Rapid Publication

Hormone-Sensitive Lipase, the Rate-Limiting Enzyme in Triglyceride Hydrolysis, Is Expressed and Active in β-Cells

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Triglycerides in the β-cell may be important for stimulus-secretion coupling, through provision of a lipid-derived signal, and for pathogenetic events in NIDDM, where lipids may adversely affect β-cell function. In adipose tissues, hormone-sensitive lipase (HSL) is rate-limiting in triglyceride hydrolysis. Here, we investigated whether this enzyme is also expressed and active in β-cells. Northern blot analysis and reverse transcription–polymerase chain reaction demonstrated that HSL is expressed in rat islets and in the clonal β-cell lines INS-1, RINm5F, and HIT-T15. Western blot analysis identified HSL in mouse and rat islets and the clonal β-cells. In mouse and rat, immunocytochemistry showed a predominant occurrence of HSL in β-cells, with a presumed cytoplasmic localization. Lipase activity in homogenates of the rodent islets and clonal β-cells constituted 2.1 ± 0.6% of that in adipocytes; this activity was immunoinhibited by use of antibodies to HSL. The established HSL expression and activity in β-cells offer a mechanism whereby lipids are mobilized from intracellular stores. Because HSL in adipocytes is activated by cAMP-dependent protein kinase (PKA), PKA-regulated triglyceride hydrolysis in β-cells may participate in the regulation of insulin secretion, possibly by providing a lipid-derived signal, e.g., long-chain acyl-CoA and diacylglycerol. *Diabetes* 48:228–232, 1999

In NIDDM, hyperglycemia is accompanied by abnormalities in lipid metabolism. The elevation of circulating free fatty acids (FFAs), in particular, has received much attention and is widely considered a possible pathogenetic factor in the disease (1,2). FFAs perturb insulin action in peripheral tissues, but have also specifically been implicated in the impaired glucose-stimulated insulin secretion (GSIS) in NIDDM, another hallmark of the disease. For instance, in rats infused with lipids, an initial augmentation of GSIS is followed by a blunted response (3); similar observations have been made in perifused islets (4). Moreover, in islets from Zucker diabetic fatty (ZDF) rats, an increase in content of islet triglycerides directly parallels the onset of diabetes, and if this accumulation is prevented, the rats remain normoglycemic (5).

On the other hand, despite the apparent adverse effects of chronically elevated levels of FFAs on insulin secretion, sometimes termed "lipotoxicity" (1), there are data to suggest that a lipid-derived signal is normally involved in the stimulus-secretion coupling in the β-cell (6). Candidates for this signal are, among others, malonyl-CoA and long-chain acyl-CoA (LC-CoA) (7), as well as diacylglycerol (DAG), which may also be generated in a separate pathway from membrane-bound phospholipids. These lipid-derived signals may be involved at several levels in the insulin secretory process (6). In agreement with this concept, islets depleted of triglycerides, and consequently incapable of forming such a signal, display an abrogated GSIS (8).

Although it has become increasingly clear that lipids play a critical role in the β-cell, it is not known if and how FFAs are mobilized from intracellular stores in the β-cell. Such mobilization would represent an initial step in events leading to formation of the aforementioned lipid-derived signals. On the other hand, perturbed control of intracellular lipid stores may precipitate impaired insulin secretion, as is apparent in the ZDF rat (5). In adipose tissue, hormone-sensitive lipase (HSL) is responsible for hydrolysis of tri- and diglycerides, the first step of which is rate-limiting in breakdown of triglycerides (9). HSL is unique among lipases in that it is regulated by hormones and neurotransmitters. In adipocytes, by means of reversible cAMP-dependent protein kinase (PKA)-mediated serine phosphorylation, hormones raising intracellular cAMP, e.g., epinephrine, activate HSL, while insulin, which decreases intracellular cAMP, reduces HSL activity. Thus, as well as controlling the re-esterification of fatty acids, insulin and other hormones regulate the release of lipids from adipose tissues through inhibition/activation of HSL. Preliminary observations from Corkey and associates have indicated that HSL is expressed in clonal β-cells (10). Here, we report that the enzyme is expressed in rodent islets and clonal β-cells and that it exerts lipase activity in these islets and cells.

