Hyperinsulinemia and Hyperglucagonemia Following Pancreatic Islet Transplantation in Diabetic Rats

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SUMMARY

Fasting blood glucose (FBG), serum immunoreactive insulin (IRI), plasma immunoreactive glucagon (IRG), body weight, and caloric intake were measured in long-term islet-isografted rats eight to 10 months following intraperitoneal islet transplantation and in age-matched, sham-operated, concurrently followed normal and diabetic controls. Islet recipients had normal body weights, but they were significantly polyphagic, hyperinsulinemic, and hyperglucagonemic when compared with normals. Fasting blood glucose levels were reduced by 10 per cent. Several factors may be related to the occurrence of these abnormalities in long-term islet-isografted rats, including (1) the mass of islets transplanted, (2) the age of donor tissue, (3) the heterotopic location of islet grafts, and (4) the lack of normal innervation of transplanted islet cells. DIABETES *25*:944-48, October, 1976.

Recent studies from this and other laboratories[1-6] have shown that intraperitoneal transplantation of isogeneic neonatal pancreatic islets into adult diabetic rats restores normal weight gain, intravenous glucose tolerance, and epididymal fat cellularity. This communication reports the occurrence of hyperinsulinemia, hyperglucagonemia, polyphagia, and reduction of fasting blood glucose in long-term islet-isografted rats studied eight to 10 months following transplantation and compared with age-matched normal controls.

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MATERIALS AND METHODS

Inbred Lewis rats five to nine days old were donors of pancreatic islets. Islet recipients (N=18) and concurrently followed normal (N=10) and diabetic (N=20) controls consisted of 200-300-gm. adult Lewis rats (Micro. Assoc.). Experimental animals were housed separately in metabolic cages and fed standard commercial rat food (CAMM) and water ad libitum. Diabetes was induced by intravenous streptozotocin, 6.5 mg./100 gm. body weight. Islet recipients and controls were chosen from a larger group of diabetic rats maintained in our laboratory. An animal was defined as diabetic only when there were at least four stable fasting blood glucose (FBG) levels > 300 mg./100 ml. during at least 10 (mean=25) days, stable 3+ urine glucose (Dextrostix), and stable 24-hour urine outputs at least five times normal (> 40 ml./24 hours).

Pancreatic islets were prepared for transplantation by a technique modified from Leonard.[4] For each exper-

iment, pancreases from 25-35 donors were minced in Hanks' solution at 4° C., with repeated washing to remove fibrous and acinar debris, and dispersed with collagenase, 10,000 units (type IV, lot 44A193, Worthington) in Puck's saline-A (6.0 ml.), with trypsin (0.5 mg./ml.) and EDTA (0.2 mg./ml.) (Gibco) in a shaker bath, 35.5°-36.5° C., 80 cycles per minute for 30 minutes and 95 cycles per minute for 10 minutes. Hanks' solution, 50 ml., 4° C., was added. Fragments were further dispersed manually by pipetting 40-55 times with a Pasteur pipette, washed, and centrifuged twice (600 rpm × two minutes). In 12 experiments (group I), the pellet was suspended in 2.0 ml. of Hanks' solution and implanted intraperitoneally via a small midline abdominal incision immediately after dispersion. In six experiments (group II), the preparation was cultured in vitro for 24 hours prior to implantation. After dispersion, washing and centrifugation, the pellet was resuspended in 60 ml. of Medium 199 (Gibco), supplemented with 10 per cent fetal calf serum, 400 U./ml. of penicillin-G, and 200 mg./100 ml. of D-glucose, and placed in two plastic Petri dishes in a servo-regulated air-CO_2 (95-5 per cent) incubator, 37° C., humidification 98 per cent. After 24 hours, cultures were harvested, centrifuged, and washed as described, resuspended in 2.0 ml. Hanks' solution, and implanted intraperitoneally. Implant preparations were 80-90 per cent viable (estimated by trypan blue exclusion) and weighed 500-700 mg., of which approximately 5 per cent was islet tissue.[4] This represented about two and one-half times the total estimated islet mass of a normal adult rat.[4]

