Hormone-sensitive Lipase Is a Cholesterol Esterase of the Intestinal Mucosa*

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The identity of the enzymes responsible for lipase and cholesterol esterase activities in the small intestinal mucosa is not known. Because hormone-sensitive lipase (HSL) catalyzes the hydrolysis of acylglycerols and cholesterol esters, we sought to determine whether HSL could be involved. HSL mRNA and protein were detected in all segments of the small intestine by Northern and Western blot analyses, respectively. Immunocytochemistry experiments revealed that HSL was expressed in the differentiated enterocytes of the villi and was absent in the undifferentiated cells of the crypt. Diacylglycerol lipase and cholesterol esterase activities were found in the different segments. Analysis of gut from HSL-null mice showed that diacylglycerol lipase activity was unchanged in the duodenum and reduced in jejunum. Neutral cholesterol esterase activity was totally abolished in duodenum, jejunum, and ileum of HSL-null mice. Analysis of HSL mRNA structure showed two types of transcripts expressed in equal amounts with alternative 5’-ends transcribed from two exons. This work demonstrates that HSL is expressed in the mucosa of the small intestine. The results also reveal that the enzyme participates in acylglycerol hydrolysis in jejunal enterocytes and cholesterol ester hydrolysis throughout the small intestine.

Hormone-sensitive lipase (HSL)† is a multifunctional enzyme with broad substrate specificity (1). It hydrolyzes tri-, di-, and monoacylglycerols, cholesterol esters, and retinyl esters. The activity against diacylglycerol is higher than the activity toward tri- and monoacylglycerols. The enzyme also exhibits cholesterol esterase activity, which is almost twice the activity toward triacylglycerols. Much has been learned in the recent years about the domain structure of HSL. Sequence compari-

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§§ The abbreviations used are: HSL, hormone-sensitive lipase; ABC, ATP-binding cassette; ALBP, adipocyte lipid-binding protein; I-FABP, intestinal fatty acid-binding protein; RT, reverse transcription.

sons revealed that HSL belongs to a family of esterases which is mainly represented by prokaryotic enzymes (2, 3). From a structural point of view, HSL is the most complex protein of the family. Sequence alignments together with biochemical experiments suggest that adipocyte HSL is composed of two structural domains (4, 5). The first 315 amino acids make up the N-terminal domain, which shows very little sequence similarities to other known proteins. The region responsible for the interaction with adipocyte lipid-binding protein (ALBP) was mapped to this domain (6, 7). In adipose tissue, ALBP could increase the hydrolytic activity of HSL through its ability to bind and sequester fatty acids and through specific protein-protein interactions. The C-terminal domain is divided in two functional parts, a catalytic core and a regulatory module. The latter is composed of 150 amino acids, including all of the known phosphorylation sites of HSL. Unlike other known mammalian triacylglycerol lipases, the activity of HSL is regulated by phosphorylation. The phosphorylation sites of protein kinase A, extracellular signal-regulated kinase, and AMP-dependent protein kinase have been mapped (8–10). The catalytic core is the region that shows homology with the other members of the family. Modeling of the part revealed that it adopts an α/β-hydrolase fold that harbors the catalytic triad constituted by Ser583, Asp703, and His731 (4, 11).

Several forms of HSL transcripts and the exon-intron organization of the HSL gene have been characterized in humans. The 88-kDa adipocyte HSL is translated from a 2.8-kb mRNA that is encoded by 9 exons (12, 13). The transcription start site was mapped in a short noncoding exon called exon B. In the adenocarcinoma cell line HT29, two mRNA species are found, the adipocyte HSL mRNA and a mRNA with a different 5’-end transcribed from exon A. Two testicular forms of HSL have been characterized. The 3.9-kb mRNA encodes a 120-kDa protein that contains a unique N-terminal region encoded by exon T1, a region that presumably forms a third structural domain in this isoform (14). The 3.3-kb mRNA encodes a protein that is identical to the adipocyte HSL form (15). However, the mRNA species differ in their 5’-ends. Exon usage is mutually exclusive, exon T2 being only transcribed in testis and exon B being transcribed in adipose tissue. The nature and role of lipases and esterases participating in the digestion of dietary lipids in the lumen of the gastrointestinal tract are well established. In addition to the enzymes in the lumen, there is evidence of lipase activity in enterocytes (16). The presence of cholesterol esterase activity is more elusive. The exact identity of the enzymes responsible for the hydrolysis of intracellular acylglycerols and cholesterol esters is still unclear. Pancreatic triacylglycerol lipase, microsomal
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Primer localization defined from translation start site is indicated in parentheses according to murine HSL genomic organization (GenBank accession number AF179427). Primers used in real time quantitative PCR are shown in bold.