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DAG, diacylglycerol; FFA, free fatty acid; GSIS, glucose-stimulated insulin secretion; HSL, hormone-sensitive lipase; LC-CoA, long-chain acyl-CoA; PCR, polymerase chain reaction; PKA, cAMP-dependent protein kinase; RT, reverse transcription.

RESEARCH DESIGN AND METHODS

Cell culturing, islet isolation, and tissue preparation. RINm5F, HIT-T15, and INS-1 cells were grown in monolayers in RPMI-1640 medium supplemented with 10% fetal calf serum and, in the case of INS-1, 50 µmol/l β-mercaptoethanol; the passage numbers used were RINm5F, 73–76; HIT-T15, 76–79; and INS-1, 84–87. Rat primary adipocytes were isolated as described (11). Islets from NMRI mice and Sprague-Dawley rats were isolated by collagenase digestion after overnight culture in RPMI-1640 medium supplemented with 10% fetal calf serum and 11.1 mmol/l glucose. For immunocytochemistry, the pancreases were immersed overnight in 2% paraformaldehyde and 0.2% picric acid in phosphate buffer (pH 7.2), followed by repeated rinsing in 10% sucrose in phosphate buffer (pH 7.2) for cryoprotection before freezing on dry ice.

RNA isolation and Northern blot analysis. Total RNA was isolated, electrophoresed under denaturing conditions, and blotted to a nylon membrane using standard techniques. Blots were probed with a partial full-length α-^{32}P-labeled rat HSL cDNA (nt 595-2489) (12).

Reverse transcription–polymerase chain reaction. Using 200 U of Moloney–Murine Leukemia Virus transcriptase (Clontech, Palo Alto, CA), 1 µg of total RNA was reverse-transcribed by oligo-dT priming. The single-strand cDNAs were subjected to 40 cycles of amplification (94°C for 45 sec, 55°C or 60°C for 45 sec, and 72°C for 1 min) using AmpliTaqGold (Perkin-Elmer, Warrington, U.K.). Oligonucleotides from exon 5 (5′-TCT CCA TCG ACT ACT CCC TGG C-3′) (12,13), the testis-specific exon (5′-CCA TCA TGA AAG ATC TGT G-3′) (14), and the noncoding exons A (5′-TCG AAG AAT GCC ATA GAG GGC TCA A-3′) and B (5′-AGC CAC AGA CCT GCT GTG CCA GC-3′) (15) were used as sense primers. Antisense primers were oligonucleotides from exon 7 (5′-AAG GAG TTG AGC CAT GAG GAG GC-3′), exon 4 (5′-GAT GCC ATG TTG GCC AGA GAC-3′), and exon 1 (5′-TGT CTC AGT GTC CAG GTC GAA ATG GT-3′) (12,13).

Immunoprecipitation. For immunoprecipitation, lysates of cells and tissues in 100 mmol/l Tris-HCl (pH 7.5) 0.3 mol/l NaCl, 3% Triton X-100, and 0.1% N-lauroylsarcosine were incubated for 2 h at room temperature with an affinity-purified rabbit anti-rat HSL antibody, followed by incubation for 30 min at room temperature with Protein A Sepharose (Pharmacia Biotech, Uppsala, Sweden). Precipitates were washed 5 times in 1 ml of 10 mmol/l Tris-HCl (pH 7.4), 0.5 mol/l NaCl, and 0.1% N-lauroylsarcosine, resolved by SDS-PAGE, and analyzed by Western blotting.

Western blot analysis. Immunoprecipitates and cell and/or tissue extracts prepared directly in SDS sample buffer were resolved by SDS-PAGE followed by electroblotting to nitrocellulose membranes. Western blot analysis was performed by chemiluminescence (SuperSignal Ultra; Pierce, Rockford, IL), using an affinity-purified rabbit anti-rat HSL primary antibody and a horseradish peroxidase-conjugated goat anti-rabbit IgG secondary antibody (Amersham, Abingdon, U.K.).

