Selective Proteolytic Processing of Rat Hepatic Sterol Regulatory Element Binding Protein-1 (SREBP-1) and SREBP-2 During Postnatal Development*

Daniela Botolin and Donald B. Jump‡

From the Departments of Physiology, Biochemistry, and Molecular Biology, Michigan State University, East Lansing, Michigan 48824

Sterol regulatory element-binding protein-1c (SREBP-1c) plays a major role in hepatic lipogenic gene expression. In adult animals, insulin and oxysterols induce SREBP-1c gene transcription, whereas polyunsaturated fatty acids suppress the nuclear content of SREBP-1c through pre-translational regulatory mechanisms. A decline in nuclear SREBP-1c is associated with suppression of hepatic lipogenesis. In contrast to adult rats, hepatic lipogenesis in preweaned neonatal rats is low. Ingestion of milk fat by the neonate may contribute to low hepatic lipogenesis. In this report, we tested the hypothesis that low lipogenic gene expression prior to weaning correlates with low mRNAsREBP-1c, as well as low precursor and nuclear forms of SREBP-1. In contrast to expectations, levels of mRNAsREBP-1c and the 125-kDa SREBP-1 precursor in livers of preweaned rats was comparable with adult levels. Despite high levels of SREBP-1 precursor, mature (65 kDa) SREBP-1 was not detected in rat liver nuclei prior to 18 days postpartum. Weaning rats at 21 days postpartum was accompanied by a rise in nuclear SREBP-1 levels as well as increased lipogenic gene expression. In contrast, SREBP-2 was present in rat liver nuclei, and its target gene, HMG-CoA reductase, was expressed above adult levels prior to weaning. These studies indicate that, prior to weaning, SREBP-2 but not SREBP-1 is proteolytically processed to the mature form. As such, SREBP-2-regulated genes are active, whereas dietary polyunsaturated fatty acids suppress the nuclear content of SREBP-1c (12–14). Clarke and co-workers (20) have reported that the principal mechanism for suppressing SREBP-1c but not of SREBP-2 are suppressed by PUFAs (18–20). Clarke and co-workers (20) have reported that the principal mechanism for suppressing SREBP-1c but not of SREBP-2 are suppressed by PUFAs (18–20). Clarke and co-workers (20) have reported that the principal mechanism for suppressing SREBP-1c but not of SREBP-2 are suppressed by PUFAs (18–20). Clarke and co-workers (20) have reported that the principal mechanism for suppressing SREBP-1c but not of SREBP-2 are suppressed by PUFAs (18–20). Clarke and co-workers (20) have reported that the principal mechanism for suppressing SREBP-1c but not of SREBP-2 are suppressed by PUFAs (18–20). Clarke and co-workers (20) have reported that the principal mechanism for suppressing SREBP-1c but not of SREBP-2 are suppressed by PUFAs (18–20). Clarke and co-workers (20) have reported that the principal mechanism for suppressing SREBP-1c but not of SREBP-2 are suppressed by PUFAs (18–20). Clarke and co-workers (20) have reported that the principal mechanism for suppressing SREBP-1c but not of SREBP-2 are suppressed by PUFAs (18–20).
mary hepatocytes or in vivo eliminates the PUFA effects on several lipogenic genes, indicating that SREBP-1c is a key target for PUFA suppression of de novo lipogenesis (19, 21).

During postnatal development of rats and mice, hepatic lipogenesis is low prior to weaning at 21 days postpartum (22–29). The activities of key enzymes, as well as their mRNAs, are very low during the suckling phase. Where examined, low lipogenic gene expression is due to low transcription rates (24). Based on studies with adult animals (2), low lipogenic gene transcription in newborns has been attributed to the ingestion of a high fat milk diet (26).

Because PUFA controls nuclear SREBP-1c levels through a pre-translational regulatory mechanism (18–20), we were interested in determining whether low hepatic lipogenic gene expression in preweaned animals correlates with low mRNA_{SREBP-1c}. Contrary to expectations, our studies indicated that both mRNA_{SREBP-1} and pSREBP-1c are well expressed in rat liver prior to weaning. Only nSREBP-1c levels are low in neonatal liver, reflecting abrogated maturation of pSREBP-1 to nSREBP-1.

