Expression and Distribution of Lactate/Monocarboxylate Transporter Isoforms in Pancreatic Islets and the Exocrine Pancreas

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Transport of lactate across the plasma membrane of pancreatic islet β-cells is slow, as described by Sekine et al. (*J Biol Chem* 269:4895–4902, 1994), which is a feature that may be important for normal nutrient-induced insulin secretion. Although eight members of the monocarboxylate transporter (MCT) family have now been identified, the expression of these isoforms within the exocrine and endocrine pancreas has not been explored in detail. Using immunocytochemical analysis of pancreatic sections fixed in situ, we demonstrated three phenomena. First, immunoreactivity of the commonly expressed lactate transporter isoform MCT1 is near zero in both α- and β-cells but is abundant in the pancreatic acinar cell plasma membrane. No MCT2 or MCT4 was detected in any pancreatic cell type. Second, Western analysis of purified β- and non–β-cell membranes revealed undetectable levels of MCT1 and MCT4. In derived β-cell lines, MCT1 was absent from MIN6 cells and present in low amounts in INS-1 cell membranes and at high levels in RINm5F cells. MCT4 was weakly expressed in MIN6 β-cells. Third, CD147, an MCT-associated chaperone protein, which is closely colocalized with MCT1 on acinar cell membranes, was absent from islet cell membranes. CD147 was also largely absent from MIN6 and INS-1 cells but abundant in RINm5F cells. Low expression of MCT1, MCT2, and MCT4 contributes to the enzymatic configuration of β-cells, which is poised to ensure glucose oxidation and the generation of metabolic signals and may also be important for glucose sensing in islet non–β-cells. MCT overexpression throughout the islet could contribute to deranged hormone secretion in some forms of type 2 diabetes. *Diabetes* 50:361–366, 2001

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ANT, adenine nucleotide translocase; LDH, lactate dehydrogenase; MCT, monocarboxylate transporter; mGPDH, mitochondrial glycerol phosphate dehydrogenase; MRC, Medical Research Council; PBS, phosphate-buffered saline.

Glucose-stimulated insulin secretion involves glycolytic breakdown of the sugar and its oxidation within mitochondria (1). This leads to an increase in the concentration of intracellular free MgATP (2,3), causing the closure of ATP-sensitive K^+ channels (4), Ca^{2+} influx, and the activation of exocytosis. Well-differentiated β-cell lines and purified primary β-cells are able to oxidize a remarkably high proportion (>80%) of glucose carbon to CO_2 and H_2O (5,6). We have previously reported that islet β-cells contain very low levels of lactate dehydrogenase (LDH) and lactate (monocarboxylate) transporter (MCT) activity. Recently, eight isoforms of MCT, each predicted to be an integral membrane protein with 12 well-conserved transmembrane-spanning regions, have been identified and cloned (7). MCT1 and MCT4 are closely associated with the monotopic membrane protein CD147 (8,9). This protein is a widely distributed member of the immunoglobulin superfamily (10,11) and appears to act as a chaperone that is essential for the correct targeting of MCT1 and MCT4 to the plasma membrane.

The tissue distribution of MCTs 1–4 has been studied quite extensively at both the protein and mRNA level and is both complex and to some extent species dependent (7). MCT1 (the only form in erythrocytes and probably also gut cells) is present in the majority of tissues, whereas MCT4 predominates in those cells requiring high rates of lactic acid efflux, such as white muscle fibers and white blood cells (12). Thus, MCT1 is the major isoform present in red muscle fibers whereas MCT4 predominates in white fibers (13). The distribution of MCT2 is more restricted and is expressed in liver, heart, kidney, testis, and the brain. Expression of MCT2 is also species dependent, particularly in the liver and heart. In hamsters, MCT2 is the major isoform in the liver and is present in the heart. In the rat, no MCT2 is found in the heart and MCT1 is present at higher levels than MCT2 in the liver (14,15). In humans, little MCT2 is found in any tissue (12). MCT3 expression appears to be exclusive to the basolateral surface of retinal pigment epithelial cells (7). Distribution of the other MCT isoforms has only been explored at the mRNA level because no suitable antibodies are currently available. It is also unknown whether these higher MCT isoforms transport lactate, because this has only been demonstrated directly for MCT1–MCT4 (7). Isoforms 5–8 are more distantly related members of the MCT family (7). Indeed, we have currently been unable to demonstrate lactate or pyruvate transport activity for isoforms 5, 6, and 8 after expression of the

