The three-dimensional microanatomy of the pancreatic duct system in the Japanese monkey, *Macaca fuscata*, with special reference to fine proximal passages of a high species-specificity

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ABSTRACT

We have previously shown the duct system in the rat pancreas to consist of two parts: a fine proximal (intercalated) duct and thicker distal (intralobular and interlobular) duct, with the latter part displaying morphological signs indicative of a bicarbonate-rich fluid secretion. In this study the pancreatic duct system in the Japanese monkey *Macaca fuscata* was observed by scanning electron microscopy after the hydrolytic exposure of cell surfaces as well as by transmission electron microscopy of ultrathin sections. Cellular expression of the water channel aquaporin 1 (AQP1) was also examined immunohistochemically. In contrast to the segmented duct system in the rat, all the duct cells in the monkey pancreas consistently displayed rich mitochondria in the cytoplasm, elaborate interdigitations of cell processes, and an intense immunoreactivity for AQP1 on the apical and basolateral cell membrane to favor active ion transport and osmotic water movement across the epithelium. Both the existence of secretory canaliculi and basal trabeculae in the duct epithelium and randomized localization of primary cilia on the luminal cell surfaces were demonstrated for the first time in monkeys, and the physiological implications of these phenomena are discussed.

The pancreatic duct system is known as the site of a secretin-stimulated secretion of a bicarbonate-rich fluid which solubilizes acinar enzymes and neutralizes acidic chyme in the duodenum (3). Micropuncture studies with intact gland preparations and isolated duct segments directly demonstrated the secretory ability of the interlobular ducts (10, 20). However, the functions of smaller ducts in the pancreatic lobules remain unclear because of their inaccessibility by fine analytical techniques.

By fuchsin staining of the guinea-pig pancreas, Bensley (1) reported centroacinlar and intercalated duct cells in the lobules to contain considerable amounts of mitochondria that can energize active transport. His finding was subsequently corroborated in dogs and humans by transmission electron microscopy (TEM) (9, 13, 15). By scanning electron microscopy (SEM), Fujita et al. (5) demonstrated dense microvilli on basal surfaces of intercalated duct cells in the dog pancreas, indicating the transcellular permeation of large amounts of substances across the duct epithelium. However, our previous TEM and SEM observations have shown the rat pancreatic duct system to consist of two parts: a fine proximal (intercalated) duct which lacks morphological specialization for active transport, and a thicker distal (intralobular and interlobular) duct characterized both by the accumulation of mitochondria and elaboration of the cell membrane (21, 25). More systematical analyses are called for concerning the duct cell morphology in different animal species.

Employing physiological experiments and gene-expression analyses, Ko et al. (14) identified the
water channel aquaporin 1 (AQP1) as the main water pathway for the pancreatic fluid secretion. In the rat pancreas, interlobular ducts, but not intralobular ducts, were observed to immunostain for AQP1 (6), while in humans, both duct categories were reported to express this molecule (2). Little is known about other animal species concerning the expression of AQP1.

We examined pancreatic duct segments in the Japanese monkey, *Macaca fuscata*, both by combined SEM and TEM observation and by immunohistochemistry for the water channel AQP1. The data showed that both the proximal and the distal parts of the monkey pancreatic duct system were lined by an epithelium with clear morphological signs of fluid secretion: rich mitochondria in the cytoplasm, elaborate interdigitations of cell processes, and an intense immunoreactivity for AQP1 on the apical and basolateral cell membrane. The existence of secretory canaliculi and basal trabeculae in the duct epithelium and randomized localization of primary cilia on duct cell apices were demonstrated for the first time in this study, and the physiological implications of these phenomena are discussed.