**MATERIALS AND METHODS**

**Animals**—Female Sprague-Dawley rats with litters were obtained from Charles River Laboratories (Kalamazoo, MI) and maintained on a Tek-Lad chow diet, ad libitum. Male rats at 15, 18, and 21 days postpartum were used for this analysis and compared with adult male rats (≥30 days postpartum).

**Cell Extracts and Western Blotting**—Extracts of rat liver were prepared by homogenizing tissue in Buffer A (0.25 M sucrose, 10 mM Tris-Cl, pH 7.5, and 3 mM MgCl₂ plus the protease inhibitors phenylmethylsulfonyl fluoride (0.1 mM), pepstatin (5 μg/ml), leupeptin (5 μg/ml), and aprotinin (2 μg/ml)). The homogenate was centrifuged (100,000 × g, 1 h) and homogenized. The pellet from the first centrifugation was resuspended in Buffer A, adjusted to 1% Nonidet P-40, and homogenized. The homogenate was centrifuged (300 × g for 5 min at 2 °C). The supernatant was then centrifuged (100,000 × g for 1 h at 4 °C) to obtain microsomes. The pellet from the first centrifugation was resuspended in Buffer B (50 mM Hepes, pH 7.4, 0.1 M KCl, 3 mM MgCl₂, 1 mM EDTA, and 10% glycerol plus the protease inhibitors phenylmethylsulfonyl fluoride (0.1 mM), pepstatin(0.1 mM), pepstatin (5 μg/ml), leupeptin (5 μg/ml), and aprotinin (2 μg/ml), adjusted to 0.4 M ammonium sulfate, and centrifuged at 100,000 × g for 60 min. The supernatant was used for analysis of nuclear proteins.

Proteins (50–100 μg) were separated electrophoretically by SDS-polyacrylamide gel electrophoresis (NuPAGE 4–10% polyacrylamide Bis-Tris, Invitrogen) and transferred to nitrocellulose membranes. Membranes were incubated with antibodies for SREBP-1 (IgG-2A4) or HNF-4 (Santa Cruz Biotechnology). The anti-mouse antibodies were detected using a horseradish peroxidase-conjugated second antibody and 3,3′-diaminobenzidine. The films were developed using the ImageQuant LAS4000 equipment (GE Healthcare).

**RT-PCR Analysis**—Total liver RNA from 15-, 18-, 21-, and 30-day-old rats was used as template. Amplified DNAs were separated electrophoretically and visualized by ethidium bromide staining.

**RESULTS AND DISCUSSION**

**Post-translational Processing of SREBP-1c Regulates Nuclear SREBP-1c Levels during Postnatal Development**—In this report, we tested the hypothesis that low levels of hepatic lipogenesis prior to weaning correlate with low mRNA_{SREBP-1c}, which leads to a decline in pSREBP-1 and nSREBP-1. Levels of mRNA encoding SREBP-1 and the lipogenesis-associated protein S14 were measured in 15- and 42-day-old rats and 42-day old rats, respectively, fed a high carbohydrate-fat-free diet for 5 days (Fig. 1). S14 was used because SREBP-1c induces S14 gene transcription (19, 31). The high carbohydrate-fat-free diet was used to illustrate the effect of a fat-free diet on hepatic SREBP-1 and S14 mRNA levels. Hepatic mRNA_{S14} is essentially absent at 15 days postpartum (Fig. 1). At this age, S14 gene transcription is not detectable (24). In contrast to mRNA_{S14}, hepatic mRNA_{SREBP-1c} in 15 day old rats is ~1.5-fold higher than that seen in 42-day-old chow-fed rats and is comparable with the level seen in rats fed a high carbohydrate-fat-free diet for 5 days. RT-PCR analysis using SREBP-1a- and SREBP-1c-specific primers indicated that SREBP-1c represents ~90% of the SREBP-1 expressed in rat liver at 15 days postpartum (not shown). The ratio of SREBP-1c to SREBP-1a was not different at 15 days postpartum and in adults. Thus, SREBP-1c is the predominant SREBP-1 transcript expressed in neonatal and adult liver.

