Hormone-sensitive lipase (HSL) is a key enzyme in fatty acid mobilization in many cell types. Two isoforms of HSL are known to date, namely HSLadi (84 kDa in rat) and HSLtes (130 kDa in rat). These are encoded by the same gene, with exons 1–9 encoding the parts that are common to both and an additional 5′-exon encoding the additional amino acids in HSLtes. HSL of various tissues, among these the islet of Langerhans, is larger than HSLadi, but not as large as HSLtes indicating that there may be other 5′-coding exons. Hence we describe the molecular basis for a novel 89-kDa HSL isoform that is expressed in β-cells, adipocytes, adrenal glands, and ovaries in the rat and that is encoded by exons 1–9 and exon A, which is spliced to exon 1 and thereby introducing an upstream start codon. The additional 5′-base pairs encode a 43-amino acid peptide, which is highly positively charged. Conglomerates of HSL molecules are in close association with the secretory granules of the β-cell, as determined by immunoelectron microscopy with antibodies targeting two separate regions of HSL. We have also determined that the human genomic sequence upstream of exon A has promoter activity in INS-1 cells as well as glucose sensing capability, mediating an increase in expression at high glucose concentration. The minimal promoter is present within 170 bp from the transcriptional start site and maximal glucose responsiveness is conferred by sequence within 850 bp from the transcriptional start site.

Because of the established association between type 2 diabetes and obesity, lipids have received much attention in research on the pathophysiology of type 2 diabetes. Lipids are believed to cause insulin resistance in tissues and to alter β-cell function. The effect of lipids on β-cells has been studied extensively, but the picture that emerges is complex. Different effects are seen depending on parameters such as duration of exposure, concentration, and fatty acid chain length and saturation. Briefly, the presence of free fatty acids is essential for normal glucose-stimulated insulin secretion (1, 2) and also has an amplifying action on glucose-stimulated insulin secretion. On the other hand, a prolonged exposure (24–48 h) to high concentrations (~1 mM) of free fatty acids is detrimental to β-cell function and survival (3, 4).

In view of this, knowledge of lipid mobilization, lipid storage, and the regulation of these in the β-cell are of great interest.

Recently, we showed that hormone-sensitive lipase (HSL) is expressed and active in islets of Langerhans and clonal β-cells (5). HSL is a key enzyme in fatty acid mobilization in the adipocytes and presumably also in several non-adipocyte cell types (see Ref. 6 for review). It is unique among known lipases in that its activity is acutely controlled by hormones. Catabolic hormones, such as glucagon and catecholamines, activate the enzyme and anabolic hormones (e.g. insulin) decrease its activity. The activity of HSL is principally determined by the intracellular level of cAMP, which controls the activity of protein kinase A that phosphorylates HSL on critical serine residues (7). In the adipocyte, this triggers translocation of HSL from the cytosol to the surface of the lipid droplet (8) and, possibly, also a change in specific activity. HSL has a broad substrate specificity, being able to hydrolyze, among others, mono-, di-, and triglycerides and cholesteryl esters. Besides β-cells and adipocytes, HSL is also present in testis, ovary, adrenal gland, heart, skeletal muscle (9), macrophages (10), and gastrointestinal tract mucosa (11). The HSL gene is located on chromosome 19 (human) (12) and spans 27 kb (Fig. 1A) (11, 13–15). All functional HSL proteins are encoded by, at least, exons 1–9. Upstream of exon 1 there are several exons that are spliced to exon 1 in a mutually exclusive manner. The 5′ exons can be coding or non-coding.

The present investigation of HSL in the β-cell concerns structure, subcellular localization, and regulation of gene expression. HSL in the β-cell is larger than the predominant HSL isoform found in the adipocyte (89 versus 84 kDa) (5). Here we show that the difference in size between the two isoforms is explained by the addition of a 5′-exon to the HSL transcript, namely exon A, which contains coding sequence. We also present evidence that the larger HSL isoform is present in other cell types besides the β-cell. Using electron microscopy, we show...
the subcellular localization of HSL in rat islet β-cells. Formerly, we have shown that HSL expression is up-regulated in rat islets of Langerhans and clonal β-cells after long-term exposure to high concentrations of glucose (16). Here we describe the genomic region responsible for this effect.

