Infant Human Pancreas

A Potential Source of Islet Tissue for Transplantation

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SUMMARY

Twelve pancreases from human infants one year old or less were analyzed for tissue insulin and amylase content before and after dispersal of pancreatic fragments by mincing and collagenase digestion. Tissue insulin and amylase content provide an index of pancreatic islet mass and exocrine digestive enzyme content, respectively. The results were compared with similar analyses performed on juvenile and adult human pancreases before and after islet isolation and on intact and dispersed neonatal rat and adult rat pancreas. Infant human pancreas has an average tissue insulin concentration of 1,128 μg./gm. of tissue and a total insulin content of 1,718 μg./pancreas, as against values of 140 μg./gm. of tissue and 7,209 μg./pancreas for adult human pancreas. Average tissue amylase concentration is 0.24 mg./gm. of tissue in infant human pancreas and 3.0 mg./gm. of tissue in adult human pancreas. The insulin/amylase ratio in infant pancreas is 4,800, as against 46 in the adult pancreas. Neonatal rat pancreas, which can be dissociated and transplanted without separation of islet and exocrine components, has a similarly high tissue insulin and low tissue amylase content when compared with adult rat pancreases. Infant human pancreas has a total islet mass 24 per cent that of an adult human pancreas, and neonatal rat pancreas has a total islet mass 11 per cent of that of an adult rat pancreas. One neonatal rat pancreas prepared by minimal collagenase digestion can cure diabetes when transplanted via the portal vein to a rat. Following dispersal of infant human pancreas by collagenase digestion, the islet content and the insulin/amylase ratio of the recovered tissue equals or exceeds that which usually can be isolated from adult cadaver pancreases. Infant human pancreas is a rich source of islet tissue that is relatively uncontaminated by exocrine digestive enzymes. After dispersal, infant human pancreas may be ideal for transplantation to selected diabetic patients. DIABETES 25:1123-28, December, 1976.

Successful experimental islet transplantation from adult donors to diabetic animals has required the development of specific techniques to separate the islets of Langerhans from the exocrine pancreas. Attempts to transplant adult pancreatic fragments as free grafts without specific islet isolation have been unsuccessful because the associated exocrine digestive enzymes either destroy the graft or injure the host.[1] Current techniques[2-4] to isolate islets from the adult pancreas (collagenase digestion, followed by either density-gradient separation or hand picking of islets under a dissecting microscope) are laborious and are associated with islet loss. In addition, the techniques are inefficient, and several adult rat donors are required to provide sufficient islet tissue for amelioration of diabetes of only one rat recipient.[4-6] It is even more difficult to isolate islets from the compact adult human cadaver pancreas,[7] and the yield is variable and unpredictable.[8]

Although a pure preparation of islets is not necessary for successful islet transplantation in the rodent,[9,10] the dissociated tissue containing the islets must not be contaminated with exocrine enzymes.

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Neonatal rat pancreas has a relatively large islet mass and low exocrine digestive enzyme content.[10] These properties allow neonatal rat pancreas to be dispersed and successfully transplanted without separation of islet and exocrine components.[10-13]

The infant human pancreas, if it possesses properties similar to the neonatal rat, could provide a rich source of islet tissue for clinical transplantation. In the work reported here, we analyzed 12 infant human cadaver pancreases for tissue insulin and amylase content before and after pancreatic dispersal and compared the infant pancreases to juvenile and adult human pancreases before and after islet isolation. Neonatal rat pancreases were similarly analyzed and compared with adult rat pancreases.

MATERIALS AND METHODS

Sources and Processing of Pancreases

Twelve fresh pancreases were obtained from infant human cadavers (age one day to one year). Each pancreas was weighed and a portion of it was homogenized in 0.25 M sucrose for analysis of tissue insulin and amylase content. The rest of the pancreas was minced into 0.5-to-1-mm. fragments, placed in Hanks' solution and digested with 20-40 mg. collagenase (Worthington, 170-210 U./mg.) per gm. of tissue. Digestion at 37° C. was continued for 10 to 30 minutes to disperse the pancreatic fragments. An aliquot of the dispersed tissue was homogenized and analyzed for total insulin and amylase content. The procedure we used is similar to the technique first described by Leonard et al. to prepare neonatal rat pancreas for transplantation.[11]

