Normal Responses of Rats with Transplanted Neonatal Islets to Stress, Epinephrine, and Cortisol

ROSY WOO AND F. XAVIER PI-SUNYER

SUMMARY

Sympathetic responses were evaluated in rats transplanted with neonatal pancreatic tissue. Transplantation was by one of two methods: mild collagenase digestion with subsequent intraportal injection, or direct placement of intact pancreatic tissue under the renal capsule. Compared with control intact animals, both groups of transplanted rats reverted to normal fasting and postglucose load plasma glucose and insulin. The hyperglycemia characteristic of stress or of injected exogenous epinephrine was present and similar in all three groups. The response to an intragastric glucose load or to tolbutamide injection during stress was similar in transplanted and in control animals. All three groups of rats responded to sustained cortisol injection with a compensatory hyperinsulinemia but maintained normal plasma glucose, water intake, and urine volume. No evidence could be detected in this study of an exaggerated "denervated" response of the transplanted islets. The normal sympathetic responses of these neonatal islet preparations in which little or no collagenase was used, in comparison with the abnormal responses previously reported for collagenase-treated individually isolated adult rat islets, suggest differences in recuperative powers of the sympathetic innervation in islets, possibly related to age and to method of preparation of transplanted tissue. DIABETES *31*:856–861, October 1982.

Pancreatic islet cells of rats respond to a variety of functional states during normal existence so that normoglycemia is maintained. In contrast, islets that have been transplanted into insulin-deficient rats may not be able to adaptively secrete hormones adequately. Certain studies have reported that islets of transplanted rats show an impaired response to stress,[1] cortisol treatment,[1] and various secretagogues, such as glucose[1,2] and tolbutamide.[1] It has been suggested that these abnormalities could be due partly to a chronically denervated state of the islets, causing a hypersensitivity to sympathetic neurotransmitters, such as has been reported for other denervated tissues.[3]

Reinnervation of parasympathetic and sympathetic fibers in transplanted islets may not occur or may take some time. Results from tests for cephalic phase insulin release in islet-transplanted rats indicate that vagal innervation is absent.[2,4–7] Information available on sympathetic fibers in transplanted pancreatic tissue[1] is limited but also suggests that reinnervation does not occur.

The importance of a chronic denervation in this tissue in overall glucose disposal is unclear. Tests for parasympathetically mediated function suggest that normal responses can occur. Even though oral glucose loads in islet-transplanted rats do not elicit an early insulin release, only some of the investigators[2,6,7] have reported a resultant abnormal oral glucose tolerance. Differences between those who find no significant impairment[4,5] and those who do suggest that other factors such as the particular islet preparation[8–10] and/or the route of transplantation (intraportal or under the kidney capsule) may determine the extent of the abnormalities seen.

Information on the influence of such factors in sympathetically mediated responses is lacking. We considered it important to test whether the adrenergic responses of islet-transplanted rats were abnormal, as previously reported.[1] If so, we also wished to test whether the abnormalities should be ascribed solely to denervation secondary to transplantation or whether they might be due to factors such as the type of islet preparation and/or route of transplantation used, as found with the cholinergic studies.

METHODS

Inbred male Lewis (LE) rats, weighing 200–240 g, were made diabetic by injection via the penile vein of 65 mg/kg body wt streptozotocin (kindly donated by the Upjohn Company, Kalamazoo, Michigan). Animals were considered diabetic if the following were present: 4-h fasting plasma glucose concentration above 300 mg/dl, 24-h water intake

From the Department of Medicine, St. Luke's-Roosevelt Hospital Center, Columbia University, New York, New York.
Address reprint requests to Dr. F. X. Pi-Sunyer, Division of Endocrinology, St. Luke's-Roosevelt Hospital Center, 114th Street and Amsterdam Avenue, New York, New York 10025.
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greater than 40 ml, 24-h urine output greater than 40 ml, stable 3+ to 4+ urine glucose (Dextrostix, Ames, Elkhart, Indiana), and weight loss. After such documentation, animals were randomly allocated to one of two groups for transplantation 10–15 days after streptozotocin administration. The first group (PT) received pancreatic islet transplants intraportally.[8] Pancreata from 1-day-old LE neonates were mildly treated with collagenase (3.4 mg/ml Hank's solution) for 25 min at 37°C. After digestion, washed tissue was injected into the portal system of the recipient as previously described,[8] using pancreata from six donor neonates for each recipient diabetic rat. In the second group (RT), intact fetal pancreata were transplanted directly under the renal capsule,[9] using five 0–2-day-old neonatal pancreata per recipient. The amount of endocrine tissue given to the PT and RT groups was estimated to be the same. Excellent results have been obtained in our laboratory using this number of donor neonatal pancreata per diabetic recipient. Information as to the interval between transplantation and achievement of normoglycemia has been reported.[11]