MATERIALS AND METHODS

Five young adult male and female Japanese monkeys, *Macaca fuscata*, weighing 3–5 kg, were used in this study. The monkeys were allowed free access to food and tap water. Under deep anesthesia by an intravenous injection of sodium pentobarbital (50 mg/kg), the animals were sacrificed by bloodletting from the cervical artery. The pancreas was removed, cut into small pieces, and immersed in the following fixatives buffered at pH 7.3 with 0.1 M phosphate: tissue pieces for electron microscopic observation were fixed with a mixture of 2.5% glutaraldehyde and 0.5% paraformaldehyde for 4 h, and those for immunohistochemical examination with 4% paraformaldehyde overnight.

SEM observation. The fixed tissue pieces were macerated in 6N NaOH at 60°C for 20 min, and rinsed in a 0.02 M phosphate buffer (pH 7.3) (23). The macerated specimens were mechanically disrupted by repeating suction and ejection with a pipette, and mounted on slide glass coated with poly-L-lysine. The mounted tissue fragments were postfixed with 1% tannic acid buffered with 0.1 M phosphate (pH 7.3) for 1 h, followed by 1% OsO4 buffered with phosphate (0.1 M, pH 7.2) for 1 h. The osmicated specimens were dehydrated through a graded series of ethanol, transferred to isooamyl acetate, and critical-point-dried with liquid CO2. The dried specimens were coated with osmium in a plasma osmium coater (Nippon Laser and Electronics Laboratory, Nagoya, Japan), and examined in a Hitachi H-4500 scanning electron microscope (Hitachi, Tokyo, Japan) at an acceleration voltage of 10 kV.

TEM observation. After fixation with the aldehyde mixture, the pancreatic tissue was postfixed in 1% OsO4 buffered at pH 7.2 with 0.1 M cacodylate for 2 h at 4°C, dehydrated through a series of ethanol, and embedded in Epon-812. Ultrathin sections were prepared with a diamond knife (Diatom, Bienne, Switzerland) on an ultramicrotome (Raichert-Nissei, Tokyo, Japan). The tissue sections were examined in a Hitachi H-7100 transmission electron microscope (Hitachi) after double staining with uranyl acetate and lead citrate.

Immunohistochemistry for AQP1. The paraformaldehyde-fixed specimens were dehydrated through a graded series of ethanol and embedded in paraffin. The paraffin sections, 4 μm in thickness, were incubated with 10% normal goat serum for 30 min and subsequently with the rabbit polyclonal antibody against AQP1 (AB3272, 1 : 800 in dilution; Chemicon International, USA) overnight. The site of the antigen-antibody reaction was made visible by the avidin-biotin peroxidase method using a Histofine SAB-PO Kit (Nichirei, Tokyo, Japan). The counterstain was performed with hematoxylin.

RESULTS

General structure of the monkey pancreatic tissue

The monkey exocrine pancreas comprised spherical acini measuring about 30 μm in diameter and an arborized system of excretory ducts (Fig. 1). The approximately 12 μm-thick intercalated ducts displayed a narrow lumen surrounded by 4 or 5 duct cells on the apical and basolateral cell membrane. The existence of secretory canaliculi and basal trabeculae in the duct epithelium and randomized localization of primary cilia on duct cell apices were demonstrated for the first time in this study, and the physiological implications of these phenomena are discussed.
The fine structure of intercalated ducts

The intercalated duct cells were elongated in shape, about 8 μm wide and 20 μm long. The cells were oriented longitudinally to the duct axis, with one or two thick conical processes extended from the proximal and the distal ends. These processes advanced