EXPERIMENTAL PROCEDURES

RNA Extraction—Total RNA was extracted from tissues or cells according to Chomczynski and Sacchi (17). Poly(A)− RNA was prepared from total RNA with the Dynabeads mRNA purification kit (Dynal) according to the manufacturer’s instructions.

cDNA Cloning—The Marathon cDNA kit (Clontech) was used to create a clonable cDNA library from 1 μg of poly(A)− RNA from INS-1, passage 90. 5′-RACE was performed on a 1:50 dilution of the cDNA library. Adapter primer 1 of the Marathon kit was used in conjunction with a primer of the following sequence: 5′-TGAGGGCAGC-3′ (EcoRI site underlined). The resulting product was cleaved with NotI and EcoRI and subcloned in pBluescript II SK (Stratagene). Plasmids were sequenced using BigDye reagents (Applied Biosystems) and comparative sequence analysis was performed using the MacVector software (Accelrys).

Animals—Rats were obtained from B&K Universal, Sweden. Studies were performed in strict accordance with the local ethical committee.

Northern Blotting—Tissues were dissected from a female rat weighing 300 g and frozen in liquid nitrogen until processing. Male genital organs were taken from a male rat of the same weight and luteal ovaries were taken from a 10-day pregnant female rat. Any adipose tissue was carefully removed from all tissues. Fifty μg of total RNA from the various tissues was electrophoresed in 0.9% agarose, 2.2 M formaldehyde gels. Nucleic acids were passively transferred to nylon membranes (Hybond N, Amersham Biosciences) and UV cross-linked.

Islet Isolation—Ten ml of cold Hanks’ balanced salt solution with collagenase P (Roche Diagnostics), 1.35 units/ml, was injected into the bile duct, filling the pancreas retrogradely. The pancreas was removed and incubated at 37 °C for 20 min. After washing four times with Hanks’ balanced salt solution, islets were manually separated from exocrine cells under a microscope.

RT-PCR—The RT step was performed with 1.5 μg of total RNA, 100 units of Moloney murine leukemia virus-RT (New England Biolabs), random hexamers, as well as a rat HSL-specific reverse primer from exon 8, at 37 °C for 60 min. A no RT reaction was run in parallel. The PCR was performed using a sense primer with the first 24 bp of exon A coding sequence and a reverse primer from exon 1 (5′-GGCTGATAGCTGCTA-GAGCACC-3′), 1 μl of RT or no RT reaction, and AmpliTaq Gold polymerase (Applied Biosystems). Product size was determined on a 1% agarose gel. The expected size of the amplified HSL DNA was 513 bp.

Creation of Antibodies—Using pBluescript containing HSL-exon A cDNA as template, a PCR fragment containing the coding sequence of exon A was created. Primers used were 5′-GTCGAGATCCATGGAGCCG-GGGCAGCTTC-3′ (BamHI site underlined) and 5′-GACCTTGCTTTCGTGGCCAC-3′ (EcoRI site underlined). The PCR product was gel purified (Qiagen, Qiagen) and restriction cleaved with BamHI and EcoRI. The resulting molecule was ligated into the same sites of pGex-2T (Amersham Biosciences) containing the Schistosoma japonicum glutathione S-transferase (GST) cDNA. The resulting plasmid was sequenced across the inserted HSL cDNA to verify that the GST-HSL-A fusion protein, immobilized according to the manufacturer’s instructions.

