Effect of retinoids on nuclear retinoic acid receptors mRNA in adipose tissue of retinol-deficient rats

Riaz-ul-Haq and Frank Chytil[1]

Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN 37232

Abstract In this study, we examined effects of retinol deficiency and three retinoids, all-*trans*-retinoic acid, 13-*cis*-retinoic acid, and etretin, the aromatic derivative of retinoic acid, on nuclear retinoic acid receptor isoforms α, β, and γ mRNA in rat adipose tissue. Retinol deficiency caused an almost 50% decrease in isoform β mRNA levels of adipose tissue, whereas little change occurred in the abundance of α and γ isoforms transcripts in this tissue. Intragastric administration of all-*trans*-retinoic acid to retinol-deficient rats increased the adipose tissue retinoic acid receptor isoforms β and γ mRNA levels sixfold and twofold, respectively, in 4 h as compared to adipose tissue of retinol-deficient rats that were administered cottonseed oil. In contrast to this, 13-*cis*-retinoic acid and etretin at equimolar doses were not effective in inducing β or γ isoforms mRNA in retinol-deficient rats. These results show that adipose tissue isoform β responds to retinol deficiency, and all-*trans*-retinoic acid rapidly induces β and γ mRNA isoforms in this tissue. Thus, retinoic acid may regulate expression of specific genes through its interaction with retinoic acid receptors in adipocytes.—**Haq, R. U., and F. Chytil.** Effect of retinoids on nuclear retinoic acid receptors mRNA in adipose tissue of retinol-deficient rats. *J. Lipid Res.* 1992. **33:** 381–384.

Supplementary key words α, β, γ isoforms • all-*trans*-retinoic acid • 13-*cis*-retinoic acid • etretin

Although the effects of vitamin A on lipid metabolism were described several decades ago, the molecular mechanisms involved in this process remain to be elucidated (1). More recently, retinoic acid (RA), the product of oxidation of retinol (vitamin A), has been shown to induce differentiation of many cells (for reviews, see 2–4). In contrast, in vitro differentiation of preadipocytes to adipocytes is inhibited by RA (5–8). This inhibition is accompanied by a decrease in activity of lipogenic enzymes and in levels of fatty acid-binding protein (9–11). It has been established that the molecular mechanism by which RA exerts its action involves its interaction with nuclear retinoic acid receptors (RARs) which in turn regulate expression of specific genes (12, 13). Three main RAR isoforms, α, β, and γ, have been characterized (14–16).

In a preliminary study we reported that rat adipose tissue contains all three isoforms of RARs (17). This suggested for the first time that differentiation of adipocytes may be regulated by RA in the whole animal. In order to define whether or not RA action in adipose tissue is mediated by RARs, we examined first whether vitamin A status affects levels of mRNA for these receptors and then whether intragastric administration of RA, 13-*cis*-RA, and etretin, a synthetic derivative of RA, rapidly influence expression of genes coding for RARs.

MATERIALS AND METHODS

Male Sprague-Dawley rats (21 days old from Sasco) were made retinol-deficient as described previously (18). Serum retinol levels of deficient rats were 0.7–2.7 μg/100 ml and those of retinol-sufficient rats were 32–45 μg/100 ml as determined by a fluorometric method (19). Retinol-deficient rats were randomly divided into four groups of three rats per group. Each group was fed by stomach intubation either 0.2 ml cottonseed oil or cottonseed oil with 333 nmol (0.3 μg/g/BW) all-*trans*-RA, 13-*cis*-RA (Sigma Chemical Company), or etretin (Ro-10-1670), an aromatic derivative of RA (a generous gift from Dr. S. Shapiro of Hoffmann-La Roche). Control retinol-sufficient rats were also fed 0.2 ml cottonseed oil. Animals were killed by decapitation after 4 h and epididymal fat pads were excised for RNA preparation.

RAR probes

RAR specific probes were obtained from cDNA clones for RAR-α, β, and γ as described (20). All fragments used as probes spanned the ligand/carboxy ter-

Abbreviations: RA, retinoic acid; RAR, retinoic acid receptor.

[1]To whom reprint requests should be addressed.

minal domain of RAR-α, β, or γ. The β-actin probe used to normalize RNA levels was a human-β actin cDNA (Clontech). cDNA probes were labeled with [^{32}P]dCTP (3000 Ci/mmol, Dupont) using a random primer extension labeling system (NEN) according to manufacturer instructions.