| Sense primer | Anti-sense primer | Size (bp) |
|--------------|-------------------|-----------|
| Exon A (-6918) | 5'-atgaggaaacgagcttgc-3' | Exon 1 (+2) | 5'-atgctgttgagcaagctg-3' | 69 |
| Exon A (-6918) | 5'-atgaggaaacgagcttgc-3' | Exon 1 (+16) | 5'-gtcaccagaacgttgc-3' | 102 |
| Exon A (-6918) | 5'-atgaggaaacgagcttgc-3' | Exon 1 (+234) | 5'-ctgtgctcacaacctagg-3' | 302 |
| Exon B (-1463) | 5'-cagactgcgactgctg-3' | Exon 1 (+2) | 5'-atgctgttgagcaagctg-3' | 74 |
| Exon B (-1463) | 5'-cagactgcgactgctg-3' | Exon 1 (+16) | 5'-gtcaccagaacgttgc-3' | 107 |
| Exon 1 (+28) | 5'-ttggctcaagctcagacg-3' | Exon 1 (+234) | 5'-ctgtgctcacaacctagg-3' | 307 |
| Exon 8 (+2294) | 5'-ggtctcaacgagcagact-3' | Exon 8 (+2360) | 5'-ctgagggctgttgctg-3' | 67 |

Experimental Procedures

Preparation of Intestinal Mucosa—French guidelines for the use and care of laboratory animals were followed. Male Swiss mice were fed ad libitum a standard chow (UAR A04, Usine d’Alimentation Rationnelle). To study the expression of HSL along the gastro-colic axis of the gut, the intestine and could contribute to the hydrolysis of intracellular lipids.

EXPERIMENTAL PROCEDURES

Preparation of Intestinal Mucosa—French guidelines for the use and care of laboratory animals were followed. Male Swiss mice were fed ad libitum a standard chow (UAR A04, Usine d’Alimentation Rationnelle). To study the expression of HSL along the gastro-colic axis of the gut, the intestine and could contribute to the hydrolysis of intracellular lipids.
RESULTS

Northern Blot Analysis of HSL mRNA—The distribution of HSL mRNA along the gastro-colic axis was analyzed by Northern blot (Fig. 1A). HSL mRNA is present in the five segments of the small intestine (Fig. 1B). No expression of HSL mRNA was detected in colon (data not shown). Using quantitative RT-PCR, the HSL mRNA level was 9.8 ± 0.7-fold higher in adipose tissue than in intestinal mucosa (n = 5).

To determine that the specificity of the detected HSL mRNA signal did not derive from contaminating visceral adipose tissue, a series of hybridization was performed with different probes that were tested on intestinal and adipose tissue samples. As shown in Fig. 1C, ALBP mRNA that encodes an adipocyte-specific fatty acid-binding protein was only detected in the adipose tissue sample. There was no hybridization of the probe in the intestinal mucosa sample. Considering the quantities of total RNA loaded (15 μg for adipose tissue and 30 μg for intestine) and the intensity of the ALBP mRNA signal obtained in adipose tissue, a weak contamination of intestinal sample by visceral adipose tissue would thus have been detected. On the contrary, I-FABP, which encodes the intestinal fatty acid-binding protein, was only detected in the intestinal mucosa sample.

Western Blot Analysis of HSL—Western blot analysis with supernatants from the five parts of small intestinal mucosa was performed and developed with specific antibodies against rat HSL. A protein with similar apparent molecular mass (82 kDa) as the adipocyte murine HSL was detected in the different intestinal parts (Fig. 2). A lower molecular band that may correspond to a proteolytic fragment was present in intestinal samples.

Immunocytochemistry Analysis of HSL—Immunocytochemistry experiments were performed on jejunal (Fig. 3, A and B) and ileal (Fig. 3, C and D) sections. HSL protein was detected in the differentiated cells of the villi and was absent in the undifferentiated cells of the crypt.

Enzyme Activity Assays—Cholesterol esterase activity was measured with cholesterol oleate. Using a diacylglycerol analog in which only the first ester bond can be hydrolyzed, diacylglycerol lipase activity can be determined without simultaneously measuring monoaacylglycerol lipase activity. Enzymatic activity determination on intestinal musosa cytosolic fractions indicated the presence of a cholesterol esterase and a lipase along the small intestine (Fig. 4). Cholesterol esterase and diacylglycerol lipase activities were 9- and 4-fold lower in jejunum than in white adipose tissue, respectively. Diacylglycerol lipase activity was inhibited using 100 μM diethyl-p-nitrophenyl phosphate by 75 ± 3% (range from 68.2 to 82.5) in all intestinal segments and by 94 ± 1% in white adipose tissue.

