Insulin Released from Fetal Rat Pancreases Transplanted into Normal and Diabetic Animals During Perfusion

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SUMMARY

Insulin release from the pancreas in response to glucose appears at the end of gestation in the fetal rat and develops completely in the neonatal period. To determine when this response begins to occur in fetal pancreases transplanted into adult animals, in vitro perfusions were performed in normal and diabetic rats after varying periods of growth. Responsivity to a 300 mg/dl glucose as opposed to a basal medium (50 mg/dl) was not present in the transplanted organ after 3 or 5 wk of growth in normal rats, despite a rise in total insulin content from 88 ± 10 mU to 197 ± 10 mU per two pancreases. Transfer of the pancreases—after 3 wk in the normal—to a diabetic rat for 2 wk resulted in a greater insulin content (343 ± 15 mU) and more insulin being released during perfusion with the basal medium (1863 ± 56 vs. 1374 ± 113 μU/h). Insulin release from these pancreases doubled during perfusion with the high glucose medium rather than with the basal medium. When the transplanted pancreas was left in the normal rats for 5 wk before being transferred to the diabetic animal for 2 wk, the insulin content increased (450 ± 10 mU) but there was no response to the glucose challenge during perfusion, despite good function, indicated by a response of all transplanted animals in the diabetic state. We conclude that glucose-induced insulin release will develop in the fetal rat pancreas transplanted into an adult animal after exposure to hyperglycemia, but optimal function requires a period of prior normoglycemia. Careful control of blood glucose with insulin may provide these conditions and will be required when this method is applied to diabetic patients. DIABETES 29:566–570, July 1980.

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A new method has been developed to completely reverse experimental streptozotocin-induced diabetes in the rat.[1] When the whole pancreas from a fetal rat is transplanted under the kidney capsule of a syngeneic adult diabetic recipient and there are optimal conditions for growth and function, complete reversal of the diabetic state follows.[2]

The normal glucose homeostasis and the normal plasma insulin response to glucose injection present in successfully transplanted diabetic rats[3] suggest that the fetal pancreas develops the mechanism for release of insulin in response to glucose. In the fetal and neonatal rat, pancreatic glucose uptake, phosphorylation, oxidation, and insulin synthesis occur before the beta cells release insulin in response to glucose.[4] The release of insulin is stimulated from the immature pancreases by such phosphodiesterase inhibitors as caffeine or theophylline[5] and by arginine;[6] however, these pancreases are almost totally unresponsive to glucose until the end of gestation and the postnatal period. The precocious appearance of insulin release in response to glucose appears in the fetal pancreases of a diabetic rat,[7] monkey,[8] and man,[9] suggesting that exposure to an elevated blood sugar induces the response. This thesis is supported by experiments demonstrating insulin release to glucose from fetal pancreases removed from pregnant rats that were infused with glucose during the last 5 days of gestation.[10]

In our previous experiments, we demonstrated that the optimal conditions needed for growth and function of the transplant are either 3 wk of growth in a normal animal before transfer to the diabetic[2] or prolonged insulin treatment after transplantation.[11] We observed that only 2 wk of growth in the normal rat before transfer to the diabetic did not affect the diabetic state. These results suggested that the duration of the growth period in the normal rat and the time of transfer to the diabetic animal are critical to the function of the transplanted pancreas.

In an effort to explain these findings, two considerations are important—the increasing capacity of the growing fetal

organ for insulin synthesis and the development of the secretory response to glucose. The experiments described here were designed to measure the insulin release from fetal rat pancreases removed after varying periods of growth in normal and diabetic recipients in response to perfusion in vitro with glucose, theophylline, and arginine.

MATERIALS AND METHODS

The induction of diabetes and the methods of transplantation were described previously by Brown et al.[3] The whole fetal pancreas, placed beneath the kidney capsule, becomes vascularized within 24 h, deriving an arterial supply via the renal artery. Venous drainage bypasses the kidney and passes either directly into the renal vein or first into the spermatic vein. In our experiments, each animal in four groups of normal rats was transplanted with two syngeneic pancreases, removed from fetal rats after between 16 and 17½ days of gestation, and they were placed beneath the capsule of the right kidney (Table 1). In groups III and IV, the kidney and undisturbed fetal pancreases were transplanted —at the times presented in Table 1—from the normal carrier into diabetic rats, as described by Mullen et al.[2] This procedure eliminates all blood vessel connections to the kidney and pancreases, except for the renal artery and vein. Groups I and II remained as normal carrier rats.