Fig. 1 Scanning electron micrographs of monkey pancreatic tissue. a. An intercalated duct (D) connected with five acini (A). The duct epithelium has been fractured along cell boundaries in the boxed area to expose a narrow lumen. b. High magnification of the box in a. The duct lumen displays sparse short microvilli and a primary cilium (long arrow). Lateral surfaces of duct cells are bordered with numerous microvilli along the basal margin (short arrows). Arrowheads, luminal cell boundaries; C, intercellular canaliculi. c. Transitions from acini (A) to an intercalated duct (D). Duct cells extend lateral processes (arrowheads). Centroacinar cells expose narrow bases ruffled with microvilli (arrows). d. An acinus (A) fractured along cell boundaries. Its narrow secretory lumen (S) continues into an intercalated duct lumen (D). A centroacinar cell (asterisk) extends numerous microvilli to the acinar base (arrows). Bars 10 μm (a), 5 μm (b–d).
Fig. 2  Transmission electron micrographs of intercalated ducts. a. Duct cells (D) display fine interdigitations of microplicae (arrows). The arrowhead indicates a thicker cell process interlocked with a corresponding groove in neighboring cell bases. A, acinar cells; L, duct lumen. b, c. Two from a series of consecutive sections of an intercalated duct. Short intercellular canaliculi (arrows) open into the main lumen (L). A, acinar cell. Bars 5 μm (a), 2 μm (b, also applied to c).
into corresponding grooves on the neighboring cell bases to form primary cellular interdigitation (Fig. 1a–b). The duct cells additionally displayed fine secondary interdigitations of flap-like microvilli on the contact surfaces of the conical processes and the grooves as well as along the basal edges (Figs. 1b–d). Similar microvilli occasionally occurred on individual cell bases to form fine basal infoldings.

On the luminal aspect, the duct epithelium displayed sparse microvilli and numerous indentations of intercellular canaliculi which were sealed from lateral intercellular spaces by tight junctions (Figs. 1b, 2b–c). The canaliculi, no longer than 1.0 μm, were bordered with dense microvilli. Primary cilia originated from margins of the apical cell surfaces without any reference to the duct axis (Fig. 1b).

The centroacinar cells were wedge-like in shape with long tongue-like processes reaching the base of acini (Fig. 1d). The basal margins of the processes were ruffled with numerous microvilli and interdigitated via the microvilli with the intercalated duct cells. The apical surfaces of the centroacinar cells displayed sparse short microvilli.

The nucleus of the centroacinar and the intercalated duct cells was mostly centered in the main body, as demonstrated by TEM. The cells contained relatively rich mitochondria and numerous fine membrane tubules and vesicles in the entire cytoplasm (Fig. 2). The basal cell membrane was attached to a thick continuous basal lamina by numerous adhesion plaques about 100 nm wide. Flask-like membrane indentations of caveolae about 80 μm deep frequently occurred among the adhesion plaques on the basal cell membrane.

The fine structure of intralobular and interlobular ducts

These ducts consisted of cuboidal or columnar epithelial cells which displayed a polygonal base ranging in size from 8 to 15 μm (Figs. 3, 4a). The duct cells radiated long lateral processes from the basal edges (Fig. 3b). Some processes slid into corresponding grooves on neighboring cell bases to establish the primary cellular interdigitation. Other processes interlocked with those of neighboring cells to constitute trabecular protrusions on the basal aspect of the duct epithelium (Figs. 3a, 4a). These trabeculae measured 2–4 μm in thickness and 12–18 μm in length, with epithelial basal lamina continuously encircling them. The basal trabeculae frequently connected with one another via short side branches to exhibit a reticular appearance.

The intralobular and interlobular duct cells displayed the secondary interdigitation of microvilli on the basal half of the lateral surfaces as well as on the intercellular surfaces of the lateral processes (Figs. 3b, 4a). The central duct lumen, sparsely covered with short microvilli, continued into the intercellular canaliculi lined by dense microvilli. These canaliculi measured 1–2 μm in length and sometimes branched into short intracellular canaliculi covered with microvilli. Some apical primary cilia occurred near cell boundaries, while others were centered on the cell apex (Figs. 3b, 4c).

Cross sections of the intralobular and the interlobular ducts by TEM presented duct cell nuclei in the basal two thirds of the epithelium. Both the main body and the lateral processes of the duct cells contained considerable amounts of mitochondria and fine membrane tubules and vesicles (Fig. 4). Basal cell surfaces displayed numerous adhesion plaques and caveolae (Fig. 4b).