recombinant proteins (the activity of MCT7 is unexplored). Within the whole pancreas, the only MCT isoforms detected by Northern analysis are MCT7 and MCT8 (12), but neither the pattern of expression of the MCT isoforms and CD147 nor their presence at the protein level has been explored within the endocrine pancreas. Using heterologous expression in *Xenopus* oocytes, detailed characterization of the kinetics and substrate/inhibitor specificity has been performed for MCT1, MCT2, and MCT4 in which K_m values for L-lactate are respectively 4, 0.7, and 28 mmol/l (16–18).

In our earlier studies, islet β-cells were reported to express somewhat lower levels of LDH and lactate transport activities than islet non–β-cells, whereas both cell types contained dramatically lower levels of these activities compared with nonislet tissue, including liver (5). Subsequent quantification of LDH and mitochondrial glycerol phosphate dehydrogenase (mGPDH) activity in islet non–β-cells (19) has suggested that the difference in the LDH and mGPDH activities of islet β- and non–β-cells may be smaller than earlier estimates (5). Stable overexpression of LDH-A activity perturbs glucose-stimulated insulin secretion in MIN6 β-cells (20) and interferes with oxidative glucose metabolism and ATP generation after acute infection of cells with LDH-A–expressing adenoviral vectors (21). Furthermore, increases in LDH activity in the islets of rodents rendered diabetic by 95% pancreatectomy (22) are associated with impaired glucose-stimulated insulin secretion. Finally, cooverexpression of LDH and MCT activities confers sensitivity to lactate of insulin secretion in islets (23).

The present study was undertaken to assess, using immunocytochemistry, the intraislet expression of LDH and MCT and CD147 activities and to compare this with the level of these proteins in the exocrine pancreas and in other rat tissues. The use of in situ fixation of the pancreas, which reduces problems of degradation of mRNA and protein, has allowed us to compare MCT and CD147 levels simultaneously within the islet and nonendocrine pancreas. The findings confirm that the common MCT isoforms are essentially absent from islets but indicate that both MCT1 and CD147 are abundant on the acinar cell plasma membrane. The role of this pattern of transporter expression in glucose sensing by islet cells is discussed.

RESEARCH DESIGN AND METHODS

Tissue preparation. Male Wistar rats weighing 220–250 g were supplied by B & K Universals (Bristol, U.K.) and housed in the Bristol Medical School animal house with free access to standard pellets and water. The animals were deeply anesthetized by an intraperitoneal injection of sodium pentobarbital and then perfused via the ascending aorta, first with 150 ml ice-cold normal saline followed by 300 ml Zambonis fixative solution (4% paraformaldehyde, picric acid in phosphate-buffered saline [PBS], and PBS 0.1 mol/l, pH 7.4). Pieces of tissue from the pancreas were immediately dissected out and immersed in the same fixative for 2 h and moved into 30% sucrose solution overnight before cryostat sectioning. The tissue was embedded in Bright Cryo-M-Bed (Bright Instrument, Huntingdon, U.K.) and frozen in isopentane/liquid nitrogen. Cryostat sections were cut at a thickness of 5 or 8 μm and mounted on poly-L-lysine–coated glass slides (BDH, London) and air-dried for 1 h at room temperature. The sections were used for immunostaining immediately or stored at –70°C for up to 8 weeks.