Western Blotting—Tissues were dissected from Sprague-Dawley rats. Tissues and INS-1 cells were homogenized with a glass Teflon homogenizer in homogenization buffer (0.25 mol/liter sucrose, 1 mmol/liter ethylenediamine tetraacetic acid, 10 μg/ml antipain, 10 μg/ml leupeptin, and 1 μg/ml pepstatin). A fat-depleted infantrant of adipose tissue was obtained after centrifugation at 50,000 × g, 4 min, 4 °C. Islets were isolated as described above and lysed in SDS-PAGE sample buffer. Determination of protein concentration was performed using a BCA protein kit ( Pierce). Samples were subjected to SDS-PAGE on 8% or 12% acrylamide gels and then electrophoretically transferred onto nitrocellulose membranes (HyBond-C extra, Amersham Biosciences).

Primary antibodies used were affinity purified anti-rat HSL antibodies, 1:5000 (raised against sequence emanating from exons 1 through 9 of the HSL gene, i.e. recombinant HSL C-term (18)) and the affinity purified rabbit antibody directed toward the GST-HSL-A fusion protein (1:100), a horseradish peroxidase-conjugated donkey anti-rabbit IgG (Amersham Biosciences) used as secondary antibody (1:2000) and the blots were developed by enhanced chemiluminescence. Detection was performed using a CCD camera (LAS1000, Fuji).

Tissue Processing and Immuno-electron Microscopy—Isolated islets were incubated in HEPES-balanced salt solution (114 mM NaCl, 4.7 mM KCl, 1.2 mM KH₂PO₄, 1.16 mM MgSO₄, 20 mM HEPES, 2.5 mM CaCl₂, 25.5 mM NaHCO₃, 0.2% bovine serum albumin, pH 7.2) supplemented with 3 mM glucose during 1.5 h and then stimulated during 30 min at RT in HEPES-balanced salt solution containing 3 mM glucose, 15 mM glucose or 15 mM glucose with 5 μM forskolin. After washing with Sörensen buffer, the islets were fixed in 3% paraformaldehyde and 0.5% glutaraldehyde in Sörensen buffer during 1 h. The islets were then washed in pure Sörensen buffer and pre-embedded in 2% agar in distilled water. The pre-embedding was followed by dehydration in an ethanol series with increasing concentration of alcohol. The final embedding was performed in Lowicryl HM20 at −50 °C, which was UV-polymerized at −45 °C during 48 h followed by 32 h at RT. Ultrathin sections were cut with an ultramicrotome (Leica UC7) and deposited on Formvar-coated nickel grids.

All washing steps were performed in a 400-ml vessel and the reactions were carried out in 100 μl-drop on Parafilm. Between all steps excess liquid was blotted off by touching the edge of the grids with a filter paper. The grids were first incubated, during 30 min at RT, in a buffer containing a phosphatase-coating HSL antibody diluted with 0.5% acetylated bovine serum albumin (bovine serum albumin-c, Auron) and 0.1% fish gelatin (Amersham Biosciences). All the grids, except the controls, were then incubated overnight at room temperature with the primary antibody rabbit anti-HSL (20) or rabbit anti-GST-HSL-A diluted 1:25 in the blocking buffer. The controls were incubated in the blocking buffer overnight.

After washing in a buffer (phosphate-buffered saline supplemented with 0.1% Tween 20) for 15 min the grids were incubated during 2 h at room temperature with a secondary antibody (goat anti-rabbit IgG conjugated with 6-nm gold particles (Auron), diluted 1:10 in the blocking buffer). Finally the grids were washed during 15 min in washing buffer followed by washing with 1% uranyl acetate for 20 min. Counterstaining was performed with 3% uranyl acetate at 40 °C for 20 min followed by lead citrate (Reynolds’ recipe) at room temperature for 4 min. The sections were then observed in a Philips CM 120 Biotwin instrument at 120 kV.

Screening of Human Genomic Sequence—A cosmids containing human genomic sequence was cleaved with BamHI and BspEII. An exon A containing fragment was identified using the exon A probe described above in Southern blotting. Southern blotting was performed using a nylon membrane (Hybond N, Amersham Biosciences) and ExpressHyb hybridization solution (Clontech) according to the manufacturer’s instructions.