After transplantation, criteria for reversion from the diabetic state to normal were as follows: fasting plasma glucose levels less than 120 mg/dl, negative urine glucose, 24-h urine output below 20 ml, 24-h water intake below 40 ml, and sustained weight gain.

Once the animals had reverted to normal by the above criteria, intragastric glucose tolerance tests were done on the RT and PT animals and on an age-matched group of normal control animals obtained at the same time as the transplanted ones and carried as controls throughout the experiment. After a 4-h fast, glucose (125 mg/100 g body wt) was given by gavage and blood taken 1 h later. Blood for these and subsequent tests to be described was obtained from the tail vein in tubes containing heparin, EDTA, and trasylol. Each animal was previously trained to the technique and always placed in a comfortable padded box for the collections.[12] No anesthesia was used. The plasma was separated and frozen at −20°C until analysis. Plasma glucose was assayed on a Beckman glucose analyzer[13] (Beckman Instruments, Inc., Fullerton, California). Plasma insulin was measured using a single-antibody charcoal separation technique[14] with rat insulin standards. Plasma glucagon was measured by a single-antibody charcoal separation technique[15] using antibody 30K of Unger (Diabetes Research Foundation, Dallas, Texas). This antibody is specific for pancreatic glucagon. Pork glucagon was used as the standard.

The two groups of experimental animals plus the controls were taken through the test procedures described below from 11- to 20-wk post-transplantation.

Cortisol and stress studies. A 16-day protocol was used. On day 1, 24-h urine output, 24-h water intake, and weight were measured. On day 2, after a 4-h fast, intragastric glucose tolerance testing was done, with blood being collected before and at 20, 40, 60, and 90 min. On day 4, after a 2-h fast, a stress test was done by tightly restraining the animals for 2 h.[1] Blood samples were collected at the end of the stressful period and then an intragastric glucose load was given with samples collected after 30 and 60 min. On days 6–10, each animal was given cortisol (5 mg/100 g body wt) subcutaneously once daily. Daily weight, water intake, and urine volume were measured. A 4-h fasting plasma glucose was determined on the last day of cortisol administration. Gastric glucose tolerance was repeated twice, once on day 11, 21 h after the last cortisol dose had been given, and again on day 15, that is, 5 days after the last injection.

After a 3–4-wk rest from this first protocol, the animals underwent a second series of studies. For this, a polyethylene cannula was permanently placed into the right atrium through the right jugular vein. The catheter was run subcutaneously to the head and anchored to the top of the skull with stainless steel screws and dental cement. The catheter was kept patent by daily change of a heparin-polyvinylpyrrolidone solution. This permanent catheter technique allows for frequent sampling or injection of material without disturbing the awake animal and has been described in detail by Steffens.[16]

Tolbutamide and epinephrine tests. After the animals had recovered from the catheter surgery (at least 1 wk), the effect of exogenous catecholamine was tested by injecting 3 μg of epinephrine through the indwelling catheter and withdrawing blood before and at 4, 10, 15, and 30 min after injection. After each blood sample was taken, an equivalent volume of saline was introduced through the sam, ; catheter into the animal to maintain a constant blood volume.

On a subsequent test day at least 2 wk after the first, tolbutamide was given through the indwelling catheter and blood was collected before and at 5 and 30 min after injection. At 5 min, only insulin levels were determined. This was done after the animal had been tightly restrained for 2 h, as described under the stress protocol above.