Sixty-eight pancreases from human brain-dead cadavers greater than one year of age were obtained for analysis of tissue insulin and amylase content. Ten pancreases were 17 months to five years old, 12 were six to 15 years old, and the remaining 46 were 16 to 60 years old. These pancreases were processed by the collagenase digestion-Ficoll density-gradient separation technique, and islet recovery and purity were assessed as previously described.[7,8]

The tissue insulin and amylase contents of 90 neonatal Lewis rat pancreases (age six to nine days) and 30 adult Lewis rat pancreases were determined and compared with infant and adult human pancreases. Neonatal rat pancreases were analyzed following dispersal by mincing and collagenase digestion for either 40 minutes or for one and one-half to two minutes. The latter technique of minimal collagenase digestion has also been shown to effectively disperse neonatal rat pancreases for transplantation.[14]

Tissue Insulin and Amylase Assays

We modified the double-antibody method of Morgan and Lazarow[15] to assay the homogenized tissue for insulin.[7] Human insulin standards (gift of Dr. Mary A. Root, Eli Lilly Company) were used for human-insulin assays, and rat insulin standards (gift of Novo Company, Copenhagen) were used for rat-insulin assays.

Amylase content was measured by the technique of Jamieson et al.[11] Soluble starch (2 per cent) was used as the substrate. Hog pancreatic alpha amylase (Sigma Chemicals, 900 U./mg.) was the standard.

Results were expressed as μg. insulin/gm. of tissue, mg. amylase/gm. of tissue, and μg. insulin/mg. of amylase. The total insulin and amylase content of individual pancreases and of recovered tissue were calculated from the known weights of the pancreases prior to and following processing. Islet mass is assumed to be proportional to total tissue insulin content, and exocrine enzyme content is assumed to be proportional to tissue amylase content.[10] The insulin/amylase ratio reflects the purity of islet tissue relative to exocrine enzyme contamination.

Insulin Secretory Capability of Dispersed Human Infant Pancreatic Fragments

Using the perifusion method of Lacy et al.[17] we tested dispersed pancreatic fragments from an infant (one year old) for their ability to secrete insulin in response to glucose stimulation. A baseline of insulin release was established by first perfusing the fragments for 60 minutes at 37° C. in a Millipore chamber with Hanks' solution and a glucose concentration of 30 mg./100 ml. The concentration of glucose was then increased to 300 mg./100 ml. and the perfusion continued for an additional 60 minutes. Effluent fractions were collected every minute and assayed for insulin.

RESULTS

The insulin and amylase content and insulin/amylase ratio of each infant human pancreas are presented in table 1. Table 2 compares the mean tissue insulin and amylase content of infant and adult human pancreases and of neonatal and adult rat pancreases. In the human pancreases, extremely high tissue insulin and extremely low tissue amylase concentrations were seen only in pancreases from infants one year old or less. In the 17-month-to-five-year-old age group, mean values of 162 ± 142 μg. insulin/gm. of

TABLE 1

Insulin and amylase content of infant human pancreases

Age	μg. Insulin / gm. Tissue	Total pancreatic insulin (μg.)	mg. Amylase / gm. Tissue	Total pancreatic amylase (mg.)	μg. Insulin / mg. Amylase
1 day	1,132	966	0.056	0.048	20,214
1 day	1,986	1,132	0.001	0.001	198,600
2 days	2,151	2,065	0.036	0.035	58,900
3 days	1,080	1,134	0.015	0.016	72,000
4 days	1,584	1,885	0.021	0.025	75,400
7 weeks	792	1,670	0.046	0.098	17,200
2 months	848	1,282	0.132	0.200	6,400
2 months	460	874	0.053	0.101	8,700
2 months	1,386	2,096	0.017	0.026	81,500
3 months	706	3,700	1.398	7.325	500
1 year	1,188	2,518	0.887	1.880	1,300
1 year	221	1,298	0.173	1.015	1,300

tissue and 4.4 ± 4.0 mg. amylase/gm of tissue were obtained. In the six-to-15-year-old age group, mean values of 178 ± 141 μg. insulin/gm. of tissue and 4.3 ± 2.9 mg. amylase/gm. of tissue were obtained. The values in both of these age groups are similar to those of adult pancreases 16 years or older (table 2).