The identified fragment of 2.5 kb was gel purified (Qiagen II, Qiagen) and subcloned into the BamHI and BspEII sites of the pTriAmp vector. The subcloned fragment was sequenced in both directions using the BigDye reagent (Applied Biosystems). The sequence was found to be essentially identical to a sequence with GenBank™ accession number AC011497 (human chromosome 19).

Determination of Transcriptional Start Site (TSS)—Poly(A)+ RNA (2 μg) from rat tissues (adipose tissue or ovary, respectively, were treated with the FirstChoice RLM-RACE kit (Ambion). 5′-RACE was performed according to instructions of the manufacturer, using a gene-specific primer of the following sequence: 5′-GGCTGATAGCTGCTAGAGACC-3′. Two μl of this PCR was used for nested PCR using a gene-specific primer of the following sequence: 5′-GATGCACTCAAGGCTCTGCTG-3′ (EcoRI site underlined). Both primers contain antisense primer from exon 1 of the rat HSL gene. The resulting products were subcloned in pBluescript II SK and sequenced. Alternatively, the nested PCR was performed using an exon A-specific reverse primer (5′-TGAGTTTCTTGAGCTGCTT-
TCGCCACC-3'; EcoRI site underlined). The size of the resulting products was determined on a 3% MetaPhor-agarose gel (FMC BioProducts).

Creation of Luciferase Expressing Vectors—Sense and antisense primers that were complementary to sequence at the stated distances from the exon A TSS were created (DNA Technology, Aarhus, Denmark). Primers contained NheI or HindIII restriction sites in the 5'-end for cloning of PCR products into these sites of the pGL3basic vector (Promega). PCR was performed using proofreading DNA polymerase (Vent, New England Biolabs; or Platinum Pfx, Invitrogen).

Dual Luciferase Reporter Assay—INS-1 cells (p85–98) were grown in 24-well plates and transfected at 80% confluence with empty pGL3basic vector (which contains a Firefly luciferase gene) or pGL3basic containing sequence upstream of exon A (10 ng/well) and pRL-CMV (which contains a Renilla luciferase gene) (10 ng/well). Transfection was performed using 2 µl of FuGENE 6 (Roche). Each pGL-construct was added to three wells for each experimental condition in each experiment. The cells were incubated for 24 h. After incubation, the cells were washed with phosphate-buffered saline and lysed. Twenty µl of cell lysate was used in the subsequent assay performed in a Wallac 1420 luminometer with dual luciferase reporter assay reagents (Promega). Lysate of uninfected cells was used as blank.

RESULTS

HSL of the pancreatic β-cell is slightly larger (89 versus 84 kDa) than the predominant isoform found in adipocytes, referred to as HSLadi, as evident when lysates of clonal β-cell or islets from mouse and rat are subjected to immunoblot analysis (5). To resolve the sequence of the HSL transcript of the β-cell responsible for the difference in protein chain length, INS-1 cell mRNA was subjected to 5'-RACE using a gene-specific anti-sense primer with HSL exon 1. Stars denote identical residues and dots denote residues with similar chemical properties in the same position. Sequence comparisons were made with MacVector software (Accelrys).

Fig. 1. Exon A of the HSL gene in rat, mouse, and human. A, organization of the HSL gene in human. Black bars annotate coding sequence and white bars untranslated exons or parts of exons. The genomic organization is very similar between mouse and human (13, 21). The organization of the HSL gene in rat is not known, but is likely to be similar. B, ClustalW alignment of HSL-exon A sequence from rat and mouse (19) and the corresponding human genomic sequence (GenBank™ AC011497). The translation start codon is printed in bold. 20 bp of exon 1, translated in the novel isoform, but not in HSLadi, are included (italic). Stars indicate nucleotides that are identical in all three species (~73% of rat sequence) and lines, above the sequence, show the nucleotides that are identical between rat and mouse (~95% of rat sequence). C, predicted primary structure of the peptide encoded by exon A and 20 bp of exon 1. Stars denote identical residues and dots denote residues with similar chemical properties in the same position. Sequence comparisons were made with MacVector software (Accelrys).
HSLαδi (Fig. 3) islets of Langerhans, a 89-kDa band was detected by anti-