Insulin-mediated induction of S14 and SREBP-1 gene transcription accounts for the elevated levels of S14 and SREBP-1c mRNAs in livers of adult animals fed high carbohydrate-fat-free diets (12–14, 31, 32). However, high SREBP-1c mRNA levels prior to weaning cannot be ascribed to elevated insulin, because blood insulin levels are typically low, and the liver displays elevated ketogenesis (26, 28, 29). Thus, factors controlling hepatic SREBP-1c mRNA levels in the suckling animal differ from that seen in the adult rat.

Because low lipogenic gene expression cannot be explained by pre-translational suppression of mRNAs_{SREBP-1c}, we examined hepatic precursor and nuclear SREBP-1 and SREBP-2...
levels in 15- and 30-day-old rats (Fig. 2). Precursor levels of SREBP-1 and SREBP-2 were 1.5- and 2-fold higher in livers of 15-day-old rats when compared with adults. As a control, the microsomal monoxygenase CYP4A was 2-fold higher in livers derived from 30-day-old animals than in those from 15-day-old animals. Although nSREBP-2 was present in nuclear extracts obtained from both 15- and 30-day-old animals, nSREBP-1 was not detected in hepatic nuclei isolated from 15-day-old rats. However, nSREBP-1 was present in nuclear extracts from 30-day-old animals. HNF-4a levels were 2-fold higher in 30- versus 15-day-old rats.

These studies indicate that SREBP-2 matures to the nuclear form, but SREBP-1 maturation is abrogated, leading to little or no nSREBP-1 accumulation in hepatic nuclei of 15-day-old rats. The absence of nSREBP-1c in nuclei correlates with low hepatic de novo lipogenesis in the neonate. Thus, the principal mechanism accounting for low lipogenic gene expression in hepatic nuclei prior to weaning is due, at least in part, to low nSREBP-1 levels. The absence of nSREBP-1 but not low mRNASREBP-1 and pSREBP-1. The mechanism controlling hepatic nSREBP-1 levels in neonatal and adult liver is clearly different.

Developmental Regulation of SREBP-1c Maturation Correlates with the Induction of Hepatic Lipogenic Gene Expression—Lipogenic gene expression, i.e. S14 and FAS, increases dramatically when rats are weaned (22–25). To determine whether SREBP-1 maturation followed this same time line, we examined SREBP-1 protein levels in hepatic microsomes and nuclei of animals at 15, 18, 22, and 30 days postpartum. Although these animals are normally weaned at 21 days of age, they begin to ingest solid food between 18 and 21 days postpartum. Microsomal pSREBP-1 remained unchanged over the 15-, 18-, 22-, and 30-day-old period (Fig. 3), a finding that correlates with the modest changes in mRNASREBP-1c in 15-day-old and adult animals (Fig. 1). Hepatic nuclear nSREBP-1 was not detected at 15 days postpartum (Figs. 2 and 3) but was detected at 18 days postpartum. By 22 days of age, nSREBP-1 levels are comparable with adult levels. The maturation of SREBP-1c parallels the dietary switch from a high fat milk diet to a control chow diet.

To determine whether the change in nSREBP-1 correlated with the onset of lipogenic gene expression, we measured the expression of three SREBP-1 target genes (S14, FAS, and GPAT) as well as the SREBP-2 regulated gene HMG-CoA reductase (Fig. 4A). The mRNAs encoding S14 and FAS increased progressively from very low levels at 15 days of age to high levels at 30 days of age. These changes correlated with increased nSREBP-1 levels (Fig. 3). In contrast, glycerophos-
phate acyl transferase (GPAT) mRNA levels were above adult levels at 15–22 days postpartum. Unlike de novo lipogenesis, GPAT is required for the synthesis of phospholipids as well as triglycerides. The growing liver likely requires GPAT expression throughout all phases of development. Expression of the SREBP-2-regulated transcript, HMG-CoA reductase, was also above adult values during the 15–22 day old period, a finding consistent with abundant nSREBP-2 (Fig. 2) and elevated cholesterol synthesis in neonatal liver (28).