Throughout the experiment, urine was collected and measured daily and glucose content was determined on a Beckman glucose analyzer (glucose oxidase). Weekly tail-blood glucose concentrations were measured by the same procedure. Perfusion of the pancreases was carried out at the end of the growth period in normal carrier or diabetic rats.

Perfusion. Before perfusion, the rats were deprived of food overnight. They were then anesthetized with ether, at which time the aorta was cannulated at the distal side of the right renal artery. The kidney and undisturbed fetal pancreas were perfused through the cannula with 10 ml of cold phosphate-buffered saline (PBS) containing 0.2 ml heparin, after which time they were immediately removed and placed in iced PBS until perfused.

The kidney and pancreas were placed into a double-walled glass funnel and maintained at 37°C by passing water through the glass chamber (Figure 1). The cannula in the renal artery was attached to the perfusion apparatus and was perfused with Krebs-bicarbonate buffer, containing 3% human serum albumin and 50 mg/dl glucose, at 37°C and aerated with 95% O_2 and 5% CO_2. A Buchler peristaltic pump delivered the medium at a rate of 4.5 ml/min. The perfusate delivered into the aorta entered the fetal pancreases through the renal artery, as visually observed by injection of cardiac green dye. The effluent from the renal vein was collected in 1-min fractions with a Gilson fraction collector. Alternate samples were assayed for immunoreactive insulin

TABLE 1
Times of pancreas transplantation from normal to diabetic rats

Group	Number of rats	Time in normal carrier (wk)	Time in diabetic rat (wk)
I	5	3	—
II	5	5	—
III	3	3	2
IV	3	5	2

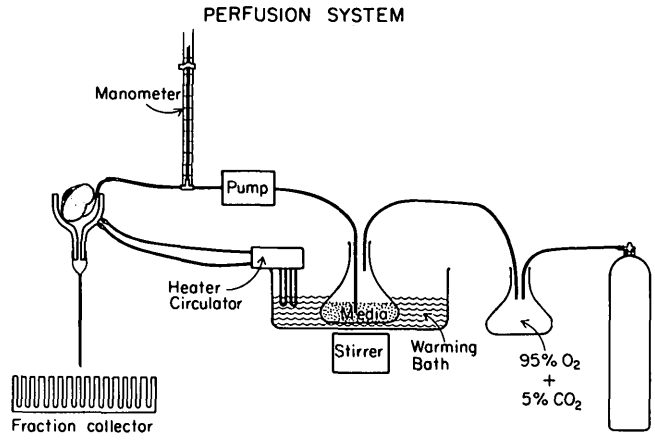

FIGURE 1. Apparatus used for perfusion of fetal pancreas and kidney via a cannula in the renal artery.

using a rat standard (Novo Industri, Copenhagen). The total perfusion time (90 min) was divided into increments, as outlined in Table 2. Glucose, arginine, and theophylline were added to the medium passing into the organ via the side arm by a Harvard infusion pump in an amount calculated to achieve the desired concentration.

Tissue insulin contents. At the end of the perfusion time, the fetal pancreases were removed from the kidney surface, immediately frozen on dry ice, and stored at −70°C. For insulin radioimmunoassay, the frozen-stored tissues were homogenized in cold acid-alcohol and extracted overnight at 4°C, and the insulin content was measured against a rat insulin standard.

Calculations. A period of 15 min was required to stabilize insulin release before the experiment was begun. Basal, unstimulated insulin release was calculated from the average insulin content in effluents collected during the second (30–45 min) and third (60–75 min) periods of perfusion with the basal medium. Since roughly 5 min was required for insulin release to stabilize after the onset and cessation of each stimulus, the insulin content of effluents collected during the last 10 min of each collection period was included in the calculation of insulin release and was expressed as microunits per hour. The paired *t* test was used to compare the amount of insulin released with the various stimuli in each animal.

RESULTS

The release of insulin from perfused fetal pancreases removed after being grown for varying periods of time in normal and diabetic rats is shown in Figure 2. Pancreases grown in normal rats for 3 or 5 wk demonstrate no increase

TABLE 2
Perfusion times for fetal pancreases

Time increment (min)	Perfusate
0–15	Medium only
15–30	Medium plus glucose (300 mg/dl)
30–45	Medium only
45–60	Medium plus arginine (20 mM)
60–75	Medium only
75–90	Medium plus theophylline (5 mM)