ovaries (Fig. 3A) bands of 84 and 89 kDa in adipose tissue, adrenal glands, and

RT) was devoid of product (Fig. 2A). The expected size was retrieved, whereas the negative control (no

primer and an exon 1 reverse primer, an amplicon of the

rat islets of Langerhans, a lesser amount of RNA from rat islets

2

ovaries with and without corpora lutea, and INS-1 cells (Fig.

A). Antisense exon A-containing probes were detected in adipose tissue, adrenal glands,

sequences were detected in adipose tissue, adrenal glands, ovaries, and INS-1 cells. B, RT-PCR analysis of islets of Langerhans using 1.5

μg of total RNA from rat islets and primers from exon A (sense) and exon 1 (antisense). The figure shows a band of the expected size (513 bp),

resolved by agarose gel electrophoresis, and no product in the no RT control.

explains the observed difference in mobility between the two

isoforms upon SDS-PAGE. Rat and mouse HSLαδi share 94%

identity at the protein level, whereas rat and human share 83%

(12, 13, 21). The novel HSL region described here consists of 43

amino acids and the level of homology between rat and mouse

is similar to that of the rest of the protein. The homology,

however, between rat and human is considerably lower (~50%). A lower degree of sequence identity (~30%) between rat and human has previously been described also for the
testis-specific domain of HSL (14).

To determine whether exon A-containing transcripts were

present in cell types other than the β-cell, Northern blot anal-
yses were performed using a radiolabeled exon A probe. Exon A

sequences were detected in adipose tissue, adrenal glands,

ovaries with and without corpora lutea, and INS-1 cells (Fig.

2A). Because of the difficulties to obtain large quantities of pure

rat islets of Langerhans, a lesser amount of RNA from rat islets

was used to perform RT-PCR. When using an exon A forward primer and an exon 1 reverse primer, an amplicon of the

expected size was retrieved, whereas the negative control (no RT) was devoid of product (Fig. 2B).

Splicing of exon A to exons 1–9 yields a transcript that

contains a translation start codon 129 bases upstream from the

ATG in exon 1. Twenty of these nucleotides are derived from

exon 1. It is not known whether the start codon in exon A is

used in tissues. To find out whether the discovered transcript is

translated into protein, affinity purified antibodies from a rab-

bit immunized with a fusion protein consisting of GST and a

peptide encoded by rat exon A (GST-HSL-A) were used. Homo-
genates of adrenal glands, ovaries, islets, liver, and adipose

tissue infranatant were subjected to Western blot analysis

using an antibody directed against HSLαδi (derived from exons

1–9) and anti-GST-HSL-A, respectively. Anti-HSLαδi detected

bands of 84 and 89 kDa in adipose tissue, adrenal glands, and

ovaries (Fig. 3A). No HSL protein was detected in liver. In

islets of Langerhans, a 89-kDa band was detected by anti-

HSLαδi (Fig. 3B). Anti-GST-HSL-A detected bands of 89 kDa in

all tissues examined, except in liver (Fig. 3, A and B). These

results demonstrate that exon A is, in fact, translated into

protein sequence and that the exon A-containing transcript

encodes a larger isoform than the predominant HSL isoform in

adipocytes.

Subcellular Localization of HSL in the β-Cell—Rat islets for

immunoelectron microscopy were incubated in 3, 15, or 15 mM

glucose with 5 mM forskolin, before fixation, to examine

whether the subcellular localization of HSL varied under these

conditions. We found no differences in localization between

these experimental groups and therefore only results from one

are shown (high glucose + forskolin). The two HSL antibodies,

used for Western blot analysis, and gold-conjugated secondary

antibodies were used to detect HSL in adjacent islet sections

(Figs. 4 and 5). Immunolabeling was detected as conglomerates

of gold particles in association with insulin granules in β-cells.