Fasting serum immunoreactive insulin (IRI) levels were measured with rat insulin standards.[7] Fasting plasma immunoreactive glucagon (IRG) levels were measured with a highly specific pancreatic antibody (Unger, 30K).[8] Serum for IRI determination was obtained from tail-vein blood samples, collected under sodium pentobarbital anesthesia (60 mg./kg., i.p.). Plasma for IRG determination was obtained from femoral-vein blood samples under ether or chloral hydrate (300 mg./kg., i.p.) anesthesia and collected in tubes containing EDTA and Trasylol.[8] Rectal temperatures were monitored during sampling, and normothermia was maintained by a heat lamp. Blood glucose was measured on tail-vein samples by the glucose oxidase technique.[9] All samples were collected in the early afternoon following a four-hour fast with free access to water. Caloric intake was calculated from the weight of food consumed by each rat in a metabolic cage over 24-hour intervals.[10]

For the present study, body weight, FBG, caloric intake, and IRI and IRG levels were measured in all surviving islet recipients (N=16) eight to 10 months after transplantation and in all surviving age-matched normal (N=9) and diabetic (N=8) controls. The experimental animals in this study are the same as those studied metabolically in previous reports.[1-3]

RESULTS

Twenty diabetic control rats had a 70 per cent mortality over 10 months and showed almost no weight gain. Throughout the period of observation (10 months, or until death), all diabetic controls showed marked hyperglycemia, glycosuria, polyuria, and polyphagia when compared with 10 age-matched normal controls[1] (table 1). One normal control rat died after induction of pentobarbital anesthesia, at nine months. Follow-up of the other nine normal control rats was uneventful during the period of observation (10 months).

All diabetic recipients of islets became normoglycemic within 10 days. One isograft recipient in group I died of pneumonia 46 days after transplantation. A second recipient in group I died at 10 months

TABLE 1

Metabolic data: islet-isografted rats and age-matched, concurrently followed normal and diabetic controls eight to ten months after transplantation or sham operation

Parameter	Normal controls (9)*	Diabetic controls (8)	Group I: immediate islet-isograft recipients (10)	Group II: 24-hour-cultured islet-isograft recipients (6)
Body weight (gm.)	400 ± 30† (9)‡	250 ± 16 (8)	410 ± 17 (10)	416 ± 63 (6)
Calories/24 hours	81 ± 9 (9)	143 ± 26 (17)	93 ± 8 (11)	99 ± 8 (6)
FBG (mg./100 ml.)	101 ± 14 (39)	444 ± 60 (38)	91 ± 13 (60)	88 ± 13 (33)
IRI (microunits/ml.)	23 ± 26 (20)	14 ± 11 (24)	146 ± 85 (22)	39 ± 25 (12)
IRG (pg./ml.)	61 ± 29 (18)	96 ± 38 (9)	93 ± 46 (14)	117 ± 49 (8)

*Number of rats.
†Mean ± standard deviation.
‡Number of determinations.

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of acute hemorrhagic pancreatitis following pancreatic biopsy. Both were normoglycemic until death. All other islet-isograft recipients (groups I and II) remained normoglycemic, with normal weight gains, urine volumes, and intravenous glucose tolerance over the first six months following transplantation. These data have been reported previously.[1] Polyphagia in islet recipients was appreciated in this early study;[1] however, its importance was not clarified until the collection of data contained in the present report.

Eight to 10 months after islet transplantation, body weights of long-term islet recipients were not significantly different (p=N.S.) from those of normal controls (table 1). However, islet recipients were found to be significantly polyphagic, hyperinsulinemic, and hyperglucagonemic when compared with normal controls (table 1). In addition, fasting blood glucose levels were reduced below those of normal controls.

Caloric intakes of islet recipients in groups I and II were significantly lower than those of diabetic controls ($p < 0.001$) but remained significantly higher than those of normal controls ($p < 0.01$) (table 1). Caloric intakes of rats in group I were not statistically different from those of islet recipients in group II.

FBG levels of long-term islet recipients were found to be unchanged from levels measured earlier in the posttransplantation period[1] (table 1). However, FBG levels in age-matched normal controls were observed to have risen over the course of the study[1] (table 1), with the result that FBG levels of long-term islet recipients were in fact significantly lower ($p < 0.001$) than age-matched normal control values (table 1) in the 8-10-month interval after transplantation.

Long-term islet recipients (groups I and II) were hyperinsulinemic in the fasting state when compared with age-matched normal controls (table 1). This was most marked in group I isograft recipients ($p < 0.001$) but was also significant in group II recipients ($p < 0.01$). IRI levels in long-term diabetic controls were somewhat lower than those of normal controls, but this was not statistically significant.

IRG levels were significantly higher in islet recipients than in normal control rats ($p < 0.02$) and were similar to those of diabetic controls (p=N.S.). Islet recipients in group II had the highest IRG levels, but differences in IRG levels between islet recipients in groups I and II and diabetic controls were not statistically significant (p=N.S.).