Analysis of HSL-null Mice Intestine—In vitro enzymatic assays were realized in HSL-null mice intestine and compared with those from wild-type littermates. Cholesterol esterase activity was totally abolished in the cytosolic fractions of duodenum, jejunum, and ileum of HSL-null mice (Fig. 5A). Although diacylglycerol lipase activity was unchanged in the duodenum of HSL-null mice, it was significantly reduced in jejunum (Fig. 5B). Enzymatic assays were also performed in whole cell homogenates. There was almost no cholesterol esterase activity in the various parts of HSL-null mouse small intestine (Fig. 6A). Diacylglycerol lipase activity was decreased in jejunum (Fig. 6B). Total esterase activity was not modified by the lack of HSL (Fig. 6C). As shown in Fig. 7, Western blot analysis performed on HSL-null mouse intestine showed complete disappearance of the 82-kDa protein. A higher molecular mass band was detected in wild-type mice. It may correspond to the 89 kDa band observed in some rat tissues expressing HSL (Fig. 24).
Analysis of the 5'-Ends of Intestinal HSL mRNA—Two forms of HSL transcripts have been characterized in the adenocarcinoma cell line HT29 (13). The 5'-ends of the two forms are transcribed either from exon B or from exon A. In an attempt to characterize intestinal HSL transcripts, different primers were used in RT-PCR (Table I) with mRNA from intestinal mucosa. As expected, the use of primers in exon 1 led to an amplification of a 206-bp PCR product in adipose tissue and intestine (Fig. 8). Using different antisense primers in exon 1 with sense primers designed either in exon A or in exon B, we could detect the different PCR products with the expected size in intestine. These results suggest that two HSL mRNA with mutually exclusive 5'-ends coexist in enterocytes. The relative abundance of exon A- and exon B-containing transcripts was determined using quantitative RT-PCR on adipose tissue and intestinal mucosa total RNA (n = 4). The ratio of exon B to exon A transcripts was 4.4 ± 0.2 in the adipose tissue and 1.1 ± 0.1 in the intestine. The data reveal that exons A and B are used equally in the enterocytes.
Hormone-sensitive Lipase Expression in Small Intestine

Lipid processing through the intestine is a complex pathway with multiple control steps. The intestine is unable to transport neutral lipids into the lymph at the rate with which they are absorbed, especially at high input rates. Nearly half of the triacylglycerol mass infused into rat intestine does not appear in the lymph (29). It is unlikely that the lipids are oxidized because β-oxidation of lipid entering the mucosa from the lumen is limited. There is no evidence that triacylglycerols are transported via the portal vein (30). These studies suggest that some triacylglycerols in the enterocyte are undergoing hydrolysis. In support of this concept, a mucosal triacylglycerol pool distinct from the chylomicron triacylglycerol precursor pool has been characterized (31). Lipolysis of the mucosal pool has been shown both in vitro and in vivo. Both acidic and alkaline lipase activities have been described in the mucosa (20, 32). Because most of the lipolytic activity was found at neutral or basic pH, the physiological importance of the acidic lipase is unclear. Here, we confirm that significant neutral lipase activity is found in the enterocyte. This activity was inhibited by diethyl-p-nitrophenyl phosphate as shown previously for mucosal lipolysis in triolein-infused rats (31). Lipase activity was found in the different parts of the small intestine. Data from HSL-null mice show that HSL contributes to lipase activity in the distal section but not in the first part of the small intestine. Recently, Mansbach and colleagues (19) showed that pancreatic lipase was expressed in the intestine with most of the enzyme detected in the first quarter (19). Altogether, the data suggest that the hydrolysis of mucosal triacylglycerols is caused by pancreatic lipase in the proximal part of the small intestine and HSL in the more distal parts.