Antibodies. Polyclonal anti-peptide COOH-terminal antibodies to human MCT1 (cross-reacts with MCT1 from most species [M.Z., A.P.H., unpublished observations]), Chinese hamster ovary MCT1 (cross-reacts with rat but not human MCT1) (24), human MCT4 (cross-reacts with rat MCT4) (12), rat MCT2 (15), and rat CD147 (9) were raised in rabbits and affinity purified as described previously.

Crude plasma membrane preparations from liver tissue homogenate and cell lines were performed as described (9,25). Samples were separated by SDS-PAGE and analyzed by Western blotting using the antibodies described and enhanced chemiluminescence detection (15,24).

Immunohistochemistry. Tissue sections were rinsed with PBS and permeabilized with 0.3% Triton X-100, then incubated in 10% swine serum (for MCTs) or 1% skim milk powder in PBS for 1 h. Incubation of the primary antibodies was performed at 4°C overnight. Briefly, sections were covered in a moist chamber with one of the following antibody solutions: rabbit anti-MCT1 at a dilution of 1:100, anti-MCT2 at 1:100, anti-MCT4 at 1:100, mouse monoclonal antibody against CD147 at 1:10, guinea pig anti-insulin at 1:500 (Dako), or rabbit anti-glucagon at 1:100 (ICN). The sections were then rinsed in PBS for 10 min three times and then incubated with fluorescein isothiocyanate (FITC)-, tetramethyl-rhodamine isothiocyanate (TRITC)-, or indocarbocyanine (Cy3)-conjugated secondary antibodies against IgG of the species from which the corresponding primary antibodies were generated. These were obtained from various suppliers including Sigma, Dako, and Jacksons.

Confocal microscopy. Specific immunoreactivities were revealed by laser scanning confocal microscopy. Confocal images were acquired using TCS NT software on an upright Leica IRBE confocal microscope equipped with an Argron/Krypton laser and using a ×63 oil immersion lens.

Islet cell purification. Purified rat β- and non–β-cells were obtained as described previously (26). Briefly, pancreatic islets were collagenase isolated from male Wistar rats. Dissociated islet cells were flow sorted on the basis of light scatter and FAD+ fluorescence into β-cells (purity >90%) and non–β-cells (70–80% α-cells, 5–10% β-cells, and 10–15% other cells) using a FACStar Plus (Becton Dickinson, Mountain View, CA) cell sorter. Freshly isolated cells were washed in PBS, frozen in liquid nitrogen, and stored at –80°C before immunoblotting.

COS7 cell culture and transfection. Cells were cultured and transfected with CD147 as described previously (9).

Statistics. Data are presented as the means ± SE for the number of observations given in parentheses.

RESULTS

Immunolocalization of MCT isoforms in pancreatic sections. Immunofluorescence confocal microscopy revealed the presence of MCT1 but not other MCT isoforms in exocrine pancreatic cells, at a level equal to or greater than that in skeletal muscle (12). In contrast, within the islet, neither MCT1 nor other MCT isoforms could be detected in insulin-containing β-cells (Fig.1 *A–F*). Weak reactivity to MCT1 was apparent in the cytosolic compartment of glucagon-containing α-cells using the anti-rat polyclonal antibody (Fig. 1 *D* and *I*). This staining, which displayed a punctate distribution, was also observed with an anti-human MCT1 antibody that cross-reacts with rat MCT1 (data not shown). Therefore, this signal in α-cells would seem most likely caused by nonspecific staining of an abundant non-MCT protein present in secretory vesicles.

Western analysis of MCT isoforms in purified islets and derived β-lines. Western analysis of either total cellular protein with anti-rat MCT1 antibody or cell membranes with antibodies against MCT1 or MCT4 failed to detect any band at the predicted molecular mass (43 kDa) (7) (Fig. 2*A*). These data confirm the absence of MCT protein in both islet β- and non–β-cells. Similarly, the highly differentiated and glucose-responsive mouse β-cell line, MIN6 (27), did not express detectable MCT1 immunoreactivity (Fig. 2*B*), whereas weak reactivity at 43 kDa was apparent in this cell line, corresponding to MCT4 (Fig. 2*B*). Another relatively responsive β-cell line, INS-1 (28), expressed low but detectable MCT1 reactivity (but not MCT4). Finally, the poorly glucose-responsive and highly glycolytic RINm5F line (5,29) expressed MCT1 at high levels (Fig. 2*B*). A closely parallel pattern of expression of CD147, a chaperone for MCT1 and MCT4 (9), was also observed in these lines with levels increasing in the order MIN6 < INS-1 < RINm5F (Fig. 2*C*).

