An Immunohistochemical Study on the Pancreatic Endocrine Cells of the Three-toed Sloth, Bradypus variegatus*

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Summary. The cellular composition and relative frequency of the occurrence of pancreatic endocrine cells were studied immunohistochemically in a primitive eutherian and arboreal folivore, the three-toed sloth, since previous histochemical and ultrastructural studies on the endocrine pancreas of the sloth have detected only a single islet cell type, the A cell.

In the sloth pancreas, four types of endocrine cells immunoreactive for glucagon, insulin, somatostatin and serotonin (5-hydroxytryptamine) were found as reported in the pancreas of human and common experimental mammals, but pancreatic polypeptide-immunoreactive cells were not detected by either avian- or bovine-pancreatic polypeptide antiserum.

The endocrine cells were distributed mainly in the islets and partly also in the exocrine tissue including the pancreatic ducts. Larger or smaller clusters consisting of glucagon- and insulin-immunoreactive cells were also found frequently in the interlobular connective tissue. In the islets, glucagon- and insulin-immunoreactive cells were the most prominent cell type, while somatostatin- and serotonin-immunoreactive cells were sparse. The most striking feature in the sloth pancreas is the high frequency of glucagon-immunoreactive cells, because these cells are by far less in number than insulin-immunoreactive cells in the islets of human and common experimental mammals. This appears to be an intriguing characteristic of the sloth pancreas in a possible relation to the animal's unique metabolic system and the phylogenetical position.

The availability of specific antisera against the pancreatic regulatory peptides has made it possible to demonstrate immunohistochemically four different pancreatic endocrine cell types: glucagon (A), insulin (B), somatostatin (D) and pancreatic polypeptide (PP) cells. These four types of endocrine cells have been established as major pancreatic endocrine cells in higher vertebrates. Noteworthy, however, previous histochemical and ultrastructural studies on the pancreas of the three-toed sloth (Bradypus trydactylus) reported the presence of only a single islet cell type, the A cell (PINHEIRO et al., 1981; ABRAHAMSOHN et al., 1981). Although immunohistochemical studies have been carried out on pancreatic endocrine cells in a large number of mammalian species, no immunohistochemical studies have been available on this unique mammal, the sloth. Thus, the purpose of the present study is to determine, by immunohistochemical methods, the composition of the pancreatic endocrine cells in the three-toed sloth.

MATERIALS AND METHODS

Six adults individuals of the three-toed sloth, Bradypus variegatus, 3 males and 3 females (3.2-4.5 kg body weight) were used in this study. They were captured under license in the forest near Recife (Pernambuco, Brazil). The animals belonged to the same species as those used in previous studies by PINHEIRO et al. (1981) and ABRAHAMSOHN et al. (1981) and were identified as Bradypus tridactylus. Since it was confirmed that the South American three-toed sloth living in the forest near Recife was Bradypus variegatus and not Bradypus tridactylus, the former scientific name was used in this study. The animals were anesthetized with 115 mg/kg body weight of Chloralose (Merck, Lab., Rahway, New Jersey) and killed by exsanguination. Samples from the head, body and tail regions of the pancreas were dissected out and fixed overnight in Bouin's fluid, then processed for embedding in paraffin routinely.

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The paraffin-embedded tissues were sectioned serially at 4 μm in thickness and stained immunohistochemically using the avidin-biotin-peroxidase complex (ABC) method (Hsu et al., 1981) to identify specific endocrine cells. Specific antisera used in this study were anti-porcine glucagon (donated by N. Yanai-Hara, Shizuoka, GL-5, 1:10,000), anti-beef-pork insulin (Immuno Nuclear Corp., Stillwater, Lot. 8622014, 1:1,000), anti-human somatostatin (donated by S. Ito, Niigata, 1:10,000), anti-avian pancreatic polypeptide (donated by J. R. Kimmel, Kansas, Lance-10/5/81 Bleed, 1:80,000), anti-bovine pancreatic polypeptide (donated by R. E. Chance, Indianapolis, Lot. 615-R-110-146-17, 1:100,000), anti-serotonin (Immuno Nuclear Corp., Lot. 16302, 1:150,000), anti-porcine motilin (donated by N. Yanai-Hara, R-1104, 1:8,000), biotinylated anti-guinea pig IgG (Vector Lab. Inc., Burlingame, Lot. 40605, 1:200), biotinylated anti-rabbit IgG (Vector Lab. Inc., Lot. 70209, 1:200) and Vectastain ABC Kit (Elite) (Vector Lab., Inc., PK-6100, 1:2). The antigen-antibody reaction was visualized with a diaminobenzidine-H₂O₂ solution.