The mRNA encoding mtHMG-CoA synthase, a PPARα-regulated gene, is high prior to weaning (Fig. 4B). High levels of mtHMG-CoA synthase are consistent with elevated ketogenesis associated with suckling rats (26, 28). In contrast, another PPARα-regulated gene, CYP4A, is low prior to weaning. This apparent paradox is explained by the fact that low blood levels of insulin prior to weaning promote ketogenesis (26, 28, 29). Long-chain PUFAs are PPARα ligands and induce CYP4A gene transcription (33, 34). Milk fats are enriched in short to medium chain saturated fatty acids. Based on structural studies, these fatty acids are likely not good ligands for PPARα or PPARγ (76). Saturated fatty acids. Based on structural studies, these fatty acids are likely not good ligands for PPARα or PPARγ (76).

Selective Proteolytic Processing of SREBP-1 and SREBP-2

Clearly, more studies will be required to establish the roles sphingolipid metabolism, INSIG-1, and INSIG-2 play in the control of hepatic SREBP-1 and SREBP-2 proteolytic processing. We anticipate that definition of the molecular basis of differential processing of SREBP-1 and SREBP-2 in neonatal rat liver will provide important clues to selective control hepatic cholesterol and fatty acid synthesis.

Acknowledgments—We thank Drs. Karl Olson and Julia Busik for critical reading of the manuscript. We gratefully acknowledge Drs. Coleman, Hobbs, and Russell for the generous gifts of cDNAs used in these studies. We thank Tim Osborne for helpful discussions.

