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Insulin and Amylase in the Postnatal Canine Pancreas (40866)[1]

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Abstract. Pancreatic fragments from 1- to 5-week-old donors have been implanted into the peritoneal cavity of diabetic dogs. Considerable improvement in glucose tolerance resulted but the dogs occasionally developed a peritonitis as a result of necrosis of acinar tissue. In order to determine if younger donors would provide more suitable tissue the insulin and amylase levels of the canine pancreas were measured during the first postnatal week. The amylase level, an estimator of acinar development, was 0.04 amylase units (AU)/mg protein at birth. It then rose for the first 4 postnatal days, reaching a maximum value of 0.84 AU/mg protein on the 4th postpartum day and then dropped by the 6th day to the original level. Adult amylase levels were not reached during the first postnatal week. Insulin levels were 10,500 micro units (μU)/mg protein at birth. They rose to a maximum of 17,700 μU/mg protein on the 6th postpartum day. The ratio of insulin to amylase was 31,000 μU/AU at birth. It dropped to a minimum of 27,800 μU/AU on the 4th postpartum day and then rose to a maximum value of 67,500 μU/AU on the 6th day. The insulin/amylase ratios imply that neonatal canine pancreas would be preferable to adult tissue for implantation into diabetic dogs. The optimum age of the donors should be 6–7 days since at this time the insulin to amylase content of the pancreas was at the maximum value.

In the postnatal rat pancreas the insulin producing islet tissue develops more rapidly than the acinar tissue (1). From 2.5 to 4.5 days postpartum the insulin to amylase ratio reaches the highest level of either the prenatal or postnatal period (1–4). This pattern of development makes the neonatal rat an ideal donor of pancreatic tissue for implantation into diabetic rats. The pancreata can be dissociated by collagenase digestion and implanted into either the abdominal cavity or the portal vein (5) without removal of acinar tissue.

We have previously used pancreatic fragments from 1- to 5-week-old canine donors for implantation into the abdominal cavity of alloxan diabetic dogs (6). These implants resulted in considerable improvement in the glucose tolerance of these diabetic dogs. In some cases, however, peritonitis resulted from the release of digestive enzymes from acinar cells of the implants.

In this study we have measured neonatal and adult insulin and amylase levels of the canine pancreas and compared insulin and amylase changes to those reported for the postnatal rat pancreas (2). The amylase level was used as an estimator of the digestive enzyme content of the pancreata.

The results of this study may be used to evaluate the potential of neonatal canine pancreata as donor tissue for implantation into diabetic dogs.

Materials and methods. Animals. Eight litters of mongrel pups were used. Litters contained from 2 to 10 pups. Pups were removed from the mother ½ hr before use. Six pups were used for each of the first 7 postnatal days. Three adult dogs of mixed breed and either sex were also used.

Preparation of pancreatic homogenates. The pups were killed with ether, and the adult dogs were killed with Diabutal (Sodium pentobarbital, Diamond Laboratories). The pancreata were removed and placed in cold 50 m*M* phosphate buffer, pH = 7.4. The tissue was homogenized in a glass to glass homogenizer using 10mg tissue/ml of cold phosphate buffer. Equal amounts of tissue from the head and tail portions of the pancreas were used. The homogenate was centrifuged at 0–5°, 3000 rpm, for 15 min. The supernate was used for subsequent assays.

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Insulin assay. Insulin content of the homogenates was determined using the Schwarz/Mann insulin assay kit. Three dilutions were made of each homogenate. For neonatal pancreata the dilutions ranged from 1:25 to 1:150; for adult pancreata the range was 1:100 to 1:300. The assay buffer was used as a diluent. In order to minimize destruction of the insulin by proteolytic enzymes present in the homogenate, samples were incubated on ice in the refrigerator for the minimum time recommended by the manufacturer, 4.5 hr.

Amylase assay. Amylase levels of the pancreatic homogenates were determined by the Caraway Method (7). Values were expressed as Caraway Amylase units/mg protein.

Protein determination. Protein content of the homogenates was determined by the Lowry method (8).

Data analysis. One-way analysis of variance over days was performed on insulin, amylase, and the insulin to amylase ratio. Multiple comparisons were made using the Duncan (9) multiple comparison test and the Student Neuman Keuls (SNK) test (10) both at an $\alpha = 0.05$ level. The SNK test is moderately conservative and the α level does not increase with repeated comparisons. The Duncan test is less conservative and therefore more likely to show differences than the SNK test but the α level increases with repeated comparisons.

A least significant difference (LSD) test ($\alpha = 0.25$) was performed on the amylase values to compare Days 1, 6, and 7. This test is the most liberal and if differences cannot be shown with this test at an α as high as 0.25, it may be assumed that the amylase values on these days are the same.

Results. The mean amylase content of the neonatal canine pancreata was 0.40 amylase units (AU)/mg protein on the first postpartum day. The amylase level rose to a maximum of 0.84 AU/mg of protein on the 4th postpartum day and then declined again to the original level (Fig. 1). Analysis of variance showed a significant difference in amylase over days at the $P = 0.05$ level. The Duncan test showed that the amylase levels on Days 1, 2, 6, and 7 were lower than Day 4 at $\alpha = 0.05$. No significant difference between postpartum Days 1, 6, and 7 was demonstrated at $\alpha = 0.25$, using the very liberal LSD test. The amylase content of adult canine pancreata was 276 ± 10 AU/mg protein.