The specificity of each immunoreaction was determined as recommended by Sternberger (1979); this included replacement of the specific antiserum with the antiserum pre-incubated with the corresponding antigen. Following immunohistochemical staining, the sections were stained lightly with Mayer's hematoxylin, dehydrated, cleared and mounted. The relative frequency of each type of immunoreactive cell in each region of the pancreas was graded subjectively by light microscopic examination into five groups according to frequency of occurrence.

Fig. 1. Four serial sections of the head region immunostained with four different antisera for somatostatin (a), insulin (b), glucagon (c) and BPP (d), comparing the distribution and frequency of occurrence of each immunoreactive cells. BPP-immunoreactive cells are absent not only in this section but also in all of the sections investigated. In a lobule located at the left of this figure are solely insulin-immunoreactive cells. A small islet (arrow) consists of insulin-immunoreactive cells only. ×80
RESULTS

The endocrine portion of the pancreas of the three-toed sloth, *Bradypus variegatus*, comprised typical islets of Langerhans as well as endocrine cells scattered singly or in small groups in the exocrine tissue. Although the islets were present throughout the pancreas, they were more frequent in the tail and body regions than in the head.

Endocrine cells immunoreactive for glucagon, insulin, somatostatin and serotonin (5-hydroxytryptamine) were detected in the three-toed sloth pancreas. Glucagon- and insulin-immunoreactive cells were the most prominent cell types. On the whole, their relative frequencies of occurrence might be approximately equal or glucagon-immunoreactive cells tending to be slightly more numerous than insulin-immunoreactive cells. Somatostatin- and serotonin-immunoreactive cells were remarkably fewer than glucagon- and insulin-immunoreactive cells. Pancreatic polypeptide (PP)-immunoreactive cells were not detected.

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<tr>
<th>Endocrine cells</th>
<th>Endocrine portion</th>
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<tr>
<td>Glucagon</td>
<td>++</td>
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<td>Insulin</td>
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<tr>
<td>Somatostatin</td>
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<td>Serotonin</td>
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<td>APP and BPP</td>
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<td>Motilin</td>
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- not detected, ± rare, + (some) few, ++ moderate, +++ numerous. APP: avian pancreatic polypeptide, BPP: bovine pancreatic polypeptide.

Fig. 2. Four serial sections of the tail region immunostained with four different antisera for somatostatin (a), insulin (b), glucagon (c) and BPP (d), comparing the distribution and frequency of occurrence of each immunoreactive cells. BPP-immunoreactive cells are absent. An islet (arrow) consists of glucagon-immunoreactive cells only. × 80
detectable by either anti-avian pancreatic polypeptide (APP) serum or anti-bovine pancreatic polypeptide (BPP) serum (Figs. 1a, 2a). Neither could motilin immunoreactive cells be found in the sloth pancreas. The relative frequencies and distributions of the four type endocrine cells identified are summarized in Table 1. Each type of endocrine cell showed similar relative frequency in the three different regions of the pancreas, except for that, in the head region, the insulin cells were more numerous than the glucagon cells.

Glucagon-immunoreactive cells were more numer-

![Fig. 3. Two serial sections of the body region immunostained with two antisera for glucagon (a) and insulin (b). Immunoreactive cells are located in the interlobular connective tissue and in the peripheral part of the lobules. ×100](image)