REFERENCES

1. Hillgartner, F. B., Salati, L. M., and Goodridge, A. G. (1995) Physiol. Rev. 75, 47–76
2. Jump, D. B., and Clarke, S. D. (1999) Am. J. Physiol. 278, C2043–C2053
3. Osborne, T. J. (2000) J. Biol. Chem. 275, 32579–32582
4. Horton, J. D., Golstein, J. L., and Brown, M. S. (2002) J. Clin. Invest. 109, 1125–1131
5. Brown, M. S., and Goldstein, J. L. (1997) Cell 88, 311–340
6. Matsuda, M., Korn, B. S., Hammer, R. E., Moon, Y-A., Komuro, R., Horton, J. D., Golstein, J. L., Brown, M. S., and Shimoni, M. (2001) J. Biol. Chem. 276, 1500–1506
7. Brown, M. S., and Goldstein, J. L. (1997) Cell 88, 311–340
8. Janowski, B. A. (2000) Mol. Cell. Biol. 73, 6571–6579
9. Wang, S., Thoolen, M., Mangelsdorf, D. J., Lustig, K. D., and Shan, B. (2000) Genes Dev. 14, 2321–2330
10. Repa, J. J., Liang, G., Ou, J., Bashmakov, Y., Lohascar, J. M., Shimoni, M., Shan, B., Brown, M. S., Goldstein, J. L., and Mangeseldorff, D. J. (2000) Genes Dev. 14, 2321–2330
11. Janovsky, B. A. (2002) J. Clin. Invest. 109, 2675–2680
12. DeRose-Boyd, R. A., Ou, J., Golstein, J. L., and Brown, M. S. (2001) Proc. Natl. Acad. Sci. U.S.A. 99, 1477–1482
13. Azzort-Marniche, D., Becard, C., Guichard, C., Foretz, M., Perre, F., and Foufelle, F. (2000) Biochem. J. 356, 389–393
14. Foretz, M., Guichard, C., Perre, F., and Foufelle, F. (1999) Proc. Natl. Acad. Sci. U.S.A. 96, 12773–12778
15. Liang, G., Yang, J., Horton, J. D., Brown, M. S., and Goldstein, J. L. (2001) J. Biol. Chem. 276, 5620–5628
16. Tobin, K. A. R., Ulven, S. M., Schuster, G. U., Steinegger, H. H., Andresen, S. M. Gustafson, A. J., and Neeb, H. I. (2002) J. Biol. Chem. 277, 15691–15697
17. Wargull, T. S., Sturley, S. L., Seo, T., Osborne, T. P., and Deckelbaum, R. J. (1998) J. Biol. Chem. 273, 25537–25540
18. Wang, S., Yi, M., Korn, B. S., Horton, J. D., Goldstein, J. L., Brown, M. S., and Shibuya, H. (2000) Proc. Natl. Acad. Sci. U.S.A. 97, 27839–27844
19. Mates, M. K., Thelen, A. P., Pan, D. A., and Jump, D. B. (1999) J. Biol. Chem. 274, 32725–32732
20. Xu, J., Nakamura, M. T., Cho, H. P., and Clarke, S. D. (1999) J. Biol. Chem. 274, 32725–32732
21. Mayer, M. K., Thelen, A. P., Pan, D. A., and Jump, D. B. (1999) J. Biol. Chem. 274, 32725–32732
22. Xu, J., Teran-Garcia, M., Park, J. H., Nakamura, M. T., and Jump, D. B. (1999) J. Biol. Chem. 274, 32725–32732
23. Jump, D. B., Tao, T.-Y., Towle, C., and Oppenheimer, J. H. (1986) J. Biol. Chem. 261, 23583–23584
24. Jump, D. B., and Oppenheimer, J. H. (1985) Endocrinology 117, 2259–2266
25. Yokoyama, T., Yuan, Y., Hamada, H., Mano, Y., Nakamura, H., and Ishibashi, S. (1999) J. Biol. Chem. 274, 35840–35844
26. Jump, D. B., Tan, Y.-T., Towle, C., and Oppenheimer, J. H. (1986) Endocrinology 117, 2259–2266
27. Jump, D. B., Veit, A., Santiago, V., Lepar, G., and Herberholz, L. (1988) J. Biol. Chem. 263, 7254–7260
28. Jump, D. B., Armstrong, M. K., and Jump, D. B. (1990) J. Biol. Chem. 265, 9222–9228
29. Pezet, J., Prieur-Bausse, C., Duse, P. H., and Girard, J. (1997) Diabetes Metab. 16, 156–160
30. Perez-Castillo, A., Schwartz, H. L., and Oppenheimer, J. H. (1987) Endocrinology 214, 300–307
31. Hatai, K., Matsuda, M., Korn, B. S., Horton, J. D., Goldstein, J. L., and Brown, M. S. (1996) J. Clin. Invest. 98, 838–845
32. Jump, D. B., Thelen, A. P., and Mayer, M. K. (2001) J. Biol. Chem. 276, 34419–34427
33. Jump, D. B., Bell, A., Lepar, G., and Hu, D. (1990) Mol. Endocrinol. 4, 1655–1660
34. Xu, H., Lamberts, M. H., Montana, V. G., Parks, D. J., Blanchard, S. G., Brown, P. J., Sernbach, D. B., Lehmann, J. M., Wisely, G. B., Wilson, T. M., Kliever, S. A., and Milburn, M. V. (1999) Mol. Endocrinol. 13, 397–403
35. Pan, D. A., Mayer, M. K., Thelen, A. P., Peters, J. M., Gonzalez, F. J., and Jump, D. B. (2001) J. Lipid Res. 42, 742–751
36. Lu, T. T., Repa, J. J., and Mangeseldorff, D. J. (2001) J. Biol. Chem. 276, 37735–37738
37. Diamond, R. H., Du, K., Lee, Y. M., Mohn, K. L., Haber, B. A., Tewari, D. S., and Taub, R. (1993) J. Biol. Chem. 268, 15185–15192
38. Yang, T., Espernshede, P. J., Wright, M. E., Yabe, D., Gong, Y., Aebbersold, R., Goldstein, J. L., and Brown, M. S. (2002) Cell 110, 489–500
39. Yabe, D., Brown, M. S., and Goldstein, J. L. (2002) Proc. Natl. Acad. Sci. U.S.A. 99, 12753–12758