Immunocytochemistry. Affinity-purified chicken anti-rat HSL antibodies (diluted 1:320) (14) or polyclonal rabbit anti-rat HSL antibodies (diluted 1:160) were used for indirect immunofluorescence in rodent islets as described elsewhere (16). Previously characterized antibodies (16) to insulin (9003; Euro-Diagnostica, Malmö, Sweden) and glucagon (7811; Euro-Diagnostica) were used for double immunofluorescence as described (16). In control experiments, recombinant rat HSL (100 µg/ml) (17) was added to the antibodies at working dilution before incubation of the sections.

Enzyme activity assays and immunoinhibition. Cell and tissue homogenates were prepared in 0.25 mol/l sucrose, 1 mmol/l EDTA (pH 7.0), 1 mmol/l dithioerythritol, 20 µg/ml leupeptin, 20 µg/ml antipain, and 1 µg/ml pepstatin A and assayed for DAG lipase activity, using as substrate the DAG analog mono-oleoyl-2-*O*-mono-oleylglycerol (17). One unit of enzyme activity is equivalent to 1 µmol of fatty acids released per minute at 37°C. Fat-depleted infranatants of rat and mouse adipose tissue were used as reference material (14,17). Immunoinhibition was performed by preincubating the homogenates in chicken anti-rat HSL antibody (whole plasma diluted 1:8) for 60 min at 25°C.

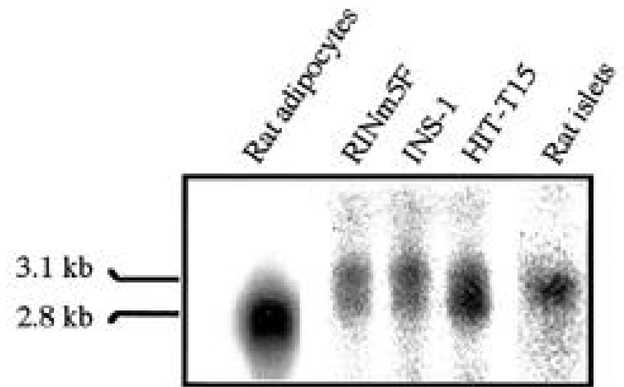

FIG. 1. Northern blot analysis of total RNA from rat adipocytes (10 µg), RINm5F, INS-1, and HIT-T15 cells (30 µg each), and rat islets (50 µg), using an HSL cDNA probe. Blots were analyzed by phosphoimaging after a 5-day exposure. The images have been digitally edited and processed to facilitate qualitative comparisons of HSL mRNA expression.

RESULTS AND DISCUSSION

To examine expression of HSL in β-cells, we performed Northern blot analysis of total RNA isolated from rat islets, clonal β-cells, and rat adipocytes. As shown in Fig. 1, HSL mRNA was detected in all sources examined, demonstrating that HSL is indeed expressed in islets and β-cells. The size of the mRNA detected in islets and clonal β-cells was slightly larger (~3.1 kb) than that in adipocytes (~2.8 kb) (15), although not as large as the previously characterized testicular HSL mRNA (14). Reverse transcription (RT)–polymerase chain reaction (PCR), using primers for amplification of sequences common to the known isoforms of HSL, confirmed expression of HSL in islets and the clonal β-cells (Fig. 2*A*). The region upstream of exon 1 in the human HSL gene contains exons that are expressed in a tissue-dependent and