Stress, tolbutamide, and epinephrine tests, plus metabolic data on water intake, urine output, and weight were all analyzed for statistical differences among control, RT, and PT groups by analysis of variance (ANOVA). All glucose tolerance tests were similarly analyzed, using areas calculated by integrating the incremental concentrations of glucose and insulin above basal levels for each animal. Paired Student's t tests were also done where mentioned in the text or figures.

RESULTS

Initial data are presented in Table 1. Weights of the three groups of animals at the time of induction of diabetes were not significantly different. After streptozotocin injection, both animals assigned to later renal transplantation (RT) and portal transplantation (PT) had abnormal fasting and post-intragastric glucose hyperglycemia. By week 2 after transplantation, fasting plasma glucose had reverted to normal. Weight was comparable to controls at both 5–6-wk and 9–10-wk post-transplantation, and fasting plasma glucose was not significantly different to controls at these times. One-hour post-intragastric glucose load plasma glucose values were comparably higher in the two transplanted groups of animals than in controls at both the 5–6-wk and 9–10-wk post-transplantation interval, though they were still within the normal range. Water intake for 24 h was 105 ± 21 SEM ml in RT and 109 ± 11 SEM ml in PT before transplantation and reverted to a normal 27 ± 3 ml in RT and 22 ± 2 ml in PT post-transplantation. A return of polyuria to normal post-transplantation also was documented.

One week later, the test procedures began. Results of the intragastric glucose tolerance test of the three groups of animals are shown in Figure 1. There were no significant differ-

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TABLE 1
Weight and plasma glucose values before and after islet transplantation

Subjects	Weight when given streptozotocin (g)	Pretransplantation 4-h fasting glucose (mg/dl)	Pretransplantation 1-h post glucose load (mg/dl)	5–6-wk post-transplantation Weight (g)	5–6-wk post-transplantation 4-h fasting glucose (mg/dl)	5–6-wk post-transplantation 1-h post glucose load (mg/dl)	9–10-wk post-transplantation Weight (g)	9–10-wk post-transplantation 4-h fasting glucose (mg/dl)	9–10-wk post-transplantation 1-h post glucose load (mg/dl)
C	202 ± 7.8*	133 ± 2.1	130 ± 3.3	335 ± 11.9	121 ± 5.6	110 ± 8.3	368 ± 11.8	113 ± 2.0	111 ± 3.2
RT	231 ± 6.7	443 ± 27.5	508 ± 22.3	360 ± 15.2	112 ± 7.8	137 ± 5.9	389 ± 13.3	115 ± 8.0	141 ± 5.2
PT	221 ± 17.8	429 ± 29.2	541 ± 22.9	336 ± 16.4	129 ± 7.8	136 ± 5.6	377 ± 13.6	113 ± 4.1	141 ± 8.8

* Mean ± SEM; C = controls, RT = renal transplants, PT = portal transplants.

ences among the three groups (RT, PT, and normal controls) in the mean integrated plasma glucose or insulin areas.

The response of the animals to stress is shown in Figure 2. The following is evident: (1) the three groups of animals started from a similar normal fasting glucose and insulin baseline; (2) all three groups manifested the hyperglycemia previously described with stress in rats,[1] but no statistical difference was evident in the response of the three groups; and (3) the response to an intragastric glucose load given immediately after the 2 h of stress was not statistically different among the groups for plasma glucose and insulin at 30 and 60 min. Thus, no evidence for an abnormally exaggerated response of plasma glucose to stress could be documented in the transplanted animals.

Figure 3 shows the fasting plasma glucose and insulin in the three groups of animals before cortisol treatment was begun and on the fifth day of cortisol treatment when blood was obtained 5 h after the cortisol injection. There was an elevated plasma glucose over pre-cortisol values, which was statistically significant for all three groups. With cortisol, a statistically significant hyperinsulinemia was documented in all three groups of animals. Measurements of body weight, 24-h water intake, and 24-h urine output during the 5-day cortisol treatment are shown in Table 2. All three groups of animals lost comparable amounts of weight during the 5 days that cortisol was being given. Urine outputs and water intake were not significantly different in RT, PT, and control animals.