RNA isolation and Northern analysis

Total RNA was isolated from 0.8–1.0 g of epididymal fat by the guanidine thiocyanate–cesium chloride procedure as described (21). The RNAs (40 μg) were subsequently size-fractioned on a 1% formaldehyde/agarose gel and transferred to Nytran membranes (Schleicher & Schuell) in 10 × SSC (1 × SSC, 0.15 M sodium chloride, 0.015 M sodium citrate). The membranes were then washed briefly in 5 × SSC, air dried, and baked in an oven for 2 h at 80°C. Nytran membranes were prehybridized (4 h) and hybridized (1×10^6 cpm/ml overnight) at 42°C in 50% formamide, 5 × SSPE, (1 × SSPE, 0.15 M NaCl, 0.01 M $Na_2HPO_4 \cdot H_2O$, 0.001 M EDTA, pH 7.4) 0.1% bovine serum albumin, 0.1% polyvinylpyrrolidone, 0.1% Ficoll, 10% dextran sulfate, 1% SDS, and 200 μg/ml sheared salmon sperm DNA. The membranes were washed (four times) in 2 × SSC at room temperature, followed by two 30-min washes in 2 × SSC/0.1% SDS at 65°C. The membranes were then exposed to Kodak XAR-5 film with intensifying screens at –70°C. After 24 or 48 h of exposure, band intensities were quantitated by densitometric scanning.

RESULTS

Total RNA from adipose tissue was subjected to Northern blot analysis with a specific RAR-β cDNA (**Fig. 1**). The same filter was again probed with β-actin cDNA to normalize the relative levels of RAR-β transcripts. Retinol deficiency caused an almost 50% decrease in RAR-β mRNA as compared to its level in retinol-sufficient rats (Fig. 1, lanes 1–3 vs. 12–14). All-*trans*-RA administration to retinol-deficient rats after 4 h led to ca. 6-fold increase in RAR-β mRNA levels (Fig. 1, lanes 4–6 vs. 12–14). This elevation of RAR-β mRNA was 3.5-fold higher than levels in retinol-sufficient rats (Fig. 1, lanes 1–3 vs. 4–6). However, the administration of 13-*cis*-RA (lanes 7–9) and etretin (lanes 10–11), the aromatic derivative of RA, to retinol-deficient rats at equimolar doses failed to induce RAR-β mRNA. In contrast, adipose tissue RAR-α mRNA levels did not respond to all three retinoids tested (data not shown).

Fig. 2 shows the effects of three retinoids on RAR-γ. RAR-γ mRNA levels decreased slightly (17%) in retinol-deficient rats (Fig. 2, lanes 1–3 vs. 12–14). All-*trans*-RA feeding to retinol-deficient rats increased

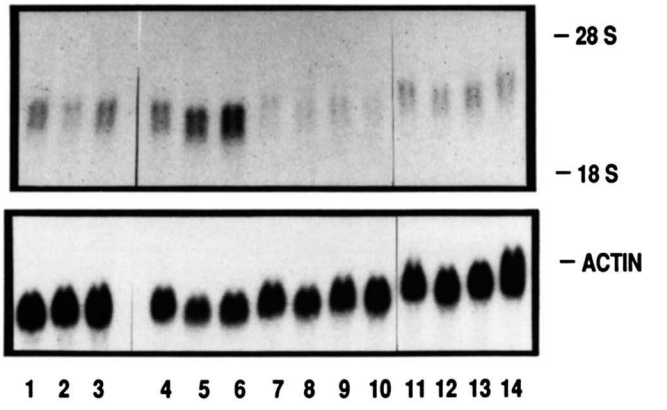

Fig. 1. Effect of retinoids on RAR-β mRNA levels in rat adipose tissue. Each lane contained 40 μg of total RNA from an individual animal. Rats were fed cottonseed oil or different retinoids and killed after 4 h. Retinol-sufficient rats fed cottonseed oil (lanes 1–3), retinol-deficient rats fed all-*trans*-RA (lanes 4–6), 13-*cis*-RA (lanes 7–9), etretin (lanes 10–11), and cottonseed oil (lanes 12–14).

RAR-γ mRNA approximately 2-fold as compared to its level in retinol-sufficient rats (Fig. 2, lanes 1–3 vs. 4–6). On the other hand, at equimolar doses 13-*cis*-RA and etretin were again not as effective as all-*trans*-RA in inducing mRNA levels of RAR-γ (Fig. 2, lanes 7–11).