The appearance of the HSL immunolabeling was virtually

identical with the two antibodies.

Determination of the Transcriptional Start Site of Exon

A—Determination of the TSS was performed using RNA ligation-

mediated RACE, a technique taking advantage of the presence

of the cap structure to discriminate between full-length and

partly degraded RNA molecules. mRNA from INS-1 cells,

ovaries, and white adipose tissue were investigated. Using

HSL-derived reverse primers and the RACE kit adapter prim-

ers, a single amplicon was obtained from each tissue at each

experimental condition and these corresponded to 5′-untrans-
lated regions between 41 and 47 bp, as detected either by

subcloning with subsequent sequencing or size determination

on MetaPhor-agarose gels (results not shown). This result was

extrapolated to the human genomic sequence, when making

constructs for the promoter studies described below.

Promoter Activity of the Genomic Region Upstream of Exon

A—A fragment of human genomic DNA containing exon A was

identified using subcloned rat exon A as a probe in Southern

blotting. The fragment was subcloned, sequenced, and used as

template to make PCR products of lengths between ~2150+38

and −30/±38. These constructs were subcloned into pGL3basic

and the resulting constructs were used to transfected INS-1 cells.

Fig. 2. Detection of HSL exon A in transcripts from rat tissues. A, Northern blot analyses were performed using 50 μg of total RNA from
each stated tissue and INS-1 cells and radiolabeled probes containing exon A sequence and 18 S sequence, respectively. Exon A-containing

transcripts were found in RNA from adipose tissue, adrenal glands, ovaries, and INS-1 cells. B, RT-PCR analysis of islets of Langerhans using 1.5

μg of total RNA from rat islets and primers from exon A (sense) and exon 1 (antisense). The figure shows a band of the expected size (513 bp),

resolved by agarose gel electrophoresis, and no product in the no RT control.
The cells were incubated for 24 h, whereafter firefly luciferase activity was measured. The results from these experiments suggested that the minimal promoter is located within 170 bp of the TSS (Fig. 6A). Full activity is reached when 288 bp are included in the construct. 2150 bp yield a slightly lower effect than the maximal measured, indicating that there may be repressing elements in the region between 2150 and 1150.

A search for transcription factor binding sites in the minimal promoter was performed, using the Genomatix MatInspector software. Two GC boxes and three antisense CCAAT boxes were found, the locations of which are indicated in Fig. 6B.

**Regulation of HSL Expression in Clonal β-Cells**—Recently, we established that HSL expression is up-regulated at both the protein and mRNA levels when clonal β-cells or islets of Langerhans are exposed to high concentrations of glucose (16). To investigate the genomic basis for this effect, the luciferase-based approach described above was used. INS-1 cells were transfected with a subset of the constructs and incubated in either 3 or 25 mM glucose for 24 h, whereafter luciferase activity was measured. In Fig. 7, the luciferase activity (relative to that evoked by empty pGL3basic) is set to one for each construct at 3 mM glucose. With the four smallest constructs (−170, −230, −288, and −343/+38) the luciferase activities were not different in high and low glucose conditions. The maximal activity measured (−200%) is obtained with −1150/+38 and −850/+38, indicating that elements responsible for mediating the effect of glucose are present between 850 bp and 343 bp from the TSS. The intervening construct (−650/+38) and the largest construct (−2150/+38) yielded intermediate effects.