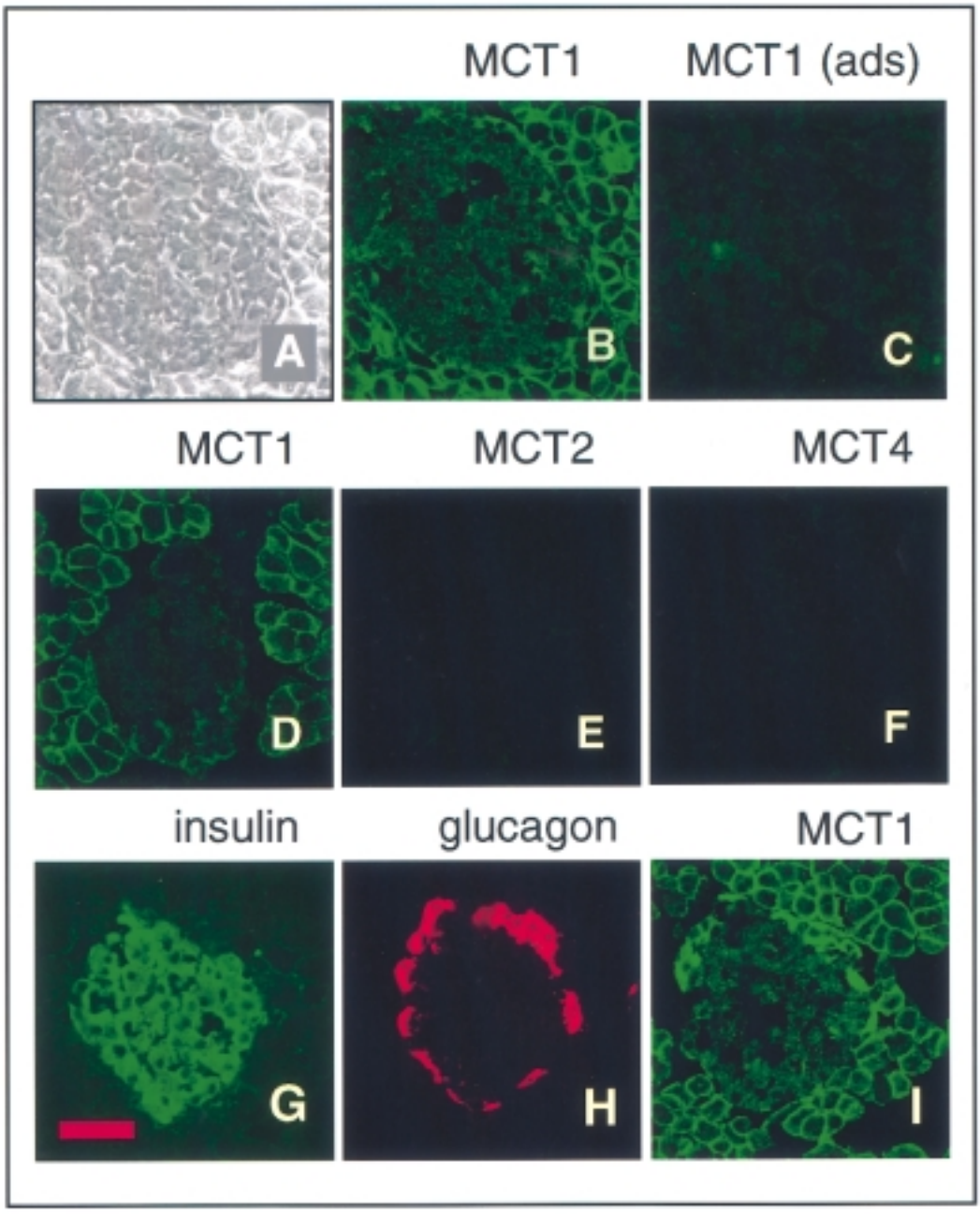

FIG. 1. Distribution of MCT1, MCT2, and MCT4 in the pancreas. Confocal microscopic immunohistochemical images of rat pancreatic islets. *A*: Shows the bright field image of an islet. *B* is the same islet as *A* stained with rat MCT1, whereas in *C* the MCT1 antibody was preabsorbed with MCT1 peptide against which the antibody was raised. *D* is another example of MCT1 staining of a smaller islet. *E* and *F* are serial sections of the same region of the pancreas, stained with anti-MCT2 and anti-MCT4 antibody, respectively. *G* to *I* are serial consecutive sections of pancreas immunostained for insulin, glucagon, and MCT1, respectively. The apparent cytosolic staining of glucagon+ cells in slide *I* was nonspecific (see RESULTS). For other details, see RESEARCH DESIGN AND METHODS. Scale bar = 50 μm.

Immunolocalization of CD147 in pancreatic sections. Given the parallelism between the expression of MCT1 and CD147 in β-cell lines, we next examined the expression of the protein in pancreatic sections. CD147 displayed a close spatial colocalization with MCT1 immunoreactivity being present at highest levels in the plasma membrane of acinar cells (Fig. 3). The remarkably similar distribution of these two proteins is demonstrated by the overlay images of Fig. 3*A*, *B*, *D*, and *E*. In contrast, weak cytosolic staining was apparent throughout the islet (Fig. 3*B* and *E*), though CD147 was essentially absent from the plasma membrane of all islet cells (observed in both large and small islets; Fig. 3), and from islet-cell membranes examined by Western blotting (data not shown). However, some CD147 staining was apparent in the cytosol of all islet cells.

DISCUSSION

We describe here the distribution of MCT isoforms and CD147 within the pancreas. We show that no MCT isoform known to transport lactate/pyruvate is expressed at the protein level on the plasma membranes or internal membrane of any rat islet cell type analyzed in situ. In contrast,

