Microanatomic features of pancreatic islets and immunolocalization of glucose transporters in tissues of llamas and alpacas

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Objective—To describe the microanatomic features of pancreatic islets and the immunohistochemical distribution of glucose transporter (GLUT) molecules in the pancreas and other tissues of New World camelids.

Animals—7 healthy adult New World camelids, 2 neonatal camelids with developmental skeletal abnormalities, and 2 BALB/c mice.

Procedure—Samples of pancreas, liver, skeletal muscle, mammary gland, brain, and adipose tissue were collected postmortem from camelids and mice. Pancreatic tissue sections from camelids were assessed microscopically. Sections of all tissues from camelids and mice (positive control specimens) were examined after staining with antibodies against GLUT-1, -2, -3, and -4 molecules.

Results—In camelids, pancreatic islets were prominent and lacked connective tissue capsules. Numerous individual endocrine-type cells were visible distant from the islets. Findings in neonatal and adult tissues were similar; however, the former appeared to have more non–islet-associated endocrine cells. Via immunostaining, GLUT-2 molecules were detected on pancreatic endocrine cells and hepatocytes in camelids. GLUT-1 molecules were detected on the capillary endothelium of the CNS, GLUT-3 molecules were detected throughout the gray matter, and GLUT-4 molecules were not detected in any camelid tissues. Staining characteristics of neonatal and adult tissues were similar.

Conclusions and Clinical Relevance—In New World camelids, microanatomic features of pancreatic islets are similar to those of other mammals. Data suggest that the poor glucose clearance and poor insulin response to hyperglycemia in adult camelids cannot be attributed to a lack of islet cells or lack of GLUT molecules on the outer membrane of those cells. (Am J Vet Res 2006;67:524–528)

In virtually all mammalian cells, most if not all glucose uptake from the blood is achieved by members of the GLUT family of molecules. The different members of this family have different characteristics with regard to affinity, preferred substrate (glucose or another sugar), directionality (preference for uptake, export, or a concentration-dependent process), and responsiveness to hormonal stimuli, which appear to be based on the host tissue’s need for glucose. Thus, the characteristics of glucose transport in a specific tissue can be estimated by determining which GLUT molecules populate the outer membranes of its cells.

Of the more common GLUT molecules, insulin-independent transporters can be widely distributed (eg, GLUT-1 molecules) or concentrated on tissues with important roles in glucose synthesis or regulation (eg, GLUT-2 molecules located on pancreatic beta cells, hepatocytes, and renal tubular cells) or high glucose requirements (eg, GLUT-3 molecules located on nervous tissue). Because these molecules represent constitutive uptake of glucose to provide basal requirements, their numbers and activity are fairly constant in these tissues. Their absence or inactivity would likely result in severe tissue dysfunction before or simultaneous to any noticeable effect on glucose clearance.

The insulin-dependent GLUT-4 molecule is most common on tissues with glucose requirements that vary considerably with activity (eg, skeletal muscle) or those in which the rate of glucose uptake is altered considerably in response to availability (eg, skeletal muscle and adipose tissue). Except in instances of overt obesity, skeletal muscle is a greater user of systematically available glucose, compared with adipose tissue. Thus, slow glucose clearance can often be linked directly or indirectly to slow glucose uptake by skeletal muscle. The rate of glucose clearance in camelids is slower than rates in many other domestic mammals. This may be attributable in part to a smaller insulin response to exogenous glucose and in part to peripheral insulin resistance in camelids. The smaller insulin response could potentially be a result of a lack of insulin-producing beta islet cells, a lack of GLUT molecules on these cells (a function ascribed to GLUT-2 in other species), a defect in intracellular signaling, or some other defect in intracellular insulin synthesis or release. Peripheral insulin resistance could be attributable to a lack of insulin receptors or a lack of insulin-sensitive GLUT-4 molecules, among other causes. Slow glucose clearance may be contributory to some of the hyperglycemic conditions detected in camelids, including hyperosmolar disorder, stress hyperglycemia, and persistent hyperglycemia (a diabetes mellitus–like condition).

Some of these aspects of glucose metabolism have been investigated in camels, which in evolutionary terms are the closest relatives to New World camelids.