Pancreatic Tissue Insulin Content

Infant human pancreases contained 1,128 ± 580 μg. insulin/gm. of tissue, as against 140 ± 120 μg. insulin/gm. of tissue in adult cadaver pancreases. Total insulin content was 1,718 ± 808 μg. per infant human pancreas and 7,209 ± 5,788 μg. per adult human pancreas.

Neonatal rat pancreases contained 526 ± 201 μg. insulin/gm. tissue, as against 168 ± 93 μg./gm. tissue in adult rat pancreases. Total insulin content was 11.5 ± 4.3 μg. per neonatal rat pancreas and 101 ± 55 μg. per adult rat pancreas.

A striking parallel exists between pancreases of the two species. Neonatal rat pancreas, though only 3.5 per cent by weight of the adult rat pancreas, contains 11 per cent as much total insulin. Infant human pancreas, though only 4 per cent by weight of the adult human pancreas, contains 24 per cent as much total insulin.

Pancreatic Tissue Amylase Content

All infant human pancreases had tissue amylase concentrations of less than 1.5 mg./gm. of tissue. The amylase content of 10 of the 12 infant pancreases was below 0.18 mg./gm. (table 1). The average tissue amylase concentration in human infant pancreases was 0.24 ± 0.44 mg./gm., as against 3.0 ± 1.8 mg./gm. of tissue in adult human pancreases (table 2). Total pancreatic amylase content was less than 1 mg. in nine of the 12 infant pancreases and averaged 0.80 ± 2.1 mg. per pancreas, as against a total amylase content of 143 ± 84 mg. per adult pancreas.

Neonatal rat pancreases contained 2.0 ± 0.4 mg. amylase/gm. of tissue and 0.04 ± 0.001 mg. total amylase per pancreas. Adult rat pancreases contained an average of 23.4 ± 10.8 mg. amylase/gm. of tissue and 14.3 ± 7.5 mg. total amylase per pancreas.

Again, the parallel between neonatal rat and human infant pancreases is apparent—both have an extraordinarily low amylase content. The tissue amylase concentration in infant human and in neonatal rat pancreases is approximately 8 per cent of that in adult pancreases of each species.

Pancreatic Tissue Insulin/Amylase Ratio

Infant human pancreases had tissue insulin/amylase

TABLE 2

Comparison of average insulin and anylase content (± S.D.) of infant and adult human and neonatal and adult rat pancreases

Pancreatic source (No. of pancreases)	Weight of pancreas (gm.)	μg. Insulin / gm. Tissue	Total pancreatic insulin (μg.)	mg. Amylase / gm. Tissue	Total pancreatic amylase (mg.)	μg. Insulin / mg. Amylase
Infant human (12)	2.07 ± 1.70	1,128 ± 580	1,718 ± 808	0.24 ± 0.44	0.90 ± 2.10	4,800
Adult human (46)	53.3 ± 16.8	140 ± 120	7,209 ± 5,788	3.0 ± 1.9	143 ± 84	46
Neonatal rat (90)	0.021 ± 0.003	526 ± 201	11.5 ± 4.3	2.0 ± 0.4	0.04 ± 0.001	263
Adult rat (54)	0.595 ± 0.150	168 ± 93	101 ± 55	23.4 ± 10.8	14.3 ± 7.5	7

ratios ranging from 500 to 198,000, and the ratio was over 1,000 in 11 of the 12 pancreases examined (table 1). The average insulin/amylase ratio, calculated from the mean of the insulin/gm. of tissue and amylase/gm. of tissue, was 4,800 for infant human pancreases and 46 for adult human pancreases.

The average insulin/amylase ratio in neonatal rat pancreases was 263, as against a ratio of 7 in the adult rat pancreases. The insulin/amylase ratios in neonatal rat and infant human pancreas were similarly high when compared with adult pancreas.