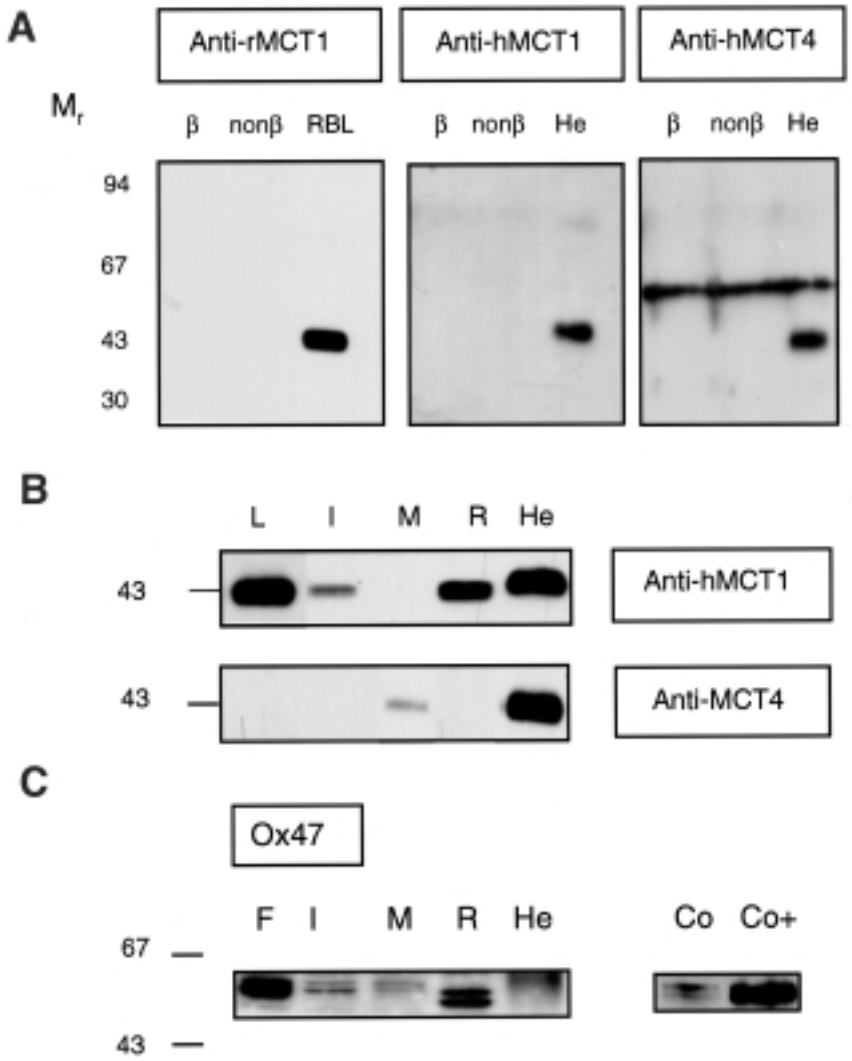

FIG. 2. Western analysis of MCT and CD147 expression in primary islet α- and β-cells and derived β-cell lines. *A*: MCT1 in fluorescence-activated cell sorter–purified islet β- and non–β-cells (5 µg total cellular protein) with polyclonal antibodies raised to rat (rMCT1) or human (hMCT1 and hMCT4) sequences (5 µg membranes). RBL, rat retinoblastoma cells; He, HeLa cells (human). The band of M_r ~60 kDa represents nonspecific binding not removed by preabsorbing the antibody with peptide. *B*: MCT1 and MCT4 expression in membranes of derived β-cell lines, I, INS-1; M, MIN6; R, RINm5F; or L, rat liver membranes. Relative intensities of MCT-1 expression (arbitrary units) were L, 230,000; INS-1, 29,000; MIN6, undetectable; RINm5F 125,000; and HeLa, 172,000. *C*: CD147/CD147 expression in: F, rat epididymal fat pads; I, INS-1 cells; M, MIN6 cells; R, RINm5F; and He, HeLa cells; Co, untransfected or Co+, Cos7 cells transfected with cDNA encoding CD147. Note that multiple bands represent differential glycosylation, which varies between cell types (9).

MCT1 was expressed strongly at the plasma membrane of acinar cells, but no staining of intracellular membranes was apparent. These data argue against a mitochondrial location of MCT1, contrary to the proposal of others (30). Indeed, we have not detected any MCT1 associated with mitochondria in any cell type or in isolated mitochondrial preparations (M.W., A.P.H., unpublished data). Furthermore, the plasma membrane preparation used here (Fig. 2) contained mitochondrial membranes because the adenine nucleotide translocase (ANT), an exclusively inner mitochondrial membrane protein, could be readily detected using specific anti-ANT antibodies (M.Z., A.P.H., unpublished data). Our current findings complement direct measurements of lactate transport into islet cells (5), which indicated that rates of transport are extremely low in both β- and islet non–β-cells. Whether other members of the MCT family are expressed in islet cells remains to be established because appropriate antibodies are currently not available. However, if they are present it seems unlikely that they will transport lactate. Interestingly, low levels of MCT4 were expressed in derived MIN6 cells and MCT1 in INS-1 cells, whereas high levels of MCT1 were present in RINm5F cells (Fig. 2*B*). These data are also consistent with direct activity measurements (5,20) and may reflect adaptations of the immortalized cell lines for growth and division that involve enhanced rates of glycolysis.