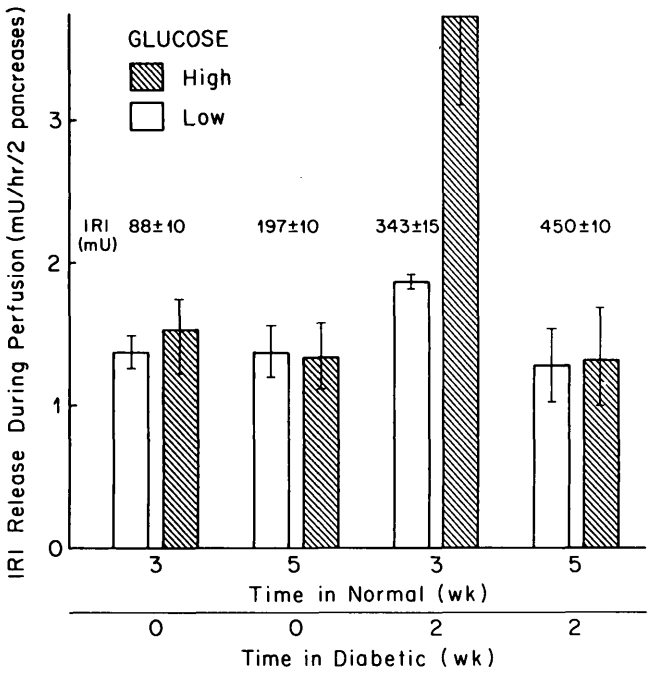

FIGURE 2. Insulin released from fetal rat pancreases during perfusion after growth in normal and diabetic rats. Insulin released during perfusion with the basal medium, containing 50 mg/dl glucose (low), is compared with that released after addition of glucose to a concentration of 300 mg/dl (high). Insulin released from pancreases perfused after 3 wk (group I) or 5 wk (group II) time in normal rats is compared with those placed into diabetic animals for 2 wk after 3 wk (group III) or 5 wk of growth in normal rats. The total insulin content ± SEM in the transplants removed from the four groups of rats is given.

in insulin release in response to the high glucose (300 mg/dl) perfusion medium compared with the low glucose medium. In contrast, after 3 wk of being grown in a normal rat, pancreases transferred to diabetic animals for 2 wk demonstrated both a greater release of insulin during perfusion with the low glucose (50 mg/dl) medium and a doubling of insulin release in response to high glucose. The pancreases grown for 5 wk in the normal rat before being transferred to the diabetic for 2 wk revealed low basal insulin release and no increase in response to the high glucose medium.

The total insulin content of the transplanted pancreases doubled (88 to 197 mU) during the interval from 3 to 5 wk of growth in the normal rat (Figure 2). In contrast, there was a fourfold increase in insulin content (88 to 343 mU) when the last 2 wk of growth took place in a diabetic recipient. There was a similar increment in insulin content of the transplants placed into diabetic recipients for 2 wk after 5 wk in the normal animal. The content of insulin in these organs removed after perfusion was not appreciably affected by the insulin released during perfusion (2–4 mU).

The amount of insulin released in response to the addition of arginine (20 mM) or theophylline (5 mM) to the perfusing medium in comparison with the medium alone, which contains 50 mg/dl glucose, is shown in Table 3. Comparison of group III with group I during perfusion with only medium reveals a significantly ($P < 0.01$) greater release of insulin in group III, but no difference was apparent between groups II and IV. After the addition of arginine to the medium, there was a statistically significant ($P < 0.01$) increase in insulin release in group III only, although the response in group IV

TABLE 3
Insulin released from two fetal rat pancreases during perfusion with medium containing 50 mg/dl glucose and followed by addition of arginine or theophylline (insulin in microunits per hour)

Medium	Group I	Group II	Group III	Group IV
Alone	1374* ± 113	1370 ± 187	1863 ± 56	1282 ± 26
+ Arginine, 20 mM	1592 ± 146	1873 ± 261	2682 ± 65	1998 ± 311
+ Theophylline, 5 mM	2100 ± 145	2158 ± 391	2664 ± 78	1836 ± 220

* $\bar{X}$ ± SEM.

was almost significant. Theophylline added to the perfusing medium resulted in a statistically significant rise in insulin release in group I ($P < 0.01$) and group III ($P < 0.01$) compared with that in the medium only, but the findings were not statistically significant in groups II or IV, because there was a large variation in response.

The temporal patterns of insulin release from a representative pancreas in groups III and IV in response to perfusion with medium alone and after the addition of glucose, arginine, and theophylline are shown in Figure 3. These findings again demonstrate the greater insulin release in group III than in group IV during perfusion with medium only and the response to glucose, arginine, and theophylline. In contrast, insulin release from the pancreas in group IV was unresponsive to glucose, and the response to arginine and theophylline was of low amplitude.