A repeat intragastric glucose tolerance test done 21 h after the end of the cortisol treatment period is shown in Figure 4. No statistically significant differences in plasma glucose- or insulin-integrated areas could be documented among the three groups. Comparing the glucose-integrated areas of rat glucose tolerances obtained before cortisol (Figure 1) with those obtained 21 h after cortisol (Figure 4), no statistically significant differences were found. Plasma insulin-integrated areas, however, were statistically greater ($P < 0.001$) after cortisol (Figure 4) than before cortisol (Figure 1) in the control and RT groups, while they were not significantly different in the PT group.

Results of another intragastric glucose tolerance test taken 5 days after the last cortisol injections were normal in all three groups for both glucose and insulin and were similar to Figure 1.

The glucose and insulin responses of the three groups of animals to tolbutamide with stress are depicted in Figure 5. With stress, RT, PT, and control groups demonstrated an elevation in plasma glucose that was significantly higher than the fasting levels ($P < 0.001$). Insulin levels fell in all groups with stress ($P < 0.01$). The 5- and 30-min insulin responses to tolbutamide after stress showed a peak at 5 min and a return toward prestress levels by 30 min.

To duplicate the symptomatic effect of stress, rats were

FIGURE 1. Gastric glucose tolerance. Glucose (125 mg/100 g body wt) was given by gavage. No significant differences in the mean integrated plasma glucose or insulin areas among control, renal-transplanted, and portal-transplanted rats were found (mean ± SEM).

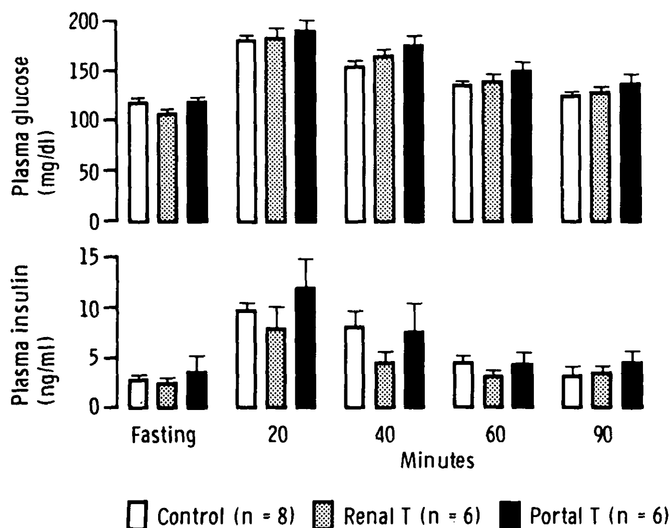
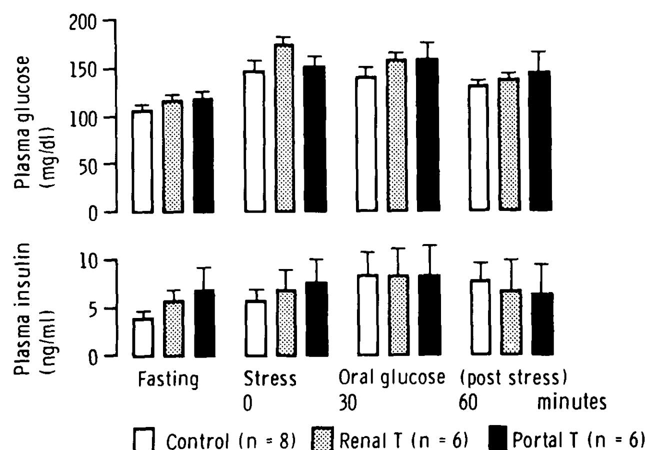


FIGURE 2. Response of animals to stress. After fasting plasma sample was taken, animals were restrained tightly for 2 h, after which sample 0 was taken; then a gastric glucose load (125 mg/100 g body wt) was given and blood was collected at 30 and 60 min. No significant differences among control, renal-transplanted, and portal-transplanted rats were found (mean ± SEM).



