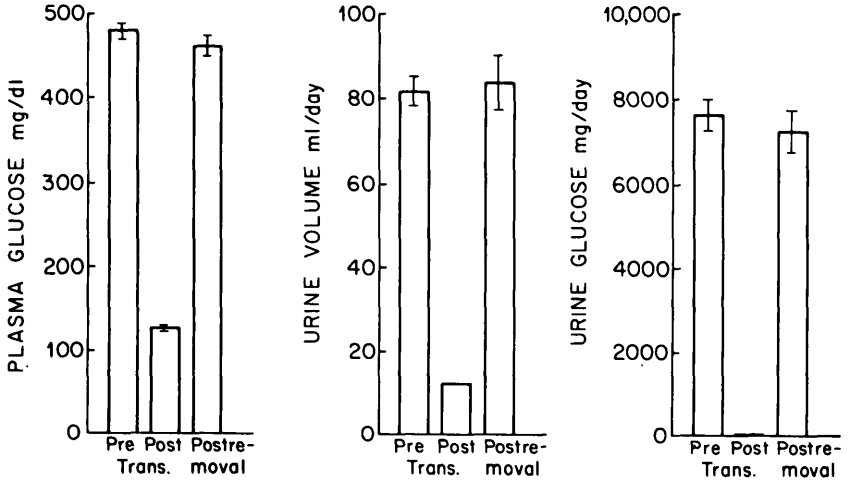

FIGURE 1. Plasma glucose, urine volume, and urine glucose in 16 streptozotocin-diabetic rats before transplantation of one fetal pancreas (Pre Trans.), after transplantation following discontinuation of insulin injections (Post Trans.), and after removal of the transplanted pancreas (Post Removal).

TABLE 1
Plasma insulin response and k values for disappearance of glucose injected in streptozotocin-diabetic control rats, transplanted diabetic rats, and normal control rats

	Diabetic		Normal
Minutes after glucose injection	Control (4)	Transplanted (11)	Control (6)
	Plasma insulin (μU/ml), $\bar{x}$ ± SEM		
0	42 ± 19	71 ± 4	44 ± 4*
10	31 ± 5	82 ± 7	62 ± 6†
20	35 ± 10	71 ± 4	59 ± 9
30	32 ± 6	65 ± 4	48 ± 6‡
k (%/min)	0.5 ± 0.07	2.78 ± 0.18	2.55 ± 0.13

* $P < 0.001$, † $P < 0.025$, ‡ $P < 0.05$.

Glucose tolerance test after stabilization (Table 1). In transplanted rats, injection of glucose into a tail vein was followed by a normal rate (k) of disappearance from the blood (2.78 ± 0.18%/min) compared with a rate of 0.5 ± 0.07%/min in untreated control diabetic rats. The plasma insulin levels before and after glucose injection in transplanted rats are higher than in the normal controls before and at most time points after glucose injection. In contrast, plasma insulin in diabetic rats of the same age and sex was totally unresponsive to glucose injection, despite a rise in mean plasma glucose from 471 ± 5 mg/dl to 621 ± 18 mg/dl.

Pregnancy in five successfully transplanted rats did not alter the clearance rate of glucose from the blood. The prepregnancy k value in this group of five rats of 2.20 ± 0.16%/min was not different from the postpregnancy value of 2.58 ± 0.29%/min.

Pancreas grafts. Measurement of the volume of the pancreas graft before removal revealed a mean value of 305 ± 30 mm^3, which consisted mainly of adipose tissue. The total content of insulin in the 15 grafts removed 77–279 days after transplantation was 818 ± 43 mU (Table 2), and there was no difference in insulin content in grafts over this age span or between male recipients (783 ± 89 mU) and female recipients (827 ± 51 mU). By comparison, the insulin content of the total pancreas removed from normal male and female rats, aged 4 mo to 2 yr, was 3725 ± 214 mU, with the contents in older rats being greater than those in younger ones. The mean insulin content in the transplanted pancreas was 22% of the mean content of pancreases removed from normal rats. By contrast, the average insulin content in grafts removed from two rats that had a partial response to transplantation was 260 mU, and in those from two rats that failed to respond was 97 mU.

TABLE 2
Total insulin content of transplanted fetal pancreases removed from diabetic rats after complete reversal of diabetes compared with pancreases removed from normal animals

Normal Rat Pancreas			Transplant	
Sex	Age (mo)	Insulin (mU)	Age of graft (days)	Insulin (mU)
M	4	2961	77	940
M	4	2572	77	780
M	5	3153	105	923
M	5	3258	105	773
F	6	4302	150	885
F	6	4333	154	818
F	6	2970	155	729
F	6	3071	157	853
F	8	5175	157	1287
F	8	4044	181	751
F	10	3990	181	617
F	10	4131	216	646
M	24	4462	216	927
			253	718
			279	630
		3725		818
$\bar{x}$ ± SEM		±214		±43

DISCUSSION

The results reported here reveal the remarkable growth and function of fetal rat pancreas following its transplantation into a diabetic rat. Diabetes is completely reversed and the insulin content of the organ increases more than 4000-fold, from 0.2 mU[6] to 818 mU. Since this is 22% of the total insulin content of pancreases removed from normal rats of the same sex, age, and strain, it suggests that the beta cell component of the fetal organ grows to at least 22% of an adult pancreas. This value probably represents an underestimation of the beta cell content of the transplant, since electron micrographs of fetal pancreases removed 6 wk after transplantation, when diabetes was completely reversed, revealed considerable degranulation of the beta cells.[3]

