Induction and Origin of Hepatocytes in Rat Pancreas

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ABSTRACT 2-[4(2,2-Dichlorocyclopropyl)phenoxy]2-methyl propionic acid (ciprofibrate), a peroxisome proliferator, induced hepatocytes in the pancreas of adult male F-344 rats when added to their diet at a dosage of 10 mg/kg body weight for 60-72 wk. These cells are morphologically indistinguishable from hepatic hepatocytes and were usually localized adjacent to islets of Langerhans with extensions into surrounding acinar tissue. A significant increase in the volume density of peroxisomes, together with immunochemically detectable amounts of two peroxisome-associated enzymes, was observed in pancreas with hepatocytes of rats maintained on ciprofibrate. Uricase-containing crystalloid nucleoids, specific for rat hepatocyte peroxisomes, were present in pancreatic hepatocytes. These structures facilitated the identification of cells with hybrid cytoplasmic features characteristic of pancreatic acinar and endocrine cells and hepatocytes. Such cells are presumed to represent a transitional state in which pancreas specific genes are being repressed while liver specific ones are simultaneously expressed. The presence of exocrine and/or endocrine secretory granules in transitional cells indicates that acinar/intermediate cells represent the precursor cell from which pancreatic hepatocytes are derived.

MATERIALS AND METHODS

Animals and Treatment: Male Fischer 344 rats, weighing 80-100 g, were purchased from Charles River Breeding Laboratories, Wilmington, MA. They were housed individually in suspended steel wire cages in a temperature (22°C) and light controlled (12 h light and 12 h dark photo period) room. 50 rats were fed powdered chow containing a hypolipidemic drug, ciprofibrate (214-(2,2-Dichlorocyclopropyl)phenoxy]2-methyl propionic acid); the concentration of the drug in the diet was adjusted periodically to ensure an estimated daily dose level of 10 mg/kg body weight. At the end of 60 wk of ciprofibrate administration, 30 of these rats were sacrificed and their pancreas processed for light or electron microscopic examination as described below. The remaining 20 rats were divided into two groups: one group consisting of 10 rats was continued on a diet containing ciprofibrate and the other group was switched to normal chow, without ciprofibrate. These animals were killed 12 wk later (i.e., 72 wk after the initiation of ciprofibrate treatment). In addition, two
FIGURE 1  Pancreas of F344 male rat fed ciprofibrate for 60 wk. Hepatocytes with characteristic eosinophilic cytoplasm and polyhedral configuration are present in the vicinity of an islet of Langerhans and extending into acinar tissue. Hematoxylin-eosin. × 250.

Morphometry:

Morphometric analysis of peroxisomes and mitochondria in pancreatic hepatocytes was performed by the method outlined by Weibel (35) as described elsewhere (18, 23). Thirty electron micrographs from three rats fed ciprofibrate for 72 wk (10 from each animal) and equal numbers from three rats that were withdrawn from ciprofibrate treatment for 12 wk were photographed at × 8,000 and magnified 2.5 times at printing.

RESULTS

Light and Electron Microscopy: For light microscopy, pancreas was fixed in neutral buffered formalin and processed for paraffin embedding. Sections 3-5 μm thick were cut and stained with hematoxylin and eosin. For electron microscopy, pancreas was minced into small pieces and fixed in cold 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, for 4 h. The tissue was rinsed overnight in 0.1 M cacodylate buffer, pH 7.4, containing 0.02 M sucrose and then incubated at 37°C for 1 h in the alkaline DAB-reaction medium of Novikoff and Goldfischer (19) for the localization of the peroxidatic activity of catalase. Controls consisted of incubations in which 0.02 M 3-amino-1,2,4-triazole was added to the medium (16). After incubation the tissue was washed in cacodylate buffer and the pieces of pancreas containing brownish islands of tissue representing clusters of hepatocytes were identified under the dissecting microscope. They were postfixed in 2% OsO₄ buffered to pH 7.4 with 0.1 M s-collidine and processed for electron microscopy. This material was embedded in Epon and sectioned on an LKB ultratome (LKB Instruments, Inc., Gaithersburg, MD); 0.5 μm thick sections were examined in a Zeiss Ultraphot III microscope, and thin sections were examined in a JEOL JEM-100CS II electron microscope.

Western Blotting:

Postnuclear fractions (25) of liver and pancreas of control rats and rats fed ciprofibrate were subjected to electrophoresis through SDS PAGE (12% polyacrylamide) (10) and transferred to nitrocellulose paper (Schleicher & Schuell, Inc., Keene, NH). The nitrocellulose paper was incubated with anti-rat catalase or anti-peroxisome proliferation-associated M₈, 80,000 polypeptide serum and then with 125I-labeled protein A (1, 33).