Consistent with the low levels of MCT immunoreactivity in both islet β- and non–β-cells, our own in situ immunocytochemical studies (C.Z., G.A.R, unpublished data) suggest that the difference in mGPDH levels between islet β- and non–β-cells may be smaller than previously measured (5), probably reflecting contamination of the non–β-cell fraction in these studies with nonendocrine cells. Indeed, more highly purified preparations reveal smaller differences (~2-fold

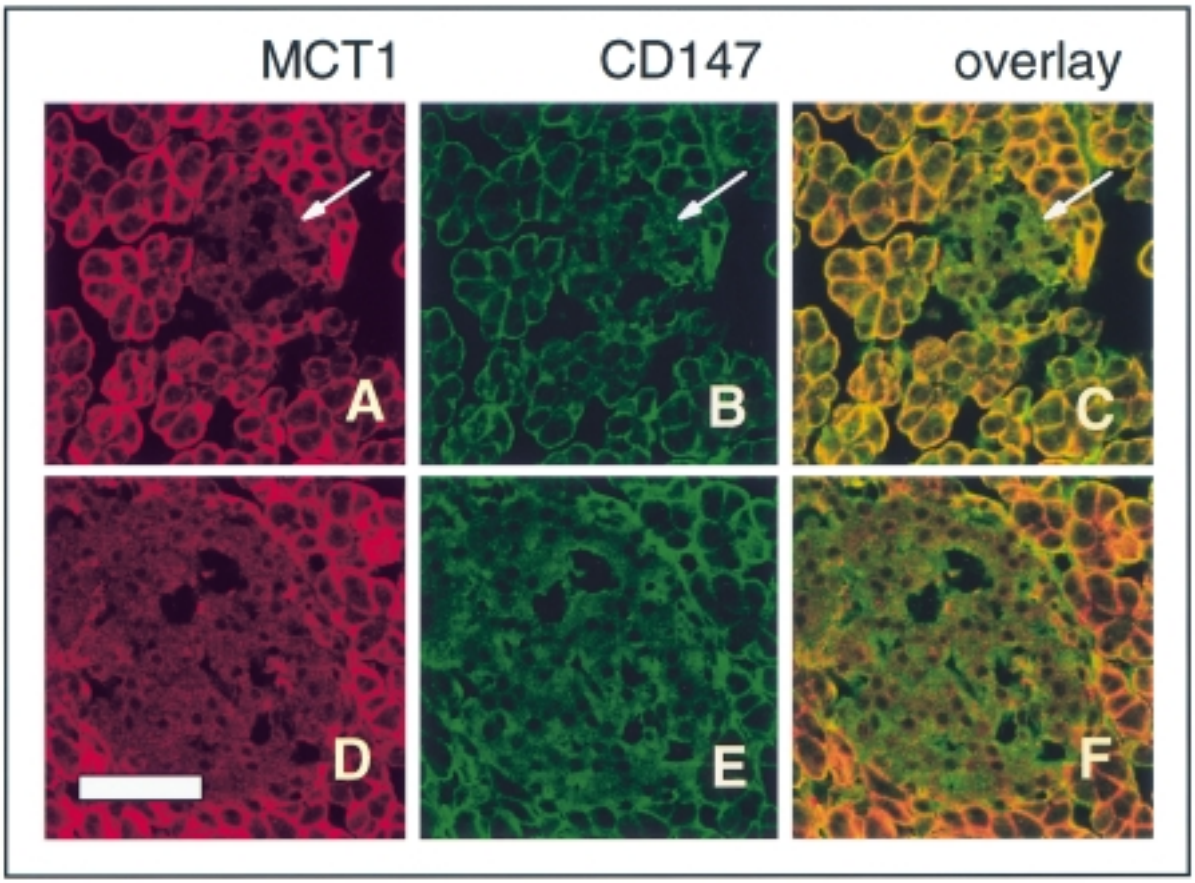

FIG. 3. Colocalization of MCT1 and CD147 in the pancreas. Two examples of colocalization of MCT1 and CD147 protein in rat pancreas. *A* to *C* and *B* to *D* represent two sections containing islets (→ in *A–C*) of different dimensions. Sections were incubated with anti-MCT1 (*A*, *D* from rabbit) and CD147 (*B*, *E* mouse monoclonal) antibodies and revealed with cy3 and FITC-conjugated secondary antibodies. *C* and *F* are overlays of *A* and *B*, and *D* and *E*, respectively. Scale bar = 50 μm.