The nature of the enzyme responsible for the hydrolysis of cholesteryl esters in the intestine has remained unclear. Pancreatic cholesteryl esterase (bile salt-stimulated lipase) is internalized upon binding to the surface of enterocytes (33). The esterase could hydrolyze intracellular cholesteryl esters or conversely participate, at acid pH, in the esterification of cholesterol (17, 34). However, the intracellular esterase activity in the absence of cofactors such as bile salts may be very low. Contribution to cholesterol esterification is also unlikely because studies on knockout mice revealed that the enzyme is responsible for mediating intestinal absorption of cholesteryl esters but does not influence free cholesterol absorption (35). In contrast, acyl-CoA:cholesterol acyltransferase 2-deficient mice are resistant to diet-induced hypercholesterolemia (36). Localization of pancreatic cholesteryl esterase in intestinal epithelium may therefore not be related to intracellular metabolism. There is evidence that the enzyme, via an apical-to-basolateral transcytotic pathway, is released at the basolateral membrane level and may contribute to serum pancreatic cholesterol esterase activity (37). Our data reveal that HSL and not pancreatic cholesterol esterase accounts for neutral cholesterol esterase activity in the small intestine.

The expression of HSL in the enterocytes may open new paths in our understanding of cholesterol intestinal absorption and metabolism. HSL-mediated hydrolysis of the intracellular pool of cholesteryl esters may contribute together with the esterification process mediated by acyl-CoA:cholesterol acyltransferase-2 and cholesterol transport mediated by ATP-binding cassette (ABC) transporters to the control of cholesterol homeostasis. Several transporters are expressed in the intestinal epithelium. ABCA1 is expressed in the small intestine and may modulate cholesterol absorption. However, data from ABCA1-deficient mice are conflicting (38, 39). Studies in patients with sitosterolemia (40, 41) and in transgenic mice over-expressing ABCG5 and ABCG8 (42) suggest that the half-transporters participate in cholesteryl efflux. Hydrolysis of
cholesteryl esters by HSL may produce free cholesterol for export through ABC transporters into the lumen. Because of the unique properties of HSL, the present work paves the way for future studies on lipid metabolism in the enterocyte.