Although partial recovery of beta cell function in islets not totally destroyed by streptozotocin was observed after a period of amelioration of diabetes from transplantation,[7] this is not likely to be a significant factor in our experiments. All rats become severely diabetic with ketosis after removal of the transplant, indicating severe insulin deficiency. The dose of streptozotocin used in nine rats was 90 mg/kg, which results in the loss of all but 2 or 3% of the normal pancreatic insulin content after 24 h:[8] this is the best indicator of beta cell destruction. Finally, we treated streptozotocin-induced diabetic rats with insulin for several months without reversal of the diabetes.

It is usually estimated that more than 90% of the pancreas must be removed to produce insulin deficiency sufficient to result in diabetes.[9] The finding of 22% of normal insulin content in the transplants, removed from rats after reversal of diabetes is consistent with this estimate and explains the maintenance of normal glucose metabolism during and after pregnancy. In four of five rats, the k value for glucose disappearance was higher after pregnancy than before it, although the mean postpartum value of 2.58%/min was not significantly greater than the 2.20%/min value determined before pregnancy. Since the metabolic and hormonal changes of pregnancy are diabetogenic,[10] it appears that the transplanted organ has a reserve capacity, which provides enough insulin for the needs of pregnancy.

Basal and glucose-stimulated insulin concentration in the blood of transplanted rats is moderately higher than normal. The reason is very likely the location of the venous drainage from the transplants into the renal vein, thus bypassing the liver. The liver usually extracts approximately 50%, in each pass, of the insulin entering the organ via the portal vein.[11] When insulin is delivered into a peripheral vein, less is retained in the liver, since, in rats, 51% of ^{131}I-labeled insulin

is recovered in the liver after injection into the portal vein,[12] compared with only 27% of that injected into a peripheral vein.

The mechanism by which insulin injection enhances the growth and function of a fetal pancreas transplanted into a diabetic rat is not clear. There is abundant evidence that supraphysiologic glucose concentrations stimulate beta cell proliferation and result in an increased beta cell mass.[13] Hyperglycemia alone is not adequate for this effect, since McEvoy and Hegre,[6,14] using the same methods as we did here, found that the beta cell mass was the same in fetal pancreases transplanted into normal and untreated diabetic rats. Thus, diabetes neither stimulated nor inhibited beta cell proliferation, but the administration of insulin to the diabetic rats after transplantation resulted in a threefold increase in beta cell mass and insulin content.[6] The question that arises is whether insulin directly stimulates beta cell proliferation or permits the proliferative stimulus of glucose to occur.

In vitro, an increase in glucose concentration from 5.5 mM to 16.5 mM produces a twofold to threefold increase within 40 h in the frequency of beta cell replication in monolayer cultures of neonatal rat pancreases.[15] This is completely inhibited by mannoheptulose, 5.5 mM, which also inhibits insulin release,[16] indicating that glucose must be metabolized by the beta cell to stimulate replication. Insulin was not added to the culture medium in these studies, but serum was present in the medium. The increased cell replication in response to 16.5 mM glucose did not occur when serum was omitted from the medium. Since serum contains growth factors,[17] these may substitute for insulin and permit the proliferative response to glucose. Evidence against a direct action of insulin to stimulate beta cell proliferation comes from the absence of this response to insulin injected into chick embryos.[18]

In man, the hyperplasia of pancreatic beta cells found in the fetus of diabetic mothers depends on the presence of an intact hypothalamus-hypophyseal system.[19] Growth hormone may be a pituitary hormone necessary for beta cell hyperplasia, since the plasma concentration is much higher in infants[20] at birth, and is still higher in the premature infant,[21] compared with adults, and the concentration responds to hyperglycemia with a rise rather than a fall, characteristic of the adult.[20] This response persists for 14 days post partum. In the blood of infants of diabetic mothers after birth (5–24 h), growth hormone levels are lower than normal and there is a subnormal rise in response to hypoglycemia.[21] This may be either due to depletion of the pituitary growth hormone content or a consequence of the lower blood glucose post partum compared with the elevated intrauterine levels resulting from maternal hyperglycemia. Additional evidence to support a role for growth hormone in beta cell growth comes from the rise in plasma insulin and the improved glucose tolerance following treatment of growth hormone-deficient dwarfs[22] and the in vitro enhancement of insulin synthesis and release from cultured islets.[23] Prolactin has the same effect in vitro,[23] but we found no increase in the insulin content of fetal pancreases removed 10 days after transplantation into normal rats who were injected with prolactin daily for 7 days.*

* Unpublished observations.

We conclude that, to achieve optimal function of the transplanted fetal pancreas, careful control of the blood sugar with insulin is necessary during the period of growth and development. Under these conditions, a fetal organ attains approximately 22% of the function of an adult pancreas and a reserve capacity to maintain normal glucose metabolism throughout a pregnancy. In applying this method to treating diabetes mellitus in human beings, an efficient mechanical device would be useful following transplantation to control blood glucose during the time of growth and development of the fetal pancreas.

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