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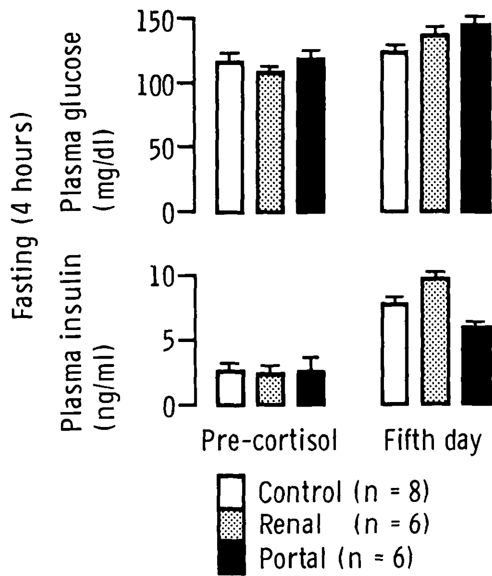


FIGURE 3. Fasting plasma glucose and insulin before and after 5 days of cortisol treatment (mean ± SEM). Blood was taken at 4-h fasting and 5 h after cortisol injection. Pre-cortisol values of glucose and insulin were significantly different for all groups. P < 0.05 or less (paired *t* test).

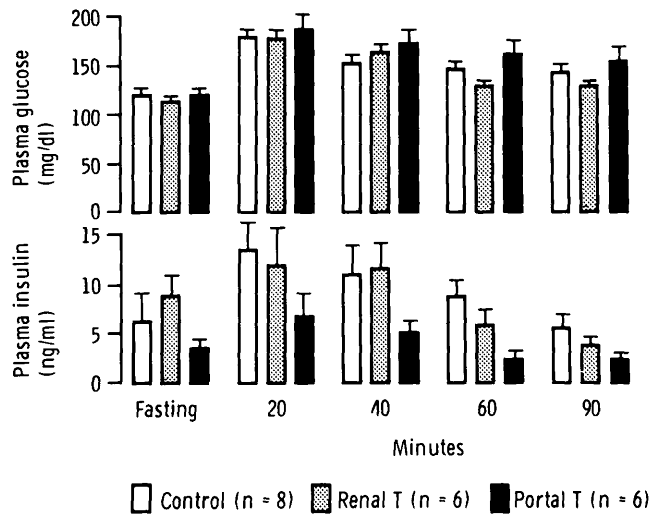


FIGURE 4. Gastric glucose tolerance. Glucose (125 mg/100 g body wt) was given by gavage after 5 days of cortisol treatment. Blood was taken 21 h after last cortisol injection (mean ± SEM). No significant differences among control, renal-transplanted, and portal-transplanted rats were found.

given 3 μg of epinephrine intravenously. Figure 6 shows the effect in the three groups of animals. There was an elevation of plasma glucose with epinephrine, gradually increasing to a peak at 10 min after the injection. The integrated glucose areas were not statistically different in the three groups of animals. There was no exaggerated glucose response in either RT or PT compared with controls. Insulin levels fell comparably at 10-min post epinephrine, with no difference among groups. Glucagon rose comparably in the three groups at the 4-min point and then fell back to baseline, again with no statistical difference between groups at any time point.

DISCUSSION

Transplantation of neonatal pancreatic tissue was accomplished in this study by two previously validated techniques. The first was to take pancreata from 1-day-old neonates, treat them with collagenase, and inject the washed but unseparated mixture of endocrine and exocrine tissue into the portal system.[8] The second was to take similar age pancreata and transplant them without any pretreatment under the kidney capsule. This was a modification of a previously described method[9] using fetal pancreata. In the present studies, intragastric glucose tolerance reverted to normal in both groups of transplanted rats. Attention was focused on tests (stress, cortisol, exogenous epinephrine, glucose- or tolbutamide-induced insulin release and on plasma glucose levels) that would require a functional adaptation by the islet cells.

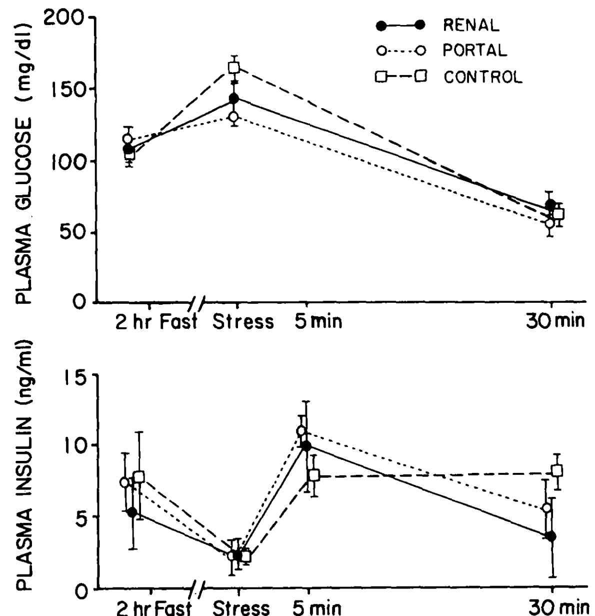
The transplanted rats, whether PT or RT, behaved like controls when under stress, showing hyperglycemia. How-