Immunohistochemistry for AQP1

Immunoreactivity for AQP1 was detected from the centroacinar cells and all the duct segments examined. The antibody labeled both the luminal and the basolateral cell membrane, with the former staining more intensely. The intercalated duct epithelium exclusively consisted of AQP1-positive cells (Fig. 5a), while the intralobular and the interlobular ducts occasionally displayed negatively-stained cells (Fig. 5b–d). Some AQP1-negative cells were identified as brush cells by a narrowed apex; others were cuboidal mucous cells (Fig. 5d).

The AQP1-positive cells displayed a clear, continuous labeling with the antibody along their free surfaces surrounding the main lumen and the intercellular canaliculi. The immunoreaction products tended to precipitate rather densely at the canaliculi (Fig. 5a–b). The basolateral surfaces of the cells were only faintly immunostained in an interrupted pattern (Fig. 5d).

DISCUSSION

Our observations have shown that, in the Japanese monkey pancreas, the secretin-induced secretion of bicarbonate-rich fluid can originate both from the intercalated portions—including the centroacinar cells—and from the intralobular and interlobular passages in the duct system. The occurrence of active ion transport accompanied by osmotic water movement across the duct epithelium is suggested by the accumulation of mitochondria, primary and secondary interdigitations of basolateral cell pro-
favorable for a rapid solubilization of their enzymes with ductal fluids secreted by the intercalated portions. The species-specific morphology of the intercalated ducts suggests species-dependent mechanisms for the primary solubilization of acinar enzymes.

There have been disagreements over whether the centroacinar cells correspond to flattened elements lying over the apices of acinar cells or wedge-shaped ones with a long process reaching the acinar

cesses, and an intense immunoreactivity for the water channel AQP1. Such morphological signs were previously reported to be absent from the intercalated ducts in the rat pancreas (6, 25) (Fig. 6). The rat pancreatic acini that are long tubular in shape (21, 22) may secrete some fluids by themselves to convey their proteinaceous outputs to the thicker distal ducts capable of fluid secretion (20). Conversely, the small round acini in the monkey pancreas appear favorably for a rapid solubilization of their enzymes with ductal fluids secreted by the intercalated portions. The species-specific morphology of the intercalated ducts suggests species-dependent mechanisms for the primary solubilization of acinar enzymes.

Fig. 3 Scanning electron micrographs of an interlobular duct viewed from a basal aspect (a) and another fractured along cell boundaries (b). Note numerous furrows and trabeculae (T) on the basal aspect of duct epithelium. Duct cells in b display dense microvilli on the basal half of their lateral surfaces (short arrows). Long lateral cell processes (arrowheads) are seen to continue into the basal trabeculae. The long arrow indicates a primary cilium projecting from a luminal surface (L) of a duct cell. Fixed materials of pancreatic secretion are clotted in the duct (asterisks) as well as in an intercellular canaliculus (C). Bars 10 μm (a), 5 μm (b).
SEM observations after the removal of extracellular matrices by NaOH maceration should effectively enable analyses of interspecies variability in the fine structure of the centroacinar cells. The intercellular canaliculi in the pancreatic duct segments were demonstrated in rats (25) and observed in monkeys for the first time in this study. These structures augment the apical cell membrane as well as elongate the tight junctions to facilitate the simultaneous transcellular transport of $HCO_3^-$ ions and paracellular movement of $Na^+$ ions across the duct epithelium (19, 20). The ductal secretory