![Fig. 4 a. Insulin immunoreactive cells in the connective tissue around the interlobular duct and peripheral part of the pancreatic lobule. ×90. b. Somatostatin-immunoreactive cells in the exocrine parenchyma. They are polygonal in shape, having long cytoplasmic processes. ×250. c and d. Somatostatin-immunoreactive cells in the epithelium of the interlobular pancreatic duct (e) and in the periductular connective tissue (d). ×500. e and f. A serotonin immunoreactive cell in the islet (e) and in the epithelium of the interlobular duct (f). ×500](image)
ous in the body and tail regions than in the head region. In some lobules of the head region, there were no glucagon-immunoreactive cells at all (Fig. 1c), whereas in the tail region some islets substantially consisted of glucagon-immunoreactive cells (Fig. 2c). Glucagon-immunoreactive cells were found both in the islets and exocrine tissue, though they were much more frequent in the islets. In the islets, they were located mainly at the periphery, forming a surrounding mantle, and along the capillaries, showing a cord-like arrangement (Figs. 1c, 2c). They were mainly columnar in shape in the islets; their cytoplasm was stained densely on its capillary side (Figs. 1c, 2c). In the exocrine portion, the glucagon immunoreactive cells were scattered singly or in small clusters inside and along the acini. They were round or oval in shape and their cytoplasm was stained evenly. They were also found frequently as larger or smaller clusters in the interlobular connective tissue and peripheral portion of the pancreatic lobules (Fig. 3). Single glucagon-immunoreactive cells were also observed in the duct epithelium.

Insulin-immunoreactive cells mostly appeared polygonal in shape (Figs. 1b, 2b). They were observed more frequently in the head region than in the body and tail regions, and were much more numerous in the islet than in the exocrine portion. In the islets, they were located mainly in the central portion. Islets without insulin-immunoreactive cells were found frequently in the tail region (Fig. 2). In the exocrine tissue, they were scattered singly or in small clusters inside and along the acini. They were also found frequently in the interlobular connective tissue and peripheral portion of the pancreatic lobules as larger or smaller clusters together with glucagon-immunoreactive cells (Fig. 3). Occasionally, small clusters of insulin-immunoreactive cells were found in the connective tissue surrounding the interlobular pancreatic ducts (Fig. 4b).

Somatostatin-immunoreactive cells were extremely fewer in number than the glucagon- and insulin-immunoreactive cells (Figs. 1, 2). There were relatively many islets lacking in somatostatin-immunoreactive cells. These cells were dispersed irregularly in the islets; they could also be detected singly or in small clusters in the exocrine portion, periductular connective tissue and pancreatic ducts (Fig. 4c, d). The single cells in the islets and exocrine tissue were polygonal in shape and equipped with one or two short or long cytoplasmic processes (Fig. 4b). Such processes appeared to have direct contact with other endocrine cells, acinar cells or capillaries.

A few serotonin-immunoreactive cells were scattered throughout the pancreas; they were very rare in the islets (Fig. 4e). These cells were also found occasionally in the epithelium of interlobular pancreatic ducts (Fig. 4f).

**DISCUSSION**

The present study is the first immunohistochemical observation to determine the composition of the pancreatic endocrine cells of the three-toed sloth, *Bradypus variegatus*. In previous studies, only a single cell type, the A cell, was identified in this species, either histologically, histochemically (PINHEIRO et al., 1981) or ultrastructurally (ABRAHAMSOHN et al., 1981). The present study, however, demonstrated four types of pancreatic endocrine cells—glucagon-, insulin-, somatostatin- and serotonin-immunoreactive cells—in the pancreas of the three-toed sloth. Among these, glucagon- and insulin-immunoreactive cells were the most prominent cell types, but their frequencies of occurrence were either equal or glucagon-immunoreactive cells seemingly slightly more numerous than insulin-immunoreactive cells. Somatostatin- and serotonin-immunoreactive cells were by far fewer than glucagon- and insulin-immunoreactive cells. In the mammalian pancreas, insulin-producing B cells generally comprise approximately 60% of the islet cells and 20% of the glucagon-producing A cells, (cf. HAZELWOOD, 1989; BONNER-WIEIR, 1991). EDWIN (1984) reported a high proportion of glucagon-producing cells and a low proportion of insulin-producing cells in the pancreases of grey kangaroos, a marsupial, and suggested that these findings might be explained by their energy source of volatile fatty acids. As folivorous sloths also utilize volatile fatty acids as their energy source, her suggestion may be worthy of note. However, since the omnivorous opossum (*Didelphis virginiana*) has been reported to have also a high frequency of glucagon-immunoreactive cells in its islets (KRAUSE et al., 1989), the possible relationship between the high frequency of glucagon cells and the use of volatile fatty acids as an energy source should be reconsidered. Some studies have demonstrated that approximately 10 times more glucagon is contained in the avian pancreas than in the mammalian pancreas (FALKMER and PATENT, 1972) and glucagon-producing A cells are dominant in the avian pancreas unlike in the mammalian pancreas (MIKAMI and ONO, 1962; HAZELWOOD, 1986). Furthermore, a similar result on the frequency of glucagon cells in the islets has been reported in some marsupials as mentioned above (EDWIN, 1984; KRAUSE et al., 1989) and in Nile crocodiles, order Crocodilia (RHOTEN, 1987). The sloth is a member of the order Edentata.
which diverged first from the main trunk of the eutherian's phylogenetical tree (Novacek, 1990). From a phylogenetical point of view, we speculated that the high frequency of glucagon cells in the pancreas might be one of the morphological characteristics of the endocrine pancreas in lower mammals. However, since there are no available reports on the endocrine pancreas of the order Monotremata, other species of sloth and other members of the order Edentata at present, it seems too early to give considerable weight to this speculation. Further studies are necessary to determine the physiological and/or phylogenetical significance of the high frequency of glucagon cells in the sloth pancreas.