**DISCUSSION**

We describe an 89-kDa isoform of HSL and its molecular structure. The novel HSL isoform is expressed in the pancreatic β-cell, adipose tissue, adrenal gland, and ovary, according to Northern and Western blot analysis. Previously, two isoforms of HSL have been described, i.e. HSLadi, an 84-kDa protein (rat) encoded by exons 1–9 of the HSL gene and HSLtes, a 130-kDa protein (rat) found exclusively in testis, encoded by exons 1–9 and a testis-specific exon (T1), which is located −16 kb upstream of exon 1 (14). The isoform presented here is encoded by exon A, in addition to exons 1–9. Exon A is located −13 kb upstream of exon 1 and has previously been shown to be incorporated in HSL transcripts in adipocytes from both hu-
man and mouse (19, 20) and enterocytes in mouse (11). In addition to the stated exons, there are several other exons in the region upstream of exon 1 in the HSL gene. These are termed exons B, C, D (19, 20), and T2 (15) and are all represented in HSL transcripts, but only exons A and T1 contain coding sequence. Thus, the HSL gene emerges as a gene that yields different transcripts by selectively adding different 5'-exons to its transcript. It seems that these 5'-exons are to a great extent mutually exclusive, but inclusion of more than one of these exons to the HSL transcript has been demonstrated (19).

Any possible difference in lipase function because of the interchange of the different N-terminal variants is presently unknown. It is not unlikely that the presence of alternative 5'-exons is a means for cell types to differentially control expression of HSL. Transcriptional regulation does not explain, however, why two of these 5'-exons contain coding sequence, altering the structure of the final protein. A search for functional motifs in the 43-amino acid peptide of the 89-kDa isoform identified a protein kinase C phosphorylation site ((ST)-X-(RK)), which is conserved in three species. The likelihood for this being of importance is low, because predicted protein kinase C sites are very common and all phosphorylation sites currently known to alter HSL activity are located within a regulatory module encoded by exons 7 and 8. It is possible that the N-terminal additions to HSL and the 89-kDa isoform are involved in protein-protein interactions. Previously, the N-terminal domain of HSL has been found to interact with adipocyte lipid-binding protein (22). Investigations are currently in progress to discern any differences in enzymatic function between recombinantly expressed HSL isoforms.

According to the presented immunoelectron microscopy images, HSL is localized in close association with the secretory granules within the β-cell. This localization is in agreement with our former studies using immunofluorescence, which revealed granular cytoplasmic labeling (5). The significance of this finding is difficult to assess. More knowledge of the role of HSL in the β-cell is needed, along with evidence of the nature and localization of the intracellular lipid stores. Free fatty acids, which can be released through the action of HSL, have been ascribed many roles in the β-cell, particularly in their active form, acyl-CoA (23). With regard to the subcellular localization of HSL, it should be borne in mind that acyl-CoA has been reported to have a stimulatory effect on the exocytotic machinery of permeabilized β-cells (24). Acyl-CoA has also been demonstrated to affect the K_ATP channel (25), which is present in the β-cell plasma membrane and in the secretory granule membrane (26). Furthermore, HSL represents the majority of neutral cholesteryl ester hydrolase activity in adipocytes.
cytes, muscle, liver (27), and islets,\(^2\) as obviated by studies of HSL null mice, whereas the diglyceride lipase activity in tissues of this model is comparatively more retained. Recently, cholesterol has been suggested to play a role in signaling leading to glucose-stimulated insulin secretion (28), opening for speculation that HSL acts, via cholesterol release, to affect insulin secretion.

The basic elements needed for transcription initiation are gathered within 170 bp upstream of the putative TSS of exon A. The promoter appears to be lacking a TATA box. The human promoter upstream of exon B has previously been found to be TATA-less and contains motifs that are common among TATA-less promoters, \(i.e.\) a GC-box, an A/T-rich domain, and an approximate Initiator (Inr) consensus sequence (YYCA\(_{11}\)1NTYY) (29), which allows for a single defined TSS (20). However, the human exon A promoter displays neither an A/T-rich domain nor an Inr consensus.