Morphology

Histologic examination of hematoxylin-eosin stained section of pancreas from animals treated with ciprofibrate for 60 and 72 wk revealed multiple foci of hepatocytes (Fig. 1). The foci of hepatocytes were also seen in the pancreas of rats that were fed ciprofibrate for 60 wk and then withdrawn from this diet for 12 wk. These clusters of hepatocytes, consisting of characteristic polyhedral cells with abundant granular eosinophilic cytoplasm and centrally placed nucleus with a prominent nucleolus, were encountered usually in the vicinity of...
the islets of Langerhans (Fig. 1). Groups of entrapped endocrine and acinar cells were also discerned. The incidence of rats with pancreatic hepatocytes was <25% in these groups, while no such foci were present in the pancreas of animals in the control group or of the historical control F344 rats in our laboratory aged 72 wk.

Examination of semithin (0.5 μm thick) sections of pancreatic tissue containing hepatocytes obtained from rats that were fed ciprofibrate showed numerous peroxisomes displaying characteristic dark brown DAB reaction product. These organelles appear as black granules in the black and white photographs (Fig. 2). Peroxisomes in pancreatic hepatocytes of rats killed 12 wk after ciprofibrate withdrawal appeared as scattered granules and were less abundant in number (Fig. 2) when compared to animals maintained on this peroxisome proliferator (Fig. 2).

At the electron microscope level, the pancreatic hepatocytes from rats that were either on ciprofibrate or discontinued after 60 wk of treatment, revealed morphological features characteristic of the parenchymal cells of adult rat liver (Figs. 3 and 4). The pancreatic hepatocytes in animals that were fed ciprofibrate for 60 wk and subsequently maintained on normal chow without the drug for an additional 12-wk period before being killed displayed centrally located nucleus, aggregates of glycogen, stacks of rough endoplasmic reticulum, round to oval mitochondria, peroxisomes, and bile canaliculi (Fig. 3). In ciprofibrate-treated rats, a remarkable proliferation of peroxisomes was observed in these pancreatic hepatocytes (Fig. 4) which was similar to that found in hepatocytes of rat liver.

**Morphometric Analysis**

Morphometric analysis (Table I) revealed that ciprofibrate treatment caused a ninefold increase in the volume density of peroxisomes in pancreatic hepatocytes as compared to hepatocytes from pancreas of rats that did not receive ciprofibrate for a period of 12 wk before they were killed. Ciprofibrate treatment also resulted in marked alterations in the size, distribution, and numerical density of peroxisomes in rat pancreatic hepatocytes. The volume density of mitochondria in pancreatic hepatocytes of rats remained unaltered with ciprofibrate treatment.
FIGURE 4 Pancreatic hepatocytes from rats fed ciprofibrate continuously until they were killed show proliferation of peroxisomes (P). Note the presence of uricase containing crystalloid nucleoids (arrowheads) in many of these proliferated peroxisomes (B). Peroxisomes react positively for catalase when incubated in alkaline D-B medium (C). Portions of pancreatic acinar cells (AC) and endocrine (EC) are seen in A and C, respectively. (A) × 5,184; (B) × 12,100; (C) × 13,630.
Hepatocyte Specific Peroxisomes of Rat

Peroxisomes in pancreatic hepatocytes displayed typical crystalloid nucleoids (Figs. 4 and 5) characteristic of peroxisomes of hepatocytes of rat liver (3, 8, 9, 30, 34). All peroxisomes, irrespective of the presence or absence of a nucleoid, stained positively for the marker enzyme catalase by the alkaline DAB cytochemical procedure (Figs. 3, 4 C, and 5). This cytochemical feature can be used to distinguish peroxisomes from zymogen as well as endocrine secretory granules (Figs. 5 B and 6).

<table>
<thead>
<tr>
<th>Table I</th>
<th>Morphometric Analysis of the Relative Volume of Mitochondria and Peroxisomes in Pancreatic Hepatocytes in Rat</th>
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<tr>
<td>Volume density</td>
<td>Mitochondria</td>
</tr>
<tr>
<td>Ciprofibrate-treated</td>
<td>23.4 ± 4.6</td>
</tr>
<tr>
<td>Ciprofibrate-withdrawn</td>
<td>28.4 ± 5.7</td>
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Male F344 rats were fed ciprofibrate for 72 wk (ciprofibrate-treated group) or fed for 60 wk and then the drug withdrawn for 12 wk before being killed (ciprofibrate-withdrawn group). 30 electron micrographs of randomly selected areas of pancreatic hepatocyte cytoplasm from each group were analyzed as described elsewhere (22) according to the method of Weibel (35). Points overlying cytoplasm, mitochondria and peroxisomes were determined to obtain the volume density of mitochondria and peroxisomes in relation to cytoplasmic volume. The values are mean ± SE.