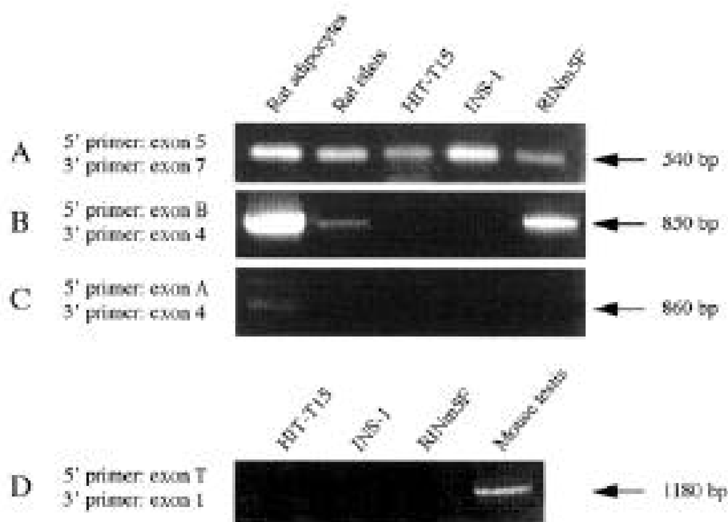

FIG. 2. RT-PCR analysis of RNA from rat islets and adipocytes, mouse testis, and RINm5F, INS-1, and HIT-T15 cells. Primers were derived from sequences located in different exons, as indicated (*A–D*). Samples (16 µl) of the 50 µl PCR reactions were fractionated in a 1% agarose gel. The ethidium-bromide stained gels are shown, with the sizes of the respective PCR products indicated.

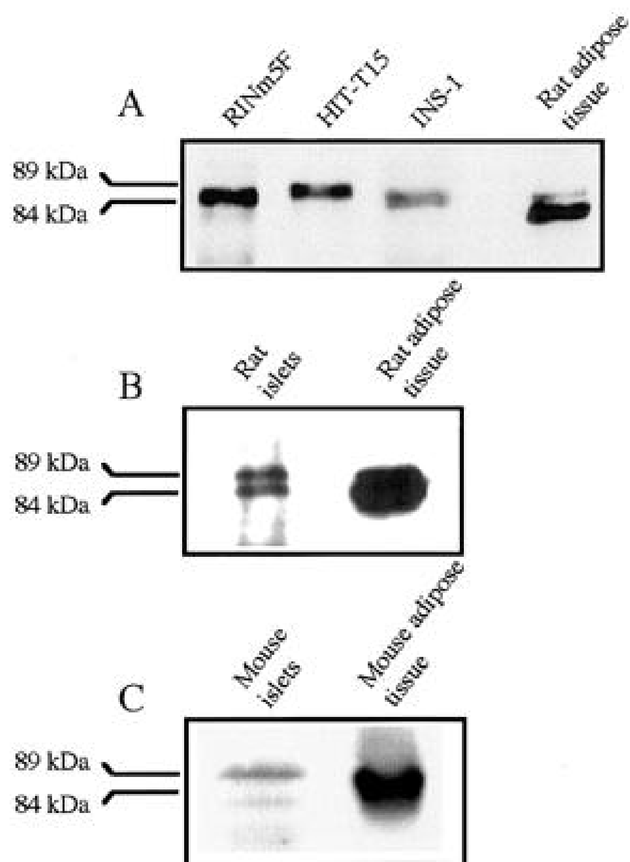

FIG. 3. Western blot analyses of HSL from RINm5F, HIT-T15, and INS-1 cells, rodent islets, and adipose tissue. Total cell lysates of the clonal β-cells (1.8 mg of total protein for each line) and rat islets (0.7 mg of total protein; 200 islets) and a fat-depleted rat adipose tissue lysate (0.1 mg of total protein) were subjected to immunoprecipitation of HSL (*A* and *B*); mouse islets were lysed directly in SDS sample buffer (*C*). In *C*, the lane containing the mouse islet sample has been exposed 10 times longer than the lane containing the mouse adipose tissue sample. HSL detected in clonal β-cells and islets is slightly larger (89 kDa) than the adipose tissue form (84 kDa); additionally, rodent islets, rat in particular, express an isoform similar in size to that of adipose tissue.

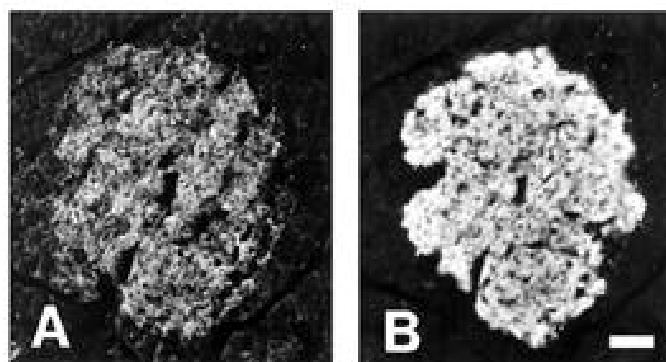

FIG. 4. Double immunofluorescence for HSL (*A*) and insulin (*B*) in a section of a rat pancreas. Bar = 50 µm.