DISCUSSION

In recent years, there have been efforts to understand the mechanism of adipocyte differentiation and its inhibition by retinoic acid in vitro (5–10). The presence of nuclear RARs in adipose tissue and rapid induction of RARβ genes by all-*trans*-RA reported here

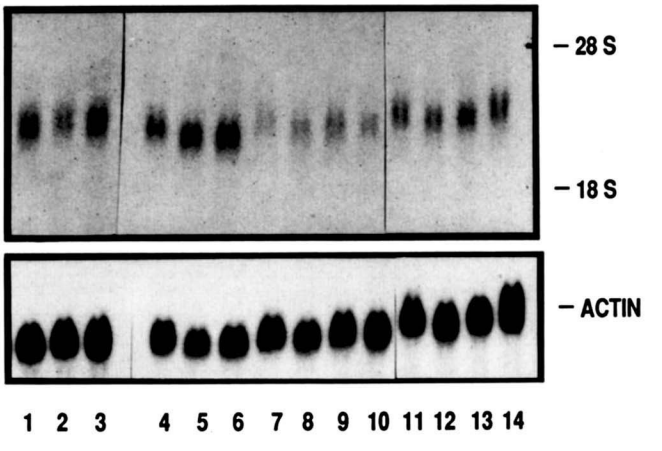

Fig. 2. Effect of retinoids on RAR-γ mRNA levels in rat adipose tissue. Each lane contained 40 μg of total RNA from an individual animal. Rats were fed cottonseed oil or different retinoids and killed after 4 h. Retinol-sufficient rats (lanes 1–3). Retinol-deficient rats fed all-*trans*-RA (lanes 4–6), 13-*cis*-RA (lanes 7–9), etretin (lanes 10–11), and cottonseed oil (lanes 12–14).

demonstrate that among the primary target genes sensitive to RA are its own nuclear receptors. These results are similar to those reported for rat liver and lung (22, 23). It appears that only RAR-β and γ are rapidly induced. However, it remains to be elucidated which genes interact with RARs as well as which specific cells of adipose tissue express the RARs.

The data presented here still strengthen the hypothesis that all-*trans*-RA dependent increase in RARs may be involved in adipocyte differentiation, which is accompanied in vitro by increased expression of C-fos (24). Addition of RA to preadipocytes inhibits this process with concomitant decrease in mRNA levels of C-Fos/Jun-B (11). RAR protein may form a complex with Jun protein, thereby inhibiting the binding of Jun protein to the target DNA (25). Thus, it appears that RA through the induction of RARs levels diminishes the activity of cellular oncogenes which in turn can affect genes involved in differentiation of adipocytes.

In summary, the rapid elevation of expression of genes coding for β and γ isoforms of RARs by all-*trans*-retinoic acid reported here suggest rather strongly that these isoforms and all-*trans*-retinoic acid are involved in regulation of differentiation in adipose cells.

The authors are thankful to Dr. M. Pfahl for the generous gift of the cDNAs for RARs. Thanks are due to M. Hunt for his able technical assistance and S. Heaver for help with the manuscript. This research was supported by NIH grants HD-09195 and HL-14214.

Manuscript received 9 September 1991 and in revised form 12 December 1991.