* (P* < 0.01).

Origin of Pancreatic Hepatocytes

Detailed examination was undertaken to determine the identity of a possible precursor cell responsible for hepatocyte conversion in rat pancreas. Cells showing ultrastructural characteristics of more than one pancreatic cell type have been described in normal rat pancreas and are known as intermediate cells (17). These cells contain zymogen granules, arrays of rough endoplasmic reticulum, and endocrine (usually β-granules) secretory granules. Since these intermediate cells and acinar cells in normal rat pancreas do not possess nucleoid containing peroxisomes (20) characteristic of liver parenchymal cells, the emergence of such peroxisomes in these cells has been viewed as an early sign of transformation into hepatocytes. Rat pancreatic hepatocytes were usually seen in close association with the islets of Langerhans and it was not unusual to find intercellular junctions between well-differentiated hepatocytes and endocrine cells. Several cells suggestive of transitional forms displaying features of both liver cell, especially the nucleoid-containing peroxisomes and acinar/endocrine cells were discerned. Peroxisomes in these cells showed uricase containing crystalloid cores (9, 15, 34) characteristic of peroxisomes of rat liver (3, 8, 30). The matrix of peroxisomes reacted positively when incubated in the alkaline DAB cytochemical medium, whereas the zymogen granules and endocrine granules present adjacent in the same cytoplasm were negative. Peroxisomes were distinguished easily from zymogen granules in these cells (Figs. 5 B and 6). Mit-
These cells contain both zymogen (Z)/endocrine (E) granules and peroxisomes (P) with uricase cores. Mitochondria in these cells are in division (arrowheads) as exemplified by the membranous partition separating the inner compartment into two units and mitochondrial doublets. Insets represent enlargement of boxed in areas. (A) × 8,625; (B) × 13,085; (C) × 19,600. (Inset to A) × 47,530; (Inset to C) × 40,640.
FIGURE 7 Mitochondrial division in a pancreatic hepatocyte. Partitions dividing the inner mitochondrial chamber into two distinct compartments are present (arrowheads). Peroxisomes (P) show DAB reaction product. × 20,600.

Chondria in these cells revealed features of division (Fig. 6) that are characterized by a membranous partition separating the inner compartment into two distinct units. These appear to separate into two organelles, which are seen as doublets (Figs. 6 and 7). Several pancreatic hepatocytes in rats maintained on ciprofibrate continuously for 60 and 72 wk contained excessive amounts of lipofuscin granules, similar to that described in hepatocytes of liver of rats on long term peroxisome proliferator treatment (27).

Detection of Catalase and Peroxisome Proliferation-associated M, 80,000 Polypeptide in Rat Pancreas with Hepatocytes

Catalase and peroxisome proliferation-associated M, 80,000 polypeptide (25) were detected by immunoblotting in pancreas containing hepatocytes induced by ciprofibrate. As expected, these proteins were also detected in the liver of normal and ciprofibrate-fed rats (Fig. 8). They were not detectable in pancreas of normal rats.

DISCUSSION

In the present study, we have described the induction of hepatocytes in pancreas of F344 rats by chronic feeding of ciprofibrate, a potent hepatic peroxisome proliferator (12). To our knowledge, this is the first detailed report of induction, characterization, and origin of pancreatic hepatocytes in rats. The incidence of rats developing hepatocytes in pancreas in this study was <25%, whereas in an earlier study similar cells were induced in a majority of hamsters following injection of a mutagenic chemical carcinogen during the height of DNA synthesis and cell proliferation in this organ (29). It is generally believed that sensitivity of cells to the effects of a carcinogen is greatly enhanced when they are replicating (2). Since heritable alterations leading to phenotypic change appear to occur only in association with mitosis (7), the regeneration stimulus as well as the potency of a mutagenic carcinogen might account for the high incidence of pancreatic hepatocyte induction in hamsters. In the rat model, a nonmutagenic chemical (26) was administered chronically to adult rats in which cell replication in the pancreas was minimal (4); this might account for the relatively low incidence of transdifferentiation. It is conceivable that ciprofibrate, if administered during either experimentally-induced pancreatic regeneration (4) or fetal or neonatal development, might enhance the incidence of pancreatic hepatocyte induction in rats.