TABLE 2
Weight, water intake, and urine output during cortisol administration

	Control	Renal-transplanted	Portal-transplanted
Day 1			
Weight (g)	401 ± 13.2*	386 ± 7.2	385 ± 9.2
Day 3			
Weight (g)	400 ± 12.9	381 ± 6.9	377 ± 8.7
24-h water (ml)	34 ± 3.1	47 ± 10.3	44 ± 7.7
24-h urine (ml)	8.0 ± 1.4	7.6 ± 3.4	6.6 ± 2.8
Day 4			
Weight (g)	397 ± 11.3	378 ± 4.9	369 ± 9.6
24-h water (ml)	36 ± 2.5	35 ± 3.9	31 ± 2.5
24-h urine (ml)	8.2 ± 1.6	4.4 ± 0.9	8.4 ± 5.4
Day 5			
Weight (g)	392 ± 11.3	374 ± 4.4	364 ± 9.7
24-h water (ml)	26 ± 2.8	45 ± 10.2	35 ± 8.9
24-h urine (ml)	3.0 ± 1.1	4.3 ± 0.8	6.0 ± 2.6

* Mean ± SEM.

FIGURE 5. Plasma glucose and insulin (mean ± SEM) after tolbutamide (5 mg/100 g body wt). Blood was taken after a 2-h fast; the animal was then restrained for 2 h, subsequent to which tolbutamide was given and blood taken 5 and 30 min later.



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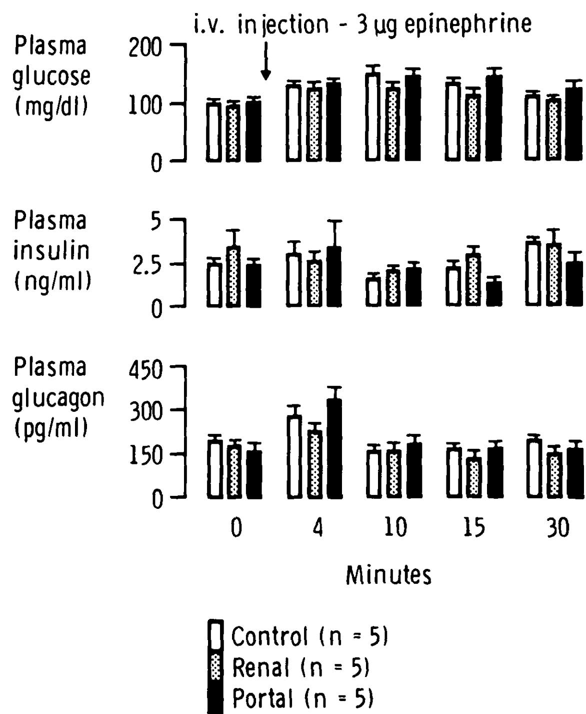

FIGURE 6. Plasma glucose, insulin, and glucagon after 3 μg epinephrine i.v. (mean ± SEM).

ever, no unusually exaggerated hyperglycemia associated with stress was noted in the transplanted animals, either fasting or after an intragastric glucose load, as has been reported by Pipeleers et al.[1] Also, no abnormally low insulin response to tolbutamide with stress, as has also been reported,[1] was documented in the present study.