mutually exclusive manner and that are either noncoding or, as in testis, encoding an NH_2-terminal extension of the enzyme (14,15). To further examine the identity of the mRNA species in rat islets and clonal β-cells, we used primers based on sequences from these exons. While a sequence from the 5′ exon expressed in white adipose tissue (exon B) was amplified from islets and RINm5F cells, we were unable to amplify this sequence from HIT-T15 and INS-1 cells (Fig. 2*B*). Sequences specific for exon A and the testis-specific exon (exon T) were not detected in islets and clonal β-cells (Fig. 2*C* and *D*). To examine the molecular identity of HSL protein, we performed Western blot analysis of cell and tissue lysates resolved by SDS-PAGE, either directly or after prior enrichment by immunoprecipitation. The main HSL form in islets and the clonal β-cells was an 89-kDa protein (Fig. 3). In addition, islets expressed an HSL protein indistinguishable in size (84 kDa) from that of the adipocyte form of the enzyme (Fig. 3*B* and *C*).

The predominant occurrence of a slightly larger HSL protein in islets and clonal β-cells compared with the main form in adipose tissue agrees with the Northern blot analyses, which detected a slightly larger transcript in rat islets and clonal β-cells. The apparent lower abundance of 84-kDa HSL in islets may explain why a smaller transcript was not detected here, but post-translational modifications may also occur. Although the known HSL sequences in different species are well conserved (17), there remains a possibility that the apparent lack of, e.g., exon A expression may be due to interspecies sequence variations, since the primers used were based on human sequences. Nevertheless, exon B was readily detected in both rat islets and RINm5F cells. The reason for exon B expression in RINm5F cells but not in the other cell lines is unclear, but may relate to the more undifferentiated nature of RINm5F cells compared with the other clonal β-cells. In rat islets, expression of exon B may be accounted for by the presence of the 84-kDa HSL, which is found in adipocytes and encoded by a transcript comprising exon B (15); a possibility also remains that different islet cell types express different HSL isoforms. Collectively, the data indicate that the main HSL isoform expressed in rodent islets and clonal β-cells is slightly larger than that in adipocytes; the extended sequence of this novel isoform may be encoded by yet unidentified upstream exon(s), in analogy to that occurring in the testis (14).

Immunocytochemical detection of HSL in sections of mouse and rat pancreases was performed to investigate the cellular localization of the enzyme. HSL-like immunofluorescence occurred in the majority of islet cells and was consistently observed in all islets present in the sections. The HSL-like immunofluorescence had a fine granular appearance, suggesting that the enzyme is localized to the cytoplasm, as opposed to the plasma membrane. In rat, double immunofluorescence showed that HSL-like immunofluorescence predominantly occurred in β-cells (Fig. 4*A* and *B*); additionally, it could also be detected in α-cells (not shown). The specificity of the results was ensured by both antibodies to HSL displaying an identical staining pattern in the islets and by preabsorption of the antibodies with recombinant rat HSL, quenching immunofluorescence.

Lipase activity was assayed to ascertain that HSL in the β-cell preparations possessed enzymatic activity. This was indeed the case, the lipase activity being ~1–4% (2.1 ± 0.6%) of that observed in homogenates of adipose tissue (Table 1). An antibody to HSL that almost abolished lipase activity in

TABLE 1
DAG lipase activity in clonal β-cells, rodent islets, and adipose tissue

Tissue/cell line	DAG lipase activity (mU/mg protein)	Immunoinhibition (% of control)
RINm5F	2.2 ± 0.2 (6)	22.2 ± 3.6 (6)*
HIT-T15	3.9 ± 0.3 (6)	76.9 ± 0.7 (6)†
INS-1	1.9 ± 0.2 (6)	32.7 ± 4.2 (3)†
Rat islets	6.6 ± 1.6 (4)	ND
Mouse islets	9.2 ± 3 (5)	ND
Rat adipose tissue	226.7 ± 12 (9)	92.9 ± 1.1 (8)†
Mouse adipose tissue	245.1 ± 20 (6)	92.9 ± 2.1 (5)†