In the sloth pancreas, glucagon- and insulin-immunoreactive cells were observed frequently as larger or smaller clusters in the interlobular connective tissue and peripheral part of the pancreatic lobules. A similar finding has been reported in the differentiating pancreas of the neonatal sheep (Titlback et al., 1985). It is not yet established whether these cells observed in the adult sloth pancreas share an endocrinial function with the islet endocrine cells or they are in a process of degeneration as suggested by Titlback et al. (1958) in the neonatal sheep. In the present findings with the sloth, there were no any pathological changes such as lymphocytes infiltration in the islets and fibrosis.

In mammalian pancreas, pancreatic polypeptide (PP)-immunoreactive cells are concentrated mainly in a part of the head region of the pancreas (PP-lobe) which is derived from the ventral primordium (Orči et al., 1976). PP-immunoreactive cells could not be detected in any regions of the sloth pancreas with the use of the antisera for avian and bovine pancreatic polypeptides. Therefore, the PP-lobe could not be identified in the sloth pancreas. However, it is known that the PP-lobe contains fewer numbers of glucagon-producing A cells than other lobes (Orči et al., 1976). Since the glucagon-immunoreactive cells were absent in some lobules of the head pancreatic region, these lobules might be derived from the ventral primordium. Pancreatic polypeptide is the fourth pancreatic hormone which is widely distributed in the pancreas from lower vertebrates such as fish to higher mammals (Van Norden and Falkmer, 1980; Falkmer et al., 1984). In the vampire bat (Desmodus rotundus) (Yamada et al., 1984), however, PP-immunoreactive cells were not detected in the pancreas by the same antisera used in this study. Since considerable species differences in the structure of the PP molecules have been reported (cf. Plisetskaya, 1989), it is unknown whether the negative immunohistochemical reaction is due to the actual absence of PP from the sloth pancreas or to a significant difference in its molecular form.

The occurrence and distribution of monoamine-containing cells or serotonin-immunoreactive cells in the pancreas have been reported in various mammals (Cegrell, 1968; Owm An et al., 1973; Ding et al., 1991). In the present study a few serotonin-immunoreactive cells were also scattered throughout the sloth pancreas. Motilin-immunoreactive cells have been demonstrated in the pancreas of caimans, Caiman latirostris (Yamada et al., 1986) and Caiman crocodilus (Yamada et al., 1991), and an egg-laying primitive mammal, the echidna (Tachyglossus aculeatus) (Yamada et al., 1990); they were not detected in the sloth pancreas.

In the previous studies on the sloth pancreas, the presence of pancreatic hormones in the acinar exocrine cells was suggested by the presence of aldehyde-fuchsin-positive granules (Pinheiro et al., 1981) and endocrine-like granules (Abrahamsöhn et al., 1981). However, the present results did not show any immunoreactivities for pancreatic hormones in the acinar cells of the sloth pancreas.

It is well known that the body temperature of the sloth is 30-34°C, changeable with external temperature, and that sloth eats only a small amount of leaves (27.2 g dry matter per day) (Montgomery and Sunquist, 1978). Although no definite conclusions can be drawn, some morphological characteristics of the sloth endocrine pancreas presented here may reflect metabolic characteristics of this arboreal folivorous mammal.

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REFERENCES