DISCUSSION

Several factors may be related to the occurrence of hyperinsulinemia in islet-isografted rats. These include (1) the mass of islets transplanted, (2) the age of donor tissue, (3) the heterotopic location of islet grafts, and (4) the lack of normal innervation of transplanted islet cells. First, it has been suggested by Leonard[4] and Steffes[6] that the functional capacity of transplanted islets is dose-related and that slightly supranormal glucose tolerance and IRI levels may be produced by transplants of greater-than-normal islet-cell mass. This may account, in part, for hyperinsulinemia in our experimental animals, since the mass of islet tissue transplanted was equivalent to two and one-half times the normal islet mass of an adult rat. While it is probable that not all transplanted islets survived, it is possible that islet recipients received an excess of functioning beta cells. The less marked hyperinsulinemia seen in recipients of cultured islets (group II) may reflect loss of some islet cells during culture and manipulation. Although the notion is highly speculative, it also is conceivable that short periods of storage of islets in tissue culture may change their functional characteristics subsequent to transplantation.

Second, late fetal and early neonatal islets have relatively high mitotic index and insulin content,[5,11,15] and it is possible that in the present study significant mitotic activity occurred in transplanted neonatal islets, yielding an even greater excess of beta cells than predicted. In addition, although in-vitro studies have shown that neonatal islets respond normally to most stimuli,[15,16] this may not be true after transplantation of neonatal islets in vivo.

Third, hyperinsulinemia also may be related to the partial systemic rather than wholly portal venous drainage of peritoneal-surface-islet grafts, since the majority of islet grafts were found adherent to parietal peritoneum. The route by which insulin enters the circulation profoundly alters both its site and mode of action. It has been observed that up to 50 per cent of insulin in the portal vein is removed in a single passage through the liver.[17,18] Recent studies of Ziegler,[19] Charles,[20] and Pipeleers[21] have shown normal IRI levels in diabetic rats receiving adult islets transplanted into the portal vein. In the present studies, partial bypass of the portal venous system may have resulted in decreased hepatic removal of insulin from the circulation and, therefore, higher insulin levels in the periphery. Bypass of the liver also could account for the failure to observe severe degrees of hypoglycemia in association with hyperinsulinemia in transplanted animals. Recent studies have shown

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that the liver is quantitatively the most important site of glucose disposal[22] as well as the most sensitive site of insulin action.[23] Loss of the normal portal-peripheral gradient thus could account for the relatively mild although significant reduction in blood glucose noted in the face of hyperinsulinemia.

The fourth factor that may contribute to the development of basal hyperinsulinemia in recipients of intraperitoneal islets is that isolated islet grafts probably are denervated tissues. Autonomic nerve endings are known to be plentiful in islets of Langerhans in the intact pancreas,[24] and endogenous autonomic modulation of basal insulin secretion has been demonstrated in man and in animals.[25,26] In addition, an insulin-sensitive glucoregulatory center has been identified in the central nervous system.[27] Therefore, it is possible that lack of reinnervation of transplanted islets may be related to the development of basal hyperinsulinemia.

It should be noted that hypoglycemia and hyperinsulinemia also have been observed following whole-organ canine[28-33] and rat[34,35] pancreatic transplantation. It has been proposed that anastomosis to systemic arterial and venous supply and graft denervation may be factors that contribute to the mild-to-moderate degrees of hyperinsulinemia observed in these studies. Occurrence of hyperinsulinemia following transplantation of one intact pancreas suggests that islet mass, per se, is not the only factor in post-transplantation hyperinsulinemia.

In addition to basal hyperinsulinemia, plasma glucagon concentration was elevated in transplanted animals to levels comparable to diabetic controls' despite normalization of blood glucose. The effect of insulin treatment on the hyperglucagonemia of diabetes has shown some degree of species variation. Restoration of normal glucagon levels has been observed in the depancreatized, insulin-treated dog.[36] On the other hand, persistent hyperglucagonemia has been observed in insulin-treated human diabetics.[37] Regarding other factors contributing to hyperglucagonemia, it should be noted that the portal-peripheral gradient for glucagon (1.3/1), is substantially smaller than the gradient (3/1) observed for insulin.[38] It is thus unlikely that systemic venous drainage of transplanted islets can account for hyperglucagonemia. The possible role of altered islet-cell mass, islet-cell denervation, or other factors in the mechanism of hyperglucagonemia remains to be established.

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