GLUT Glucose transporter
The density and distribution of beta islet cells within the pancreas of camels appear to be similar to those of other animals, except that the pancreas of camels has additional isolated insulin-immunoreactive cells that are not associated with the islets. Also, camels appear to have insulin and an insulin receptor with morphologic and functional characteristics similar to those of other mammalian species. To our knowledge, the microscopic anatomic features of the pancreas have not been examined in New World camelids and the distribution of GLUT molecules has not been assessed in any species of camelid.

Tissue distribution of GLUT molecules can be examined via immunohistochemistry, western blot analysis, or identification of mRNA. Although camelid-specific anti-GLUT antibodies and probes are not available for these investigations, GLUT molecules in most species have relative homology and cross-reactions between species in immunologic and cDNA-based assays are common. Compared with other methods of GLUT detection, immunohistochemistry has the advantage that it only detects the transporter molecule itself, rather than untranslated mRNA (which may be present in tissues that do not express the transporter protein), and can be performed on fixed tissues. The purpose of the study reported here was to describe the microanatomic features of pancreatic islets and the immunohistochemical distribution of GLUT molecules in various tissues of New World camels. By doing so, it was our intention to attempt to clarify the mechanisms of slow glucose clearance in camels. We hypothesized that New World camelids would have pancreatic microanatomic features similar to those of camels and other species, with a lack of GLUT-2 molecules on islet cells (resulting in their poor insulin response to hyperglycemia) and a lack of GLUT-4 molecules on skeletal muscle cells (resulting in their peripheral insulin resistance).

Materials and Methods

Tissue samples—This study was approved by the Institutional Animal Care and Use Committee of Oregon State University. Seven healthy New World camels (4 llamas and 3 alpacas), 2 neonatal camelids (1 llama and 1 alpaca) with developmental skeletal abnormalities, and 2 BALB/c mice were used in the study. The neonatal camelids had been euthanatized because of poor prognosis for life, whereas the healthy camelids and mice had been euthanatized as control animals in unrelated studies. Samples of pancreas (1 sample from each pole of the organ in adult camelids and 1 randomly selected sample of pancreas from crias and mice), liver, skeletal muscle, mammary gland, brain, and adipose tissue were collected from all animals. All tissues were fixed in neutral-buffered 10% formalin for 48 hours and embedded in paraffin blocks.

For examination of the microanatomic features of pancreatic tissue in camelids, one 7-μm-thick tissue section from each sample was mounted on a glass slide and stained with H&E. Entire tissue sections were assessed microscopically for the presence of lesions prior to more detailed examination of the endocrine component of the tissue. In sections of each pole of the pancreas from adults, the total number of islets of Langerhans identified in 10 randomly selected 25× fields of view was determined; in the pancreatic section from each cria, 10 randomly selected 25× fields of view were similarly examined. In all instances, islets at the edge of the field of view were included in the count. The size (maximum width) of each islet included in the count was also categorized (as ≤200 μm or >200 μm). Each pole of the pancreas was then examined again, and the total number of endocrine cells per islet observed in each of 5 randomly selected islets was counted. Random selection consisted of blindly turning both control knobs of the microscope and choosing the islet closest to the center of the new field. Mouse tissues were not examined in this manner.

Immunohistochemistry was performed by use of the avidin-biotin-peroxidase method. Briefly, tissue sections were deparaffinized in xylene, rehydrated in 100% ethanol, washed in an endogenous enzyme blocker in 100% ethanol for 5 minutes, and then washed twice in 95% ethanol and once in automation buffer diluted 1:9 in distilled water. High-temperature antigen retrieval was performed on sections slides in a pressure cooker for 5 minutes, after which the slides were allowed to cool to room temperature (approx 20°C) over a period of 20 minutes. The slides were then washed 3 times in automation buffer followed by digestion with protease solution at room temperature for 5 minutes. The slides were washed in the diluted automation buffer 3 more times, loaded into a slide rack, rinsed, and blotted with buffer to ensure good capillary flow.