The response of the diabetic state during the 2 wk following transplantation of two fetal pancreases after 3 wk of growth in normal rats (group III) and 5 wk (group IV) is shown in Table 4. Since the urine volume and glucose content were measured daily, these results are more meaningful than measurement of plasma glucose carried out once each week. Among the three diabetic rats in group III, number 1 probably responded, number 2 clearly responded, and number 3 did not respond. All three diabetic rats in group IV responded to the transplanted pancreases at the time of removal for perfusion.

DISCUSSION

The results of these experiments indicate that fetal pancreases transplanted into normal rats and removed after 3 or 5 wk do not develop the capacity to respond to a glucose challenge with an increased release of insulin during perfusion. This clearly differs from the normal development of glucose responsivity in the neonatal rat pancreas, which first appears at 18 h[12] or 48 h[13] after birth, is well developed at 3 days post partum,[13] and is apparently fully developed by 12 days post partum.[12] This lack of stimulation of insulin release by glucose in the transplanted fetal pancreas does not result from a paucity of the hormone, since the insulin content doubles in the period from between 3 and 5 wk.

The dichotomy of increased insulin synthesis without release in response to glucose is characteristic of the fetal beta cell.[14] However, these immature cells release insulin in response to theophylline and arginine.[15] We also observed that, although it did not reach statistical significance, there was an increased release of insulin during perfusion in response to arginine and theophylline in pancreases removed

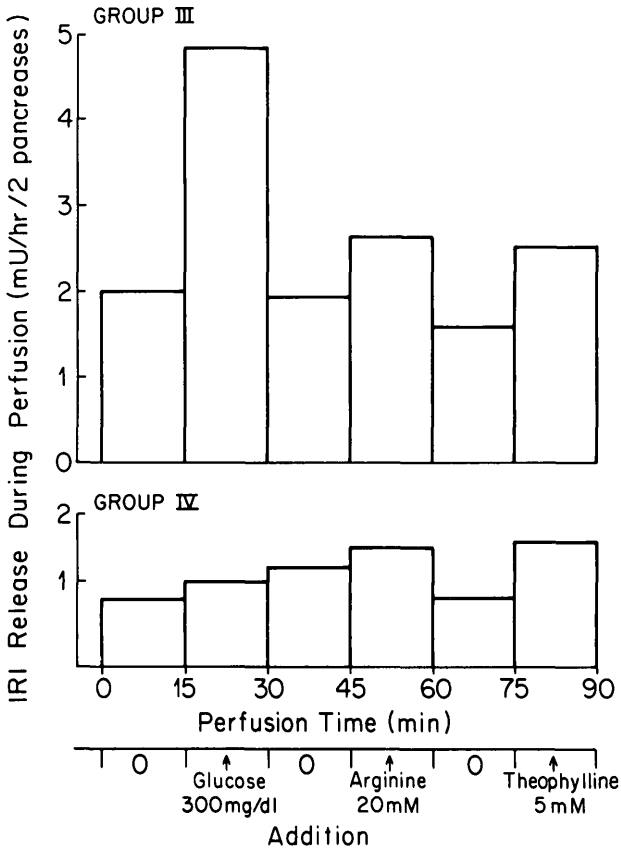

FIGURE 3. Insulin released during perfusion from a transplanted pancreas in group III (rat 2) and group IV (rat 2). The basal perfusion medium, which contained 50 mg/dl glucose, was supplemented with the indicated additions.

from normal rats. It is of interest that some diabetic patients are unresponsive to glucose, but they respond to theophylline[16] or arginine.[17]

The response to a high glucose stimulus during perfusion was clearly different in pancreases removed after 2 wk of growth in diabetic rats (group III). Insulin release doubled during perfusion with medium containing 300 mg/dl glucose. In addition, the amount of insulin released during perfusion with the basal medium was also greater from pancreases removed from diabetic rats than from those removed from normal animals. This is probably due to the greater insulin content of pancreases growing in diabetic animals compared with those growing for 3 wk (fourfold) or 5 wk (twofold) in normal animals. Other investigators who used pancreases from fetuses of the same gestational age implanted into the same site found similar insulin contents.[18] They found that the insulin content of pancreatic implants removed at about 10 wk from alloxan-diabetic rats, responding within 4 wk after transplantation of eight fetal pancreases, was 112 ± 10 mU per implant.