Islet Recovery After Pancreatic Processing

After dispersion of infant human pancreases by mincing and prolonged collagenase digestion, the recovered tissue had a mean total insulin content of 467 ± 329 μg. and an insulin/amylase ratio of 2,600. The total insulin content before and after pancreatic processing indicated that 73 per cent of islet tissue was destroyed or lost when the pancreas was dispersed by this technique. However, even after dispersal of the infant pancreas the average insulin content of the remaining tissue was 6.5 per cent of that contained in an entire adult pancreas.

Islet recovery from adult human pancreases, processed by collagenase digestion-Ficoll gradient separation, ranged from less than 1 to 51 per cent, as determined by total insulin content on the isolated material. Islet yield from adult human pancreas was usually in the lower range, and mean insulin content of purified tissue was 400 ± 661 μg., or 5.5 per cent of the total present in an average adult pancreas. Although insulin/amylase ratios up to 3,200 were achieved, the average insulin/amylase ratio of the isolated tissue was 300, with a ratio of 46 in whole pancreas prior to processing.

The data show that the quantity of islet tissue available from infant pancreas following dispersal by prolonged collagenase digestion equals or exceeds that which can usually be obtained by islet isolation from adult human pancreases. The insulin/amylase ratios indicate that islets within dispersed infant pancreases are less contaminated with amylase than are the islets isolated from adult pancreases.

Neonatal rat pancreases dispersed by collagenase digestion for 40 minutes had a final insulin content of 3.4 ± 4.2 μg. per pancreas, a reduction of 70 per cent from the original total pancreatic insulin content. However, a neonatal rat pancreas dispersed by the minimal collagenase digestion technique had a final insulin content of 6.6 ± 4.0 μg. per pancreas, a reduction of only 43 per cent. These results indicate that shortening the digestion period preserves islet tissue. The average final total tissue insulin content of one neonatal pancreas dispersed by minimal collagenase digestion was 6.5 per cent of that present in an entire adult pancreas. The minimal collagenase digestion technique was not used on the infant human pancreases analyzed.

Insulin Secretion From a Dispersed Infant Human Cadaver Pancreas

Baseline insulin release from the dispersed pancreas fragments perfused with 30 mg. glucose/100 ml. was 12 μU./minute. Insulin release increased abruptly to a peak of 108 μU./minute following stimulation with 300 mg. glucose/100 ml. This peak was followed by a sustained release of 40 μU. insulin/minute for the duration of perfusion, indicating that viable islets were present in the dispersed tissue.

DISCUSSION

We analyzed the tissue insulin and amylase content of pancreatic tissue on the assumptions that (a) tissue insulin is proportional to islet beta-cell mass, (b) tissue amylase is proportional to exocrine digestive enzyme content, and (c) the insulin/amylase ratio provides an index of islet mass and purification in relation to exocrine enzyme contamination. These assumptions are based on the work of Leonard et al.[10] They showed that the concentrations of amylase and chymotrypsinogen in rat pancreatic tissue parallel each other at various stages of development and that fetal, neonatal, and adult rat pancreatic tissue insulin concentration correlates with the percentage of pancreatic volume attributable to islet tissue. Leonard et al. quantified the percentage of islet tissue by the linear scan method of Lazarow and Carpenter.[18]

Our analysis of infant human pancreas was prompted by two facts: (a) islets cannot be consistently isolated from adult human pancreases,[8] and (b) dissociated neonatal rat pancreas can be successfully transplanted to cure diabetic rats without first separating the islets from associated exocrine tissue.[10-13] Our analyses show that the infant human and neonatal rat pancreas share two important characteristics—a high tissue insulin (and therefore islet) content and a low exocrine enzyme content. These are the same two properties that presumably render neonatal rat pancreas suitable for transplantation as a free graft.

Apart from our analyses, there is little information available on the insulin and exocrine enzyme content of human infant pancreas. Wrenshall et al.[19] reported on the extractable insulin of human pancreases and

defined a downward trend in concentrations of insulin per gram of pancreas from younger to older pancreases. However, their report includes no specific data on infant pancreas and no data at all on pancreatic exocrine enzyme content. They did provide evidence that the insulin/gm. of pancreas is an approximate index of islet mass/gm. of pancreas.