Tissue sections were blocked for 20 minutes in normal goat serum diluted with automation buffer and blotted dry. Primary antibodies (antibodies against GLUT-1, -2, -3, and -4 molecules) were applied, and sections were incubated at 4°C overnight (approx 16 hours). These antibodies were commercially available products and were developed in rabbits against the extracellular domains of rodent GLUT molecules. Antibodies were used at a dilution of 1:200, except for anti-GLUT-4 antibody, which was used at a dilution of 1:800 because of intense staining of the mouse tissues at the 1:200 dilution. Rabbit IgG at a dilution of 1:200 in 2% normal goat serum was used as an isotype control specimen, and BALB/c mouse tissues were used as positive control specimens. After incubation, the slides were washed and blotted 6 times and the biotinylated secondary antibody was applied for a 30-minute incubation at room temperature. Sections slides were washed in the diluted automation buffer solution and incubated with peroxidase complex for 30 minutes at room temperature. Nova Red was used as the chromagen, and Gill hematoxylin (diluted 1:10) was used as the counterstain. Sections were examined via light microscopy.

Data analysis—Islet counts and number of cells within islets were compared between the caudal and cranial pancreatic poles of adult camelids by use of the Mann-Whitney U test. Results were considered significant at a value of P < 0.05.

Results

Pancreatic tissue sections from only 1 adult llama had histologic evidence of disease. The pathologic changes consisted of mild multifocal peripancreatic fat necrosis in the section from each pole, with a few small aggregates of multinucleate giant cells and fibroblasts in the adipose tissue. Glandular and endocrine elements appeared normal. This llama was included in subsequent analyses because of the mild nature of the changes.

Islets were easily located in all sections of pancreatic tissue. Additionally, scattered individual cells with an endocrine morphology (round to polygonal cells with a lightly eosinophilic, nongranular cytoplasm vs the typical columnar to polygonal exocrine cells with a
granular cytoplasm) were visible between exocrine acini in all sections (Figure 1). Islets were generally located within the lobules of exocrine tissue, but 2 islets were identified in the interlobular connective tissue of 1 cria. Most islets were round, but oval and irregular shapes were also observed. There was no distinct connective tissue capsule around any islet, and many of the larger islets were traversed by large capillaries. Endocrine cell shapes were mainly polygonal, but columnar cells were often visible as a palisade along capillaries. Differential cytoplasmic staining of cells along the outer rim of many islets suggested a nonhomogeneous cell population.

The total number of pancreatic islets was determined in twenty 25X fields of view (cranial plus caudal pole sections) for each adult and in ten 25X fields of view for each neonatal camelid. The median number of islets/20 fields in adult camelids was 18 (range, 7 to 21 islets); islets were more numerous in the caudal lobe of the pancreas (median, 9 islets; range, 4 to 17 islets) than in the cranial lobe (median, 5 islets; range, 3 to 11 islets). The difference in islet counts between pancreatic poles in adults was not significant. Islet size in adult camelids ranged from 25 to 400 µm; the maximum width was < 200 µm in 89 of 94 islets examined.

The number of cells comprising individual islets in adults ranged from 5 to 198, but most contained < 50 cells. Median islet cell counts between pancreatic poles in adult camelids were not significantly different; there were 18 cells/islet in the cranial pole and 31 cells/islet in the caudal pole. In the 10 fields examined in the sections from each of the 2 neonatal camelids, the total number of islets encountered was 11 and 15, with all but 2 of these being < 200 µm wide. The largest was 250 µm wide. Considerable variation in the number of cells per islet was also detected in sections from the crias.

Anti–GLUT-1 antibody stained the vascular endothelium of the CNS intensely (Figure 2). Small amounts were visible on the capillary endothelium between adipocytes and myocytes, in the gray matter of the CNS, and on large vessels of the liver. Staining patterns were similar to those observed in mouse tissues.

In contrast, anti–GLUT-2 antibody stained the sinusoidal membranes of hepatocytes and pancreatic islets intensely (Figure 3). Isolated single pancreatic cells not associated with the islets also had strong affinity for this stain (Figure 1). Compared with findings in...
adult cameld tissues, pancreatic tissues from neonatal camels appeared to have fewer islets of high cell density and more single stained cells. Staining specific for GLUT-2 was not detected in any other tissues from adult and neonatal camels. The intensity of this staining was markedly weaker than the anti–GLUT-3 antibody staining detected in mouse CNS tissue; the staining intensity was also markedly weaker than the anti–GLUT-1 antibody staining of the same tissue from these animals. No non-CNS tissues from mice or camels had affinity for this stain.

In all cameld tissues examined, GLUT-4–specific staining was not detected. Among mouse tissues, anti–GLUT-4 antibody staining was faint throughout the skeletal muscle sections.