Data are means ± SE (number of experiments). Immunoinhibition is expressed as percent inhibition compared with a control sample incubated with a preimmune serum. Comparisons were made with a paired two-tailed t test: $^{*}P < 0.01$, $^{\dagger}P < 0.001$. ND, not determined.

adipose tissue significantly reduced enzyme activity in the clonal β-cells, albeit to a variable extent, ranging from ~20% in RINm5F to 75% in HIT-T15. This variability may reflect a diminished inhibitory potency due to structural differences in β-cell HSL or that other components of the β-cell homogenates not present in adipose tissue preparations interfere with the assay. Nevertheless, the results demonstrate lipase activity in islet cells, a significant part of which is accounted for by HSL.

The demonstration of HSL expression and activity in β-cells is an important step toward unraveling the significance of lipids in β-cells. The essentiality of triglycerides for stimulus-secretion coupling in the β-cell has been demonstrated in islets depleted of such stores (8); here, an abrogated insulin response to glucose is evident, conceivably caused by the lack of one or more lipid-derived signals. Through the action of HSL, triglycerides stored in the β-cell can be hydrolyzed and ultimately generate such signals, e.g., DAG and LC-CoA, which may exert pleiotropic effects critical for stimulus-secretion coupling. DAG is a known stimulator of protein kinase C (18), while anaplerotic metabolism of glucose, via generation of malonyl-CoA (7) and its subsequent inhibition of carnitine palmitoyl transferase I (19), can raise the cytoplasmic levels of LC-CoA. On the other hand, perturbed metabolism of lipids in β-cells may precipitate diabetes. Diabetes in ZDF rats is preceded by triglyceride accumulation in islets (5), counteraction of which would maintain euglycemia. Conversely, culture of normal islets in the presence of lipids mimics the diabetogenic events in ZDF islets, i.e., basal hypersecretion of insulin while GSIS is blunted (5). FFAs may also be involved in the destruction of β-cells in ZDF rats (20) because FFAs were found to induce islet nitric oxide (NO) production and expression of NO synthase.

The mechanisms whereby lipids affect β-cell function are not fully understood. Corkey and associates have proposed that the malonyl-CoA/LC-CoA system is a fuel sensor in the β-cell required for stimulus-secretion coupling (6). LC-CoA may affect the exocytotic machinery, various glycolytic enzymes (5,6), or transcription of critical proteins; for instance, expression of acetyl-CoA carboxylase in INS-1 cells is downregulated by FFAs (21). On the other hand, LC-CoA activates K^{+}_{ATP} channels in isolated membrane pieces from pancreatic β-cells (22). Subsequent membrane repolarization with closure of voltage-gated Ca^{2+}-channels may be the mechanism whereby protracted exposure to FFAs impairs GSIS (3–5). The particular role, however, of malonyl-CoA in β-cells has recently been both challenged (23) and supported (24) by use of molecular approaches, thus necessitating further investigations to clarify its role in the stimulus-secretion coupling.

Regardless of the role for lipid-derived signals in the β-cell, HSL may regulate, at least in part, the availability of such signals. Because HSL is known to be activated through reversible serine phosphorylation by PKA (9), a number of circulating hormones, e.g., glucagon-like peptide 1, glucose-dependent insulinotropic peptide, and glucagon, which potentiate GSIS by raising cytoplasmic cAMP levels (25), may also activate HSL. Conversely, the signaling pathway involving phosphoinositide-3-kinase, protein kinase B, and phosphodiesterase 3B, which is believed to inactivate HSL in adipocytes by reducing cAMP and PKA activity (9), is also in operation in β-cells (26,27). The expression of HSL in β-cells points to the interesting possibility that activation of this lipase, via generation of lipid-derived signals, may contribute to the overall release of insulin. This possibility, as well as the perturbed lipid metabolism that may adversely affect β-cells in the events leading to NIDDM (lipotoxicity) (1), warrants further investigation of the role of HSL in β-cells.

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Q1a: Would you like to use your US address in the footnote?
Q1: Corkey is not an author of Ref. 10. Please advise.
Q2: Correct that it was the antibody to HSL, and not HSL itself, that almost abolished lipase activity?
Q3: “would” as meant? If not, please reword for clarity.