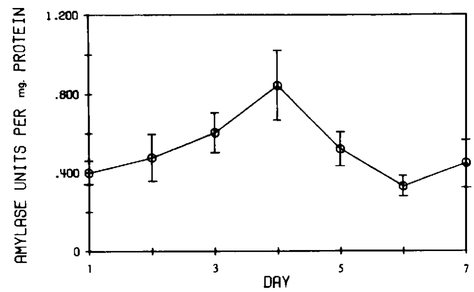

FIG. 1. Mean (±SEM) amylase content (Caraway Amylase units/mg protein) in the neonatal canine pancreas.

The mean insulin value of neonatal pancreata was 10,500 microunits (μU)/mg protein on the first postpartum day. The mean insulin content oscillated during the first week, but showed a slight tendency to increase. The highest average value of 17,700 μU insulin/mg protein occurred on the 6th day postpartum (Fig. 2). Analysis of variance of the insulin content of the neonatal canine pancreas for the first 7 postpartum days showed a significant effect due to days only at $P = 0.10$. The Duncan multiple comparison test showed that the insulin levels were lower on Day 1 than on Day 6 (α 0.05). The SNK test showed no significant differences. The mean insulin content of the adult pancreata was 17,500 ± 2,600 μU/mg protein.

The initial mean insulin to amylase ratio

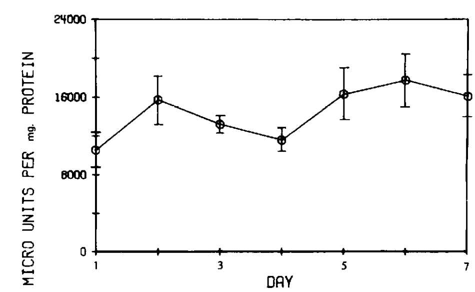

FIG. 2. Mean (±SEM) insulin content (μU insulin/mg protein) in the neonatal canine pancreas.

of neonatal pancreata was 31,000 μU insulin/AU. It then dropped slightly, reaching a minimum value of 27,800 μU insulin/AU on the 4th postpartum day, and subsequently rose to a maximum value of 67,500 μU insulin/AU on the 6th postpartum day (Fig. 3). Analysis of variance showed the effect of day to be significant at the $P = 0.03$ level. The Duncan test ($\alpha = 0.05$) showed the ratio on postpartum Days 6 and 7 to be higher than on Days 3 and 4, and the Day 6 ratio was also higher than Days 1 and 2. The SNK test showed no significant differences. In adult pancreata the insulin to amylase ratio was 61 ± 9 μU/AU.

Discussion. The pattern of insulin and amylase development in the neonatal canine pancreas was considerably different from that found in the rat pancreas (1, 2).

The amylase levels/mg protein in the canine pancreas on the first postpartum day were comparable to the levels found by Leonard *et al.* (2) in the rat pancreas at birth. In the canine pancreas the amylase levels/mg protein doubled by the 4th postnatal day whereas Leonard *et al.* (2) found that in the rat the amylase levels/mg protein declined to 0.06 times the birth level by the 3rd postpartum day. There was also considerable species difference in the relationship between birth and adult amylase levels. In the rat the levels found at birth and on the first postpartum day were equal to or greater than the adult levels (2). In the dog the adult levels were 300 to 700 times greater than levels found during the first postnatal week.

The pattern of insulin development also

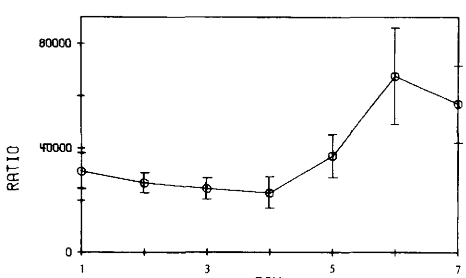

FIG. 3. Mean (±SEM) insulin/amylase ratio (μU insulin/Caraway Amylase units) in the neonatal canine pancreas.

differed considerably in the canine and rat pancreas. Insulin levels/mg protein in the canine pancreas at birth were about one-half the adult levels and increased to adult levels during the first postnatal week. Leonard *et al.* (2) found that insulin levels/mg of protein were slightly greater in the newborn than in the adult and subsequently increased 4.5 times. The maximum level was reached on the 3rd postpartum day and then decreased to adult levels by Day 20. Adult insulin/mg protein levels in the canine pancreas were comparable to adult levels found in the rat pancreas.

The increasing insulin and declining amylase levels in the postnatal rat pancreas resulted in a very high insulin/amylase ratio indicated that 2- to 4-day postpartum rat pancreata would provide an extremely good source of dissociated pancreatic tissue for transplantation into the peritoneal cavity of diabetic rats. This fortuitous pattern of endocrine and exocrine development was not found in the canine pancreas and the maximum insulin/amylase ratios in the canine pancreas were only 0.02 times as great as those found in the rat pancreas. Nevertheless, on the basis of a comparison of postnatal and adult insulin/amylase ratios in the canine pancreas it appears that neonatal pancreas would provide the more suitable tissue for transplantation. The 6th and 7th postpartum day appear to be the optimum time to obtain donor tissue. However, since pancreatic fragments from 7-day-old donors occasionally cause peritonitis when implanted into the peritoneal cavity (6) some removal of acinar tissue will be necessary if this implantation site is used. Islet purification should be kept to a minimum, however, since it has been shown in the rat (5) that each step in the purification process results in considerable islet loss. A very short period of collagenase digestion may eliminate sufficient acinar tissue to alleviate the problem of peritonitis when pancreatic tissue is implanted in the peritoneal cavity. The spleen may also provide a suitable implantation site for partially purified neonatal canine pancreatic tissue. Dispersed adult canine pancreatic tissue has been successfully im-

planted into the spleen of diabetic dogs (11, 12) without removal of acinar tissue.

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