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REFERENCES

1. Holm, C., Østergaard, T., Laurell, H., and Contreas, J.-A. (2000) Annu. Rev. Nutr. 20, 365–393
2. Langin, D., and Holm, C. (1993) Trends Biochem. Sci. 18, 466–467
3. Hemila, H., Koivula, T. T., and Palva, I. (1994) Biochim. Biophys. Acta 1210, 249–253
4. Contreas, J.-A., Karlsson, M., Østergaard, T., Laurell, H., Svensson, A., and Holm, C. (1996) J. Biol. Chem. 271, 31428–31430
5. Østergaard, T., Danielsson, B., Degerman, E., Contreas, J.-A., Edgren, G., Davis, R. C., Schatz, M. C., and Holm, C. (1996) Biochem. J. 319, 411–420
6. Shen, W. J., Sridhar, K., Bernlohr, D. A., and Kraemer, F. B. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 5528–5532
7. Shen, W. J., Liang, Y., Hong, R., Patel, S., Natu, V., Sridhar, K., Jenkins, A., Bernlohr, D. A., and Kraemer, F. B. (2001) J. Biol. Chem. 276, 49443–49448
8. Anthonisen, M. W., Rönström, L., Wernstedt, C., Degerman, E., and Holm, C. (1998) J. Biol. Chem. 273, 215–221
9. Greenberg, A. S., Shen, W. J., Muliro, K., Patel, S., Roth, R. A., and Kraemer, F. B. (2001) J. Biol. Chem. 276, 45456–45461
10. Garton, A. J., Campbell, D. G., Carling, D., Hardie, D. G., Colbran, R. J., and Yeaman, S. J. (1989) Eur. J. Biochem. 179, 249–254
11. Wei, Y., Contreas, J.-A., Sheffield, P., Østergaard, T., Derewenda, U., Kneusel, R. E., Matern, U., Holm, C., and Derewenda, S. (1999) Nature Struct. Biol. 6, 340–345
12. Langin, D., Laurell, H., Holst, L. S., Belfrage, P., and Holm, C. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 4897–4901
13. Grober, J., Laurell, H., Blaise, R., Fabry, B., Schaak, S., Holm, C., and Langin, D. (1997) Biochem. J. 328, 453–461
14. Stenson Holst, L., Langin, D., Mulder, H., Laurell, H., Grober, J., Bergh, A., Mohrenweiser, H. W., Edgren, G., and Holm, C. (1996) Genomics 35, 441–447
15. Mairal, A., Melaine, N., Laurell, H., Grober, J., Stenson Holst, L., Guillaudeux, T., Holm, C., Jegou, B., and Langin, D. (2002) Biochem. Biophys. Res. Commun. 291, 286–290
16. Shen, H., Howles, P., and Tao, P. (2001) Adv. Drug Delivery Rev. 50, S123–S125
17. Field, F. J. (1984) J. Lipid Res. 25, 389–399
18. Dolinsky, V. W., Sipione, S., Lehner, R., and Vance, D. E. (2001) Biochim. Biophys. Acta 1532, 162–172
19. Mahan, J. T., Hada, G. D., Rao, R. H., and Mansbach, C. M., II (2001) Am. J. Physiol. 280, G1187–G1196
20. Rao, R. H., and Mansbach, C. M., II (1993) Arch. Biochem. Biophys. 304, 483–489
21. Holm, C., Oliveira, G., and Ottosson, M. (2001) Methods Mol. Biol. 155, 97–119
22. Chomczynski, P., and Sacchi, N. (1987) Anal. Biochem. 162, 156–159
23. Tals, J., Brakebusch, C., and Passer, H. (1999) Methods Mol. Biol. 129, 153–187
24. Holm, C., Belfrage, P., and Fredriksson, G. (1987) Biochem. Biophys. Res. Commun. 148, 99–105
25. Laurin, N. N., Wang, S. P., and Mitchell, G. A. (2000) Mamm. Genome 11, 972–978
26. Remaury, A., Laurell, H., Grober, J., Reynisdottir, S., Dauzats, M., Holm, C., and Langin, D. (1995) Biochem. Biophys. Res. Commun. 207, 175–182
27. Blaise, R., Guillaudeux, T., Tavernier, G., Daegelen, D., Kervad, B., Mairal, A., Holm, C., Jegou, B., and Langin, D. (2001) J. Biol. Chem. 276, S109–S115
28. Smith, P., Rouet, P., Lucas, S., Mairal, A., Sengenes, C., Lafontan, M., Vaulont, S., Casado, M., and Langin, D. (2002) Diabetes 51, 293–300
29. Mansbach, C. M., II, Arnold, A., and Cox, M. A. (1985) Am. J. Physiol. 249, G642–G648
30. Sabeau, S. M., and Frase, S. (1977) J. Lipid Res. 18, 496–511
31. Tipton, A. D., Frase, S., and Mansbach, C. M. (1989) Am. J. Physiol. 257, G871–G878
32. Rao, B. H., and Mansbach, C. M., II (1990) Biochim. Biophys. Acta 1043, 273–280
33. Bruneau, N., Lombards, D., and Bendayan, M. (1998) J. Cell Sci. 111, 2665–2679
34. Ponz de Leon, M., Carrubi, F., Di Donato, P., and Carulli, N. (1985) Digest. Dis. Sci. 30, 1053–1064
35. Howles, P. N., Carter, C. P., and Hui, D. Y. (1996) J. Biol. Chem. 271, 7196–7202
36. Buhman, K. K., Accad, M., Novak, S. T., Choi, R. S., Wong, J. S., Hamilton, R. L., Turley, S., and Farese, R. V. (2000) Nature 406, 1341–1347
37. Bruneau, N., Nganga, A., Bendayan, M., and Lombards, D. (2001) Exp. Cell Res. 271, 94–108
38. McNesih, J., Aiello, R. J., Guyot, S., Turi, T., Gabel, C., Aldinger, C., Hoppe, K. L., Roach, M. L., Boyer, J. L., de Wet, J., Broccardo, C., Chimini, G., and Francone, O. L. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 4245–4250
39. Drobnik, W., Lindenthal, B., Lischer, R., Ritter, M., Christiansen, Weber, T., Liebich, G., Giesa, U., Igel, M., Brunska, H., Buchler, C., Fung-Leung, W. P., Von Bergmann, K., and Schmitz, G. (2001) Gastroenterology 120, 1203–1211
40. Berge, K. E., Tian, H., Graf, G. A., Yu, L., Grishin, N. V., Schultz, J., Kuziemko, F., Poon, B., Barns, R., and Hobbs, H. (2000) Science 290, 1771–1775
41. Lee, M. H., Lu, K., Hazard, S., Yu, H., Shen, S., Hidalgo, H., Kojima, H., Allikmets, R., Sakuma, N., Pegoraro, R., Srivastava, A. K., Salen, G., Dean, M., and Matsui, C. (2000) Nature Genet. 27, 79–83
42. Yu, L., Li-Hawksins, J., Hammer, R. E., Berge, K. E., Horton, J. D., Cohen, J. C., and Hobbs, H. H. (2002) J. Clin. Invest. 110, 671–680
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