The constant treatment with cortisol for 5 days induced a hyperinsulinemia in this study (Figure 3) as it has done in others.[1] However, the compensatory hyperinsulinemia was sufficient to maintain glucose homeostasis in the transplanted rats as well as in the controls. It seems evident that the transplanted islet tissue, whether placed under the renal capsule or into the portal system, contained enough reserve viable β-cell tissue to respond with adequate hyperinsulinemia to maintain glucose homeostasis. By 21 h after the last cortisol dose, the hyperinsulinemia was significantly higher in controls and renal transplants (Figure 4 versus Figure 1), but was not significantly different in portal transplants. The reversion to normality by 21 h after the last cortisol dose underscores the viability and resilience of the transplanted β-cells.

The response to exogenous epinephrine injection, given in an amount calculated to equal a normal physiologic response to acute stress,[1] was found to be similar in both RT and PT when compared with that in control rats. The expected hyperglycemia related to the glycogenolysis produced by epinephrine occurred, and this was associated with a modest fall of insulin. Glucagon levels rose in response to the β-adrenergic effects of epinephrine, as predicted from previous reports of others.[17,18] Again, however, no difference was found between transplanted and control animals, in contrast to the report of Pipeleers et al.,[1] where an exaggerated hyperglycemia and hyperglucagonemia were noted in the transplanted animals.

Speculation on the possible differences between results reported here and those of Pipeleers et al.[1] seems indicated. It was suggested by those investigators that the impaired responses obtained in rats with transplanted islets were secondary to denervation of islet tissue, causing a hypersensitivity of these tissues to catecholamines. Rats in the present study were tested earlier after transplantation [11–20-wk post-transplantation versus 12–40-wk post-transplantation (Pipeleers et al.)]; therefore, it would be expected that denervation should have been more evident in the present animals since less opportunity for reinnervation was given. In the present study, as with Pipeleers et al., sympathetically mediated responses were investigated.

Several investigators have attributed abnormal glucose tolerance responses in their islet-transplanted rats to parasympathetic denervation. Recent studies suggest that transplanted islets lack the parasympathetic-dependent cephalic phase of insulin release.[2,4–7] To circumvent the confounding effect of such possible parasympathetic denervation on our investigation of sympathetic innervation, intragastric rather than oral glucose loads were given, and no evidence for sympathetic abnormality was found. The absence of any significant difference between control and transplanted rats in this study suggests that sympathetic reinnervation may have occurred or that denervation, if present, did not result in functional impairment.

It is possible that the method of preparation of the islet tissue before transplantation may be responsible for the differences between this study and that of Pipeleers et al.,[1] which found impaired sympathetic responses. In that study, islets were isolated individually from adult rat pancreata after preliminary digestion with collagenase. In the present study, neonatal pancreatic tissue was either not treated at all with collagenase (renal capsule) or to a lesser degree [50–60 mg collagenase/5 ml Hank's solution for 20 min at 37°C for adult islets[10] versus 17 mg/5 ml for 25 min at 37°C in these neonatal preparations (intraportal)] before transplantation. Therefore, two major differences between the present study and the aforementioned one are: (1) the use of neonatal instead of adult tissue, and (2) the decreased collagenase digestion. It is now well established that adult rat islets individually isolated by the collagenase technique do not respond normally to a number of stimulants when incubated in vitro.[19,20] It is possible that the damage produced to the islets by the collagenase technique is severe enough to persist once the islets have been transplanted and is not reversed in the new heterotopic site, while the islets prepared by the techniques used in the present study are not so damaged and are able to respond normally to stimulants and to stress. It is also possible that less damage may also allow more rapid and complete nervous regeneration of the islets. Possibly, neonatal islet tissue has more recuperative and regenerative potential than adult islet tissue, so that once transplanted into the recipient animal it is able to develop its full potential even at a heterotopic site. Selawry et al.[21] have reported better results in rat pancreatic isotransplantation with islets from young as opposed to old rats.

Finally, it is of interest that in this study islet tissue, whether under the renal capsule, embedded in the liver parenchyma, or normally present in the pancreas, was able to respond with the adequate insulin secretion necessary to maintain normal glucose homeostasis. Although site differences have been found when marginal amounts of islet tis-

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sue have been implanted,[22] in the present study both groups of transplanted rats were given comparable and optimal amounts of tissue.[8,9] Under these circumstances, it seems that full responsivity of the islet tissue to prevailing glucose and other stimulant levels, rather than islet tissue site per se, is more important in ensuring appropriate carbohydrate homeostasis.

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