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**Fig. 4** Cross section of an interlobular duct epithelium by transmission electron microscopy. **a.** Basal trabelulae (T) are connected with their parent duct cells in the boxed area and cut apart from the main cell bodies at left. Arrows indicate microplicae of duct cells. C, intercellular canaliculi; L, duct lumen. **b.** High magnification of the box in a. Arrows, focal adhesions on the basal cell membrane; arrowheads, caveolae. **c.** An apical primary cilium arising from a cell boundary (arrow). Note dense microvilli close to the base of the cilium. C, intercellular canaliculus. Bars 2 μm (a), 1 μm (b, c).
canaliculi closely resemble the liver bile canaliculi with regard to their constitution in the narrow grooves on lateral cell surfaces, dense coverage with microvilli, and frequent continuation into intracellular canaliculi (5, 24). Recent membrane fraction analyses have shown the bile canaliculi to comprise lipid-enriched microdomains that accumulate channels and transporters essential for bile secretion (11, 26). Similar membrane microdomains may operate to compartmentalize the molecular machinery for pancreatic fluid secretion to the canaliculi of the duct epithelium, as suggested by the immunostaining for AQP1 in this study. Molecular features of the ductal secretory canaliculi and the mechanism for their development and maintenance require elucidation.

Our observations confirmed the ample expansion of the contact surface of the duct epithelium with

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Fig. 5 Immunohistochemistry for aquaporin 1 followed by a counter staining with hematoxylin. Positive immunoreaction is detectable in an intercalated duct (a), including a centroacinar cell (asterisk), in an intralobular duct (b), and in an interlobular duct (c, d). The boxed area in c is presented at high magnification in d. Immunoreaction products are precipitated both on the luminal (arrowheads) and the basolateral (arrows) membranes of duct cells. The antibody labeling is sometimes enhanced at the intercellular canaliculi (C). Vascular endothelial cells (V) are also immunostained positively. A, acini; B, brush cell; M, mucous cells. Bars 10 μm (a, b, d), 50 μm (c).
the basal lamina by virtue of the trabeculae of cell processes. Similar trabecular protrusions appear to be sectioned apart from the pancreatic duct epithelium in previous TEM reports without reference in the text (7). It is natural to regard these basal trabeculae as anchorages for the duct epithelium against the rigorous luminal flow induced by secretin (16). In support of this possibility, the basal trabeculae as well as the main body of the duct cell displayed numerous adhesion plaques along the plasma membrane apposed to the basal lamina. The basal cell membrane was additionally characterized by numerous caveolae that can disassemble and reassemble to buffer membrane tension surges during mechanical stress (18). In this regard, it is worth noting that in the dog pancreas, vesicular membrane indentations or caveolae were reported to increase in number in a basal part of the intercalated duct epithelium after stimulation with secretin (9). The physiological dynamics of the basal trabeculae require further analyses in order to clarify their functional implications.

The apical primary cilia in the duct epithelium were seen to arise from various locations on cell boundaries, in contrast to the centered apical cilia in kidney tubules (12, 27). The apical primary cilia are generally regarded as luminal flow sensors involved in the control of cell proliferation and mitotic orientation for maintenance of the tissue architecture (17).

Fig. 6 Schematic representation of the pancreatic duct system in the Japanese monkey (upper) and that in the rat (lower). Monkey pancreatic acini are uniformly small and round, while those in the rat pancreas frequently display long tubular shapes. The former structures, rather than the latter, appear favorable for the rapid joining of acinar outputs and ductal fluids. Monkey centroacinar cells, intercalated duct cells, and intralobular and interlobular duct cells are consistently characterized by rich mitochondria (M) and membrane elaborations to indicate the active transport of ions across the epithelium. In the rat pancreas such morphological specializations are confined to the intralobular and the interlobular duct cells. C, secretory canaliculi of duct cells; P, interdigitating processes of neighboring cells.
The centered primary cilia on the kidney tubule cells were reported to govern longitudinal cell division with respect to the tubule axis, thereby supporting the developmental elongation of nephrons (4, 12). The randomized primary cilia of the pancreatic duct cells could presumably detect a turbulent luminal flow and/or control variously-oriented cell division for maintenance of the arborized passages connected with numerous acini.

Comparative observations of the three-dimensional microanatomy of the pancreatic duct system combined with immunohistochemical probing for functional molecules will advance our understanding of the pancreatic secretion of bicarbonate-rich fluid, aiding in the construction and interpretation of animal models of human pancreatic diseases.

REFERENCES