The insulin contents of the human pancreases we examined indicate that infants less than one year old have an islet beta-cell mass that is approximately 24 per cent of that present in an average adult pancreas. This finding corroborates the results of histologic studies on 100 human pancreases reported by Ogilvie.[20] Using volumetric techniques, Ogilvie determined that the average weight of islet tissue in an infant pancreas less than one year old was 0.23 gm. Adult pancreases 16 years old or older had an average islet mass of 1.05 gm. According to Ogilvie's technique, total islet mass in an infant pancreas is 22 per cent of that in an adult pancreas. This figure is almost identical to the one we obtained. Ogilvie found considerable variation in the number of islets in human pancreases, but he reported an average of 506,000 in the infant and 983,000 in the adult. He calculated the islet tissue to be 4 per cent of the pancreas in the infant and only 1.6 per cent in the adult. These percentages are similar to those determined in neonatal and adult rat pancreases by histologic linear-scan methodology.[10,18]

Our analyses suggest that dispersed human infant pancreas will be a suitable preparation for islet tissue transplantation. It remains to be seen whether or not more than one donor will be required for successful transplantation. The total mass of islet tissue in an infant pancreas (24 per cent that of an adult), if successfully engrafted, should be sufficient for maintenance of normal carbohydrate metabolism.

Neonatal rat pancreases dispersed by collagenase digestion for 40 minutes can consistently cure diabetic rats when transplanted as a free graft.[10-13] We have found that dispersal by this technique is associated with loss of islet tissue.[13] Leonard et al.[11] originally used 20 to 30 donors to obtain a sufficient quantity of tissue for transplantation. They later showed that the younger the donor tissue, the less islet mass required for successful transplantation. However, intraperitoneal transplantation of islet tissue prepared from dispersed neonatal pancreas of any age still required several donors to effect a cure of diabetes.

Kemp et al.[5] originally reported that the portal vein was a more efficient site for transplantation of isolated adult rat islets. Even with this technique, several adult rat donors were required to obtain a sufficient quantity of islets. We have combined the use of rat neonates as a donor source and the portal vein as a transplant site and have cured diabetic rats by transplanting as few as two neonatal rat pancreases dispersed by collagenase digestion for 40 minutes.[13] Dispersion by minimal collagenase digestion for one and one-half to two minutes has allowed us to cure diabetic rats by transplantation of only one neonatal rat pancreas.[14] Our analyses of neonatal rat pancreases reported here indicate that a conservation of islet tissue can be achieved by minimal collagenase digestion. We calculate that diabetes in rats can be ameliorated by transplanting tissue with an islet mass that is 6 per cent of that in a normal adult rat pancreas. Although we have not yet applied the minimal collagenase digestion method to the infant human pancreas, we believe that dispersal by this technique may provide enough islets from a single infant pancreas to cure diabetes mellitus in one patient.

The progression of renal glomerular and tubular lesions associated with diabetes in the rat can be halted or reversed by curative islet transplantation.[22,23] The etiology of kidney, eye, vascular, and other lesions in diabetic patients is controversial. The development of microangiopathic lesions in animals with induced diabetes[24-26] and the clinical observations that the incidence and severity of small-blood-vessel disease is lessened when carbohydrate metabolism can be well controlled[27,28] suggests that the lesions are directly related to disordered carbohydrate metabolism itself. If this hypothesis is correct, perfect control of carbohydrate metabolism would prevent the development of these lesions. Exogenous insulin, no matter how meticulously administered by standard techniques, cannot provide such control.[29] Therefore, if the progressive vascular complications in various organs are to be prevented, dramatic and innovative changes in the therapy of diabetes mellitus are needed.

Technically, it is much more difficult to separate islets from the exocrine tissue and associated digestive enzymes of the adult human cadaver pancreas than from the adult rat pancreas. By using neonatal rat donors, specialized techniques are not required to separate islets from exocrine tissue for successful transplantation.[10-13] Infant human pancreas and neonatal rat pancreas share an important characteristic—a low exocrine digestive enzyme content. Furthermore, the infant human pancreas contains a larger percentage of islet mass relative to the adult than does the neonatal rat pancreas. The infant

human cadaver pancreas may be an ideal source of islet tissue for transplantation.

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