when expressed per unit mass of protein) between mGPDH and LDH expression in β- and in non–β-cells (19). Thus, the ratio of LDH:mGPDH may be similar in β- and islet non–β-cells (but dramatically lower than other cell types) (5), which is consistent with the important role of the glycerol phosphate shuttle in these cells. Supporting this view, LDH immunoreactivity was not significantly different in islet β- and non–β-cells, as observed in the immunocytochemical studies of Jonas et al. (22). Furthermore, these workers demonstrated that in a model of diabetes (partial pancreatectomy), LDH-A was overexpressed thoughout the islet, consistent with the inhibitory effects of LDH-A overexpression on glucose-stimulated insulin secretion (20,21). All these data fit well with the recent findings that K_{ATP} channel (31) and sulphonylurea receptor-1 immunoreactivity (32) are present in islet α- and somatostatin-secreting cells, and the demonstration of tolbutamide and diazoxide-sensitive K^+ currents in α-TC glucagon-secreting cells (33). Taken together, this would seem to be strong evidence to support the view that each of these cell types is capable of sensing changes in blood glucose (and other nutrient) concentrations via metabolic coupling mechanisms involving the enhanced oxidation of pyruvate derived from glucose. However, the mechanisms by which the two cell types then respond differently (with either enhanced [β-] or diminished [α-] hormone secretion) are unknown.

The importance of low LDH-A levels for glucose-induced insulin secretion in the β-cell type has been directly demonstrated (20,21). Importantly, overexpression of LDH interferes with mitochondrial metabolism of glucose and insulin secretion, most profoundly at submaximal but suprathreshold glucose concentrations (21). Although not examined here, it seems possible that MCT overexpression may exert a similar inhibitory effect on insulin release and could synergize with the effect of increased LDH levels under some circumstances.

Therefore, a further intriguing question is whether low LDH and MCT levels are also important for normal glucose-mediated inhibition of secretion in other islet neuroendocrine cells types (α, δ, polypeptide P). If this is the case, then alterations (increases) in LDH-A and MCT levels in α-cells could potentially contribute to the diabetic phenotype by decreasing the inhibition of glucagon release by elevated glucose. At the same time, it is also likely that low levels of LDH-A and MCT, and perhaps CD147, are important to prevent the inappropriate activation of insulin, glucagon, and other islet hormones release by lactate (23).

CD147 and MCT1 in the exocrine pancreas. The striking parallelism in the level of expression and close subcellular colocalization of CD147 and MCT1 that we report here lends further support to the view that CD147 expression is important for correct targeting of MCT1 to the plasma membrane (9). The expression of MCT1 in acinar cells is expected given the widespread occurrence of this isoform (7). While MCT2 and MCT4 protein were not detected in acinar cells, we cannot exclude the possibility that higher MCT isoforms may be

present, which is consistent with the presence of MCT7 and MCT8 mRNA in the pancreas (34).

Conclusion. Our data indicate that low plasma membrane lactate transport activity, previously described for purified β-cells and other islet cells in transport assays (5), can be correlated at the molecular level with the expression of undetectable levels of MCT protein in these cells. Thus, islet cells appear at present to be unique in mammals in not expressing the apparently ubiquitous MCT1 isoform present in all other tissues and cell types so far examined.

Because MCT1 expression is regulated in skeletal muscle during chronic stimulation (13), it will be important to show whether changes in MCT expression can also occur in the islet during altered nutritional regimes or disease states. In particular, derangement of MCT or CD147 gene expression might contribute to modifications in insulin secretion in some forms of type 2 diabetes (23), as previously reported for LDH activity (21,22). Given the large excess of lactate transport activity in most tissues (7), inhibitors of islet cell lactate transport, or of MCT gene expression, could provide a novel therapeutic target for this disease.

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