Discussion

Macroscopically, the anatomic features of the pancreas of New World camels are generally similar to those of other mammals; the pancreas is a flattened, lobulated organ that has cranial (head) and caudal (tail) lobes. The pancreatic duct joins the bile duct about 3 cm proximal to the intestinal orifice. Reports of pathologic changes in the pancreas of camels are rare. Severe peripancreatic fat necrosis has been described as a cause of vague clinical illness in llamas and alpacas. A similar but mild disease was detected microscopically in one of the llamas in the present study, although that llama had no clinical signs attributable to pancreatic malfunction.

In the camels of the present study, islets of Langerhans were easily identified in both cranial and caudal lobes of the pancreas and appeared randomly located, with no relationship to the ductal system. The distribution of islets between the 2 lobes in adult camels was not uniform, a feature common to the endocrine pancreatic tissue of many mammals. Overall, the caudal lobe of the pancreas was found to contain more islets, as is typical of many mammals. However, a more quantitative study performed previously revealed that the cranial lobe and body of the pancreas of the 1-humped camel (Camelus dromedarius) both contained slightly more total endocrine hormone activity than the caudal lobe. In our study, the pancreatic endocrine tissue of camels was located within the exocrine tissue, as reported in camels, and the islets were rarely seen in the interlobular connective tissue. This is in contrast to pancreatic tissue from cattle, which appears to have a population of large islets within the interlobular tissue. Another common feature observed in the cameld pancreas was the presence of scattered individual endocrine cells between exocrine acini, which has also been observed in Old World camels. These cells were identified in adults and neonatal camels in our study.

In the present study, the maximum width of islets in the pancreatic tissue of adult camels was extremely variable, ranging from 25 to 400 μm. Findings from 1-humped camels are similar. The distinctive dual population of islets described in cattle and fetal sheep could not be identified in the tissues examined in our study. Because large islets are reported to be more numerous in fetal pancreatic tissue and in the body of the pancreas, we cannot discount the possibility that such a dual population exists in New World camels.

In comparison with findings in adult camels, the pancreatic endocrine tissue of camel neonates was easily located but the islets were often somewhat less discrete, probably (at least in part) because of the underdevelopment of the exocrine tissue of this age group. The size range of islets in neonatal camels was similar to that detected in adults. Comparable camel data are not available.

Distributions of GLUT molecules were similar to those in other mammals, except for the low intensity of GLUT-3–specific staining of nervous tissue and the absence of GLUT-4–specific staining of skeletal muscle. Absence or difference in intensity of staining could have been a result of either lower numbers of transporter molecules in those camelid tissues or lower affinities of the primary antibodies for the transporter molecules of camels. Among the primary antibodies used, only anti–GLUT-1 antibody has cross-reactivity to ruminant GLUT-1 molecules and none have been evaluated in camels.

The presence of GLUT-2 immunoreactivity on pancreatic islet cells suggested that low insulin production in camels is not related to poor recognition of extracellular glucose concentrations by beta islet cells. Similar to clinically normal camels, mice lacking this transporter molecule have low insulin production and poor glucose tolerance, but unlike the former, these mice also have evidence of increased fat mobilization and ketone production. Immunoreactivity is poorly quantitative and does not address transporter function, so we cannot completely exclude the possibility of impaired glucose transport across beta cell membranes; further investigations of total beta cell mass and intracellular function of those cells in camels are warranted.

Although it could not be proved by findings of our study, it is an intriguing possibility that camels have substantially fewer insulin-responsive transporters in their skeletal muscle, compared with several other mammalian species. This possibility is also supported indirectly by other data. Selective disruption of skeletal muscle GLUT-4 in mice results in insulin resistance and slow glucose clearance, which are characteristics of healthy camels. Animals with skeletal muscle that contains a high proportion of type IIB fibers are prone to insulin resistance and obesity; and some camelid muscles contain > 80% type IIB fibers. Additionally, as many as half of all type IIB fibers in camels contain a myosin heavy chain type IIB isoform, which is not present in many mammals including humans, cats, horses, and cattle. Potentially, the different populations of myosin heavy chain isomers result in different rates of glucose uptake in these species. We postulate that inhibition of muscle glucose uptake provides camels with a survival advantage in the harsh natural environments where they thrive.
Poor peripheral glucose uptake spares glucose for vital survival functions, including lactation and gestation, the latter of which exceeds 11 months in camels.

References