The location of the TSS for exon A is, as mentioned above, extrapolated from that determined from rat tissues and is, thus, not certain. Analysis of 5'-RACE products has shown that transcription may start at multiple sites, even downstream of the start codon in exon A of the mouse (19). A "sliding TSS" is not uncommon among genes with TATA-less promoters, but it is not the case in rat HSL, where we have found transcription to start at a single site or within very few base pairs. Because there is no good Inr consensus sequence, within the human sequence, it is possible that conditions for a sliding transcription start are at hand.

As indicated in Fig. 6, two GC boxes (Sp1 binding) and three CCAAT boxes (NF-Y binding), in the minus orientation, have been found, when using the MatInspector software (30) to investigate the human exon A promoter. In a review of functional CCAAT boxes, the reverse orientation was found to be more common in TATA-less promoters, than in promoters with a TATA box and they are often closer to the TSS (31). The sequence that includes all the CCAAT boxes (\(-100/+37\)) is not transcriptionally active, but the fact that three such elements are found within 100 bp and in close proximity to an exon makes it tempting to speculate that they play a role. The GC boxes may be obligate for promoter function, because they are included in the smallest fragment examined that yields transcription (\(-170/+37\)). Further exploration of the singular elements and their relative contribution to transcription of exon A will be undertaken in the future.

We have previously shown that HSL expression is induced in rat islets and clonal \(\beta\)-cells during incubation with high glucose concentration (16). This has also been shown to be the case in

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\(^2\) M. Fex, C. S. Olofsson, M. S. Winzell, P. Rorsman, C. Holm, and H. Mulder, manuscript in preparation.
both primary (32) and clonal (33) adipocytes, and cis-acting elements in the sequence upstream of exon B, which is the primary 5′-exon in adipocytes, have been located (34). Here we show that glucose exerts an effect on the ability of genomic DNA upstream of exon A to instigate transcription. This effect is of the same magnitude as that detected when inducing HSL expression in β-cells and islets (−200%). DNA sequence critical for this effect is located within 850 bp upstream of the TSS, whereas it is not present in the first 343 bp. Possibly there are several elements that work in concert for this effect, because fragments with 5′-cutoffs between these two numbers exhibit a partial effect.

**Fig. 6.** Transcriptional activity and proximal transcription factor binding sites of the HSL exon A promoter. A, promoter activity assays were performed as described under “Experimental Procedures” with 11 mM glucose. The Firefly luciferase activities are expressed as multiples of that of empty pGL3basic vector. The results are mean ± S.E. Each experiment was performed with sample triplicates (n = 3). B, the MatInspector application was employed to search for binding sites of nuclear proteins in promoter sequence. Highly conserved bases in regions that share homology with known binding sites are boxed. Two possible GC boxes (Sp1 binding) and three possible minus-strand CCAAT boxes (nuclear factor Y-binding) were found in the −170/+38 fragment of the human exon A promoter sequence. A CCAAT enhancer-binding protein binding site is located in the region of the middle CCAAT box.

**Fig. 7.** Effect of glucose on exon A promoter activity. Promoter activity assays were performed as described under “Experimental Procedures,” with 3 mM glucose (■) or 25 mM glucose (▲). The mean luciferase activity at 3 mM glucose was set to one for each examined construct. The results are mean ± S.E. Each experiment was performed with triplicate samples (n = 3–7). The luciferase signals for −650, −850, and −1150/+38 were significantly different between 3 and 25 mM glucose (p = 0.008, p = 0.0009, and p = 0.034, respectively). The level of induction at high glucose was larger for −850/+38 than for −650/+38 (p = 0.036).
In conclusion, we have described a novel isoform of HSL, which is slightly larger than the adipocyte form, because of the addition of exon A to the transcript. If this has an effect on HSL function is not known today. The exon A-containing transcript addition of exon A to the transcript. If this has an effect on HSL which is slightly larger than the adipocyte form, because of the

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J. Biol. Chem. 2004, 279:3828-3836.
doi: 10.1074/jbc.M311365200 originally published online October 23, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M311365200

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