Hyperglycemia induces the glucose mechanism for insulin release in infants of diabetic mothers and can be induced precociously in offspring of normal rats by administering glucose to the mother at the end of gestation. It is not surprising, therefore, that this mechanism also occurs in the transplanted fetal pancreas. Similar events occur in vitro; fetal rat islets cultured in a medium containing 11.1 mM glucose release insulin in response to a glucose challenge after between 7 and 10 days in culture. This does not occur when the islets are cultured in a low glucose medium.[19]

Although these observations strongly suggest that a hyperglycemic stimulus is needed to induce the insulin response to glucose, it does not appear that this stimulus is present during the normal neonatal period. Rats at this stage of development are not exposed to a high, circulating, glucose level.[13] Even in overfed neonatal rats, serum glucose was 69 ± 5 mg/dl at 2 h after feeding at the second day after birth, when the insulin response to glucose was already intense; this was a sevenfold rise in vivo and a threefold rise in vitro. Underfeeding neonatal rats delays the appearance of glucose responsivity. At the second day after birth, when glucose responsivity was not yet present, un-

TABLE 4
Severity of the diabetes before and during the 2 wk after transplantation of two fetal pancreas removed from normal rats after 3 wk (group III) and 5 wk (group IV)

	Urine volume (ml/day)			Urine glucose (g/day)			Plasma glucose (mg/dl)	
Rat	Before	After	P	Before	After	P	Before	After
				Group III				
1	107* ± 6	88 ± 6	<0.05	9.6 ± 5.5	7.6 ±0.9	<0.03	513	471
2	98 ± 6	48 ± 7	<0.001	8.0 ±0.5	2.2 ± 0.5	<0.005	401	256
3	119 ± 12	147 ± 11	N.S.	11.9 ± 2.1	8.9 ± 1.7	<0.03	530	460
				Group IV				
1	144 ± 3	115 ± 5	<0.001	13.9 ±0.2	9.7 ± 1.4	<0.05	537	568
2	119 ± 5	68 ± 7	<0.001	11.4 ± 0.6	6.3 ± 1	<0.02	525	468
3	146 ± 1	22 ± 3	<0.001	12.8 ± 1	1.8 ± 0.5	<0.001	560	267

* $\bar{X}$ ± SEM.

derfed rats had a serum glucose level of 54 ± 3 mg/dl at 2 h after feeding and of 77 ± 3 mg/dl by the fourth day, when a brisk insulin response to glucose stimulation had developed. These values could be interpreted as relative hyperglycemia, since the blood glucose level in fasted newborn rats falls from 40 mg/dl at 8 h to 20 mg/dl at 16 h.[20]

There is evidence that the metabolism of glucose by the beta cell is a prerequisite for stimulation of insulin release,[21] and, since the beta cell is freely permeable to glucose,[22] phosphorylation to glucose 6-phosphate may be the rate-limiting step. Glucokinase, the major, phosphorylating enzyme, has been identified in isolated islets,[23,24] and it has been suggested that this enzyme regulates glycolysis in islets.[25] We recently measured glucokinase in fetal and neonatal rat pancreases and in transplants growing in normal and diabetic rats.[26] The results are consistent with the role of glucokinase as a regulator of glucose metabolism in neonatal rat islets.

In seeking an explanation for the lack of response to glucose during perfusion of pancreases in group IV, neither a deficiency of insulin content nor an inability to release insulin appears likely. The insulin content of these pancreases was the highest of any group, and the diabetes of all rats transplanted with these pancreases responded even at 2 wk, when the animals were killed. A possible explanation for the lack of response during perfusion is depletion of a readily releasable supply of insulin from the beta cells. Transplanted diabetic rats who are hyperglycemic have plasma insulin levels of between 100 and 200 μU/ml, and there is no rise in plasma insulin in response to glucose stimulation.[27] Human diabetics with fasting hyperglycemia but no first phase insulin release, who achieve normal fasting glucose levels by diet, exhibit a return of first phase release, suggesting that a readily releasable pool of insulin is repleted.[28]

In conclusion, these experiments reveal the fact that glucose-induced insulin release will develop in the fetal rat pancreas transplanted into an adult animal when it is exposed to hyperglycemia. Optimal function of the fetal organ requires a period of growth in a normoglycemic milieu before the hyperglycemia, and careful insulin treatment of diabetic rats provides the desired conditions for optimal growth. In considering the application of this method of treatment of diabetes to man, the results of these studies suggest that exact control of the blood sugar after transplantation will be necessary.

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