In this paper we have used the designation hepatocytes rather than hepatocyte-like cells for the ciprofibrate-induced eosinophilic polyhedral cells in rat pancreas because these cells are indistinguishable from hepatic hepatocytes by light and electron microscopic criteria or by protein markers for peroxisomes. The presence in peroxisomes of these cells of a characteristic urate oxidase-containing crystallloid nucleus, which is a unique morphological feature of peroxisomes of rat liver cells (9, 30, 34), is consistent with our interpretation of these cells in pancreas as hepatocytes. Although we are designating these cells as pancreatic hepatocytes, based on an unequivocal morphologic similarity and rat liver hepatocyte-specific peroxisomes, further studies are required to catalogue...
other hepatocyte specific properties. Peroxisomes per se are not exclusive cell markers for hepatocytes; (8, 20) but the presence of the crystalloid is the *sine qua non* for their identity as hepatocytes (8, 9, 20, 30, 34). Peroxisomes and microperoxisomes (20) in nonhepatic cells of rats lack this unique crystalloid core and the enzyme uricase that is obligatorily coupled to this structure (9, 15, 34). Accordingly, uricase is considered a hepatocyte-specific protein (8, 34). Catalase and peroxisome proliferation-associated *M*, 80,000 protein were detected in the present study by immunoblotting procedure in pancreas containing hepatocytes in rats that were fed ciprofibrate. Peroxisome proliferation-associated *M*, 80,000 protein is immunochemically identical to the bifunctional enoyl-CoA hydratase (21), a component of peroxisomal fatty acid β-oxidation enzyme system (14). It is also important to note that excessive accumulation of lipofuscin, a pigment indicative of lipid peroxidation reactions (5), was noted in pancreatic hepatocytes of rats fed ciprofibrate for 72 wk. Large amounts of lipofuscin also accumulate in parenchymal cells of liver of rats during hepatocarcinogenesis induced by peroxisome proliferators (26–28) which is attributed to excess production of H₂O₂ by peroxisomal oxidases (27, 28).

The present studies in rats as well as earlier ones in hamsters (22) clearly demonstrate that hepatocytes in pancreas also respond to peroxisome proliferative effect of a hypolipidemic compound in a manner characteristic of hepatic hepatocytes (6, 24, 28, 31). Peroxisome proliferation is inducible only in hepatic parenchymal cells of liver (28) and to a lesser extent in proximal tubular epithelium of the kidney (11). Current evidence implicates a cytosolic receptor as a mediator of drug-induced peroxisome proliferation (13). The fact that peroxisome proliferation can be induced in hepatocyte-like cells in pancreas indicates that these cells probably also possess receptor sites capable of recognizing peroxisome proliferators. The magnitude of increase in numerical and volume densities of peroxisomes in rat pancreatic hepatocytes reported in this study is comparable to the response observed in hepatocytes of host liver. The ability of these cells at extrahepatic locations to respond to a peroxisome proliferator in a similar fashion to the parenchymal cells of liver, including lipofuscin accumulation, further supports the notion that pancreatic hepatocytes possess structural and functional control mechanisms similar to those of hepatic hepatocytes.

The present study also provides some insight into the origin of hepatocytes in rat pancreas. Because uricase-containing crystalloid nucleoids in peroxisomes are the basic cellular structures peculiar to rat liver hepatocytes (8), we have chosen this as a marker to investigate the process of transdifferentiation and to identify the possible precursor cell(s) in which the change of gene expression occurs. The presence in these hepatocyte foci of transitional or hybrid forms with cytoplasmic features of pancreatic acinar/endocrine cells as well as structures that are unique to rat hepatocyte cytoplasm is a further indication that it is feasible to induce permanent changes in the state of an already well-differentiated cell and that the process can be discerned morphologically. These transitional cells prove conclusively that cells with structural and functional features of hepatocytes originate in the pancreas and represent a state in which the normally active pancreas specific genes are undergoing repression with simultaneous activation of liver-specific genes that normally remain repressed in pancreatic cells. Evidence of mitochondrial division in these cells, leading to the formation of oval to round mitochondria characteristic of hepatocytes similar to that presented by Tandler et al. (32) in the liver during recovery from riboflavin deficiency, further supports the view that transformation process encompasses all or most aspects of the synthesis of products and structural proteins that identify the transformed cells as hepatocytes. The presence of endocrine and exocrine granules in these transitional cells indicates that acinar and/or intermediate cells of pancreas are the precursors for pancreatic hepatocytes. The occurrence of cells intermediate in morphology between that of acinar and endocrine cells has been described in the pancreas of several species (17). Whether such intermediate cells arise by transformation of differentiated pancreatic acinar cell in response to a metabolic disturbance or are present in the organ *ab initio* is not clear. Intermediate cells have been observed at junctions of acinar tissue with islets (17), the location at which foci of pancreatic hepatocytes are induced in the present study. If acinar cell is the precursor for intermediate cells, it is not surprising that under appropriate conditions they can express previously repressed genetic information leading to the formation of hepatocytes. In summary, the rat model of pancreatic hepatocytes may represent a valuable system to further investigate the genetic control of transdifferentiation given the fact that this species is used extensively for embryological, developmental, carcinogenic, and molecular biological studies.

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