REFERENCES

1. Roels, O. A. 1967. Biochemical systems. *In* The Vitamins. W. H. Sebrell, Jr. and R. S. Harris, editors. Academic Press, New York, London. Vol. 1: 167–245.
2. Lotan, R. 1980. Effect of vitamin A and its analogs (retinoids) on normal and neoplastic cells. *Biochim. Biophys. Acta.* **605:** 33–91.
3. Roberts, A. B., and M. B. Sporn. 1984. Cellular biology and biochemistry of the retinoids. *In* The Retinoids. M. B. Sporn, A. B. Roberts, and D. S. Goodman, editors. Academic Press, Orlando, FL. Vol. 2: 210–286.
4. Chytil, F., and R. U. Haq. 1990. Vitamin A-mediated gene expression. *In* Critical Reviews in Eukaryotic Gene Expression. G. S. Stein, J. L. Stein, and J. B. Kian, editors. CRC Press, Boca Raton, FL. Vol. 1: 61–73.
5. Sato, M., A. Hiragun, and H. Mitsui. 1980. Preadipocytes possess cellular retinoid binding proteins and their differentiation is inhibited by retinoids. *Biochem. Biophys. Res. Commun.* **95:** 1839–1845.
6. Kuri-Harcuch, W. 1982. Differentiation of 3T3-F442A cells into adipocytes is inhibited by retinoic acid. *Differentiation.* **23:** 164–169.
7. Pairault, J., and F. Lasnier. 1987. Control of adipogenic differentiation of 3T3-F442A cells by retinoic acid, dexamethasone and insulin. A topographic analysis. *J. Cell. Physiol.* **132:** 279–286.
8. Pairault, J., A. Quignard-Boulange, I. Dugail, and F. Lasnier. 1988. Differential effects of retinoic acid upon early and late events in adipose conversion of 3T3 preadipocytes. *Exp. Cell. Res.* **177:** 27–36.
9. Murray, T., and T. R. Russel. 1980. Inhibition of adipose conversion in 3T3-L2 cells by retinoic acid. *J. Supramol. Struc.* **14:** 255–266.
10. Castro-Munozbedo, F., M. Marsch-Moreno, A. Beltran-Langarica, and W. Kuri-Harcuch. 1987. Commitment of adipocyte differentiation in 3T3 cells is inhibited by retinoic acid, and the expression of lipogenic enzymes is modulated through cytoskeleton stabilization. *Differentiation.* **36:** 211–219.
11. Stone, R. L., and D. A. Bernlohr. 1990. The molecular basis for inhibition of adipose conversion of murine 3T3-L1 cells by retinoic acid. *Differentiation.* **45:** 119–127.
12. Petkovich, M., N. J. Brand, A. Krust, and P. Chambon. 1987. A human retinoic acid receptor which belongs to the family of nuclear receptors. *Nature (London).* **330:** 444–450.
13. Giguere, U., E. S. Ong, P. Segui, and E. M. Evans. 1987. Identification of a receptor for the morphogen retinoic acid. *Nature (London).* **330:** 624–629.
14. Brand, N., M. Petkovich, A. Krust, P. Chambon, H. deThe, A. Marchio, P. Tiollais, and A. DeJean. 1988. Identification of a second human retinoic acid receptor. *Nature (London).* **332:** 850–853.
15. Benbrook, D., E. Leonhardt, and M. Pfahl. 1988. A new retinoic acid receptor identified from a hepatocellular carcinoma. *Nature (London).* **333:** 669–672.
16. Zelent, A., A. Krust, M. Petkovich, P. H. Kastner, and P. Chambon. 1989. Cloning of murine α and β retinoic acid receptors and a novel receptor γ predominantly expressed in skin. *Nature (London).* **339:** 714–717.
17. Haq, R. U., and F. Chytil. 1991. Expression of nuclear retinoic acid receptors in rat adipose tissue. *Biochem. Biophys. Res. Commun.* **176:** 1539–1544.
18. Lamb, A. J., P. Apivatanaporn, and J. A. Olson. 1974. Induction of rapid, synchronous vitamin A deficiency in the rat. *J. Nutr.* **104:** 1140–1148.
19. Thompson, J. N., P. Erdody, and W. B. Maxwell. 1974. Simultaneous fluorometric determinations of vitamin A and E in human serum and plasma. *Biochem. Med.* **8:** 403–414.
20. Pfahl, M., M. Tzukerman, X. K. Zhang, J. M. Lehmann, T. Hermann, K. N. Wills, and G. Graupner. 1990. Nuclear retinoic acid receptors cloning, analysis, and function. *Methods Enzymol.* **189:** 256–270.
21. Chirgwin, J. M., A. E. Pryzbyla, R. J. MacDonald, and W. J. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry.* **18:** 5294–5299.
22. Haq, R. U., M. Pfahl, and F. Chytil. 1991. Retinoic acid affects the expression of nuclear retinoic acid receptors in tissues of retinol deficient rats. *Proc. Natl. Acad. Sci. USA.* **88:** 8272–8276.
23. Haq, R. U., M. Pfahl, and F. Chytil. 1991. Differential effects of all-*trans* and 13-*cis*-retinoic acid on mRNA levels of nuclear retinoic acid receptors in rat lung and liver. *Biochem. Biophys. Res. Commun.* **180:** 1137–1144.

24. Stumpo, D., and P. J. Blackshear. 1986. Insulin and growth factors effects on c-fos expression in normal protein kinase C-deficient 3T3-L1 fibroblasts and adipocytes. *Proc. Natl. Acad. Sci. USA*. **83:** 9453–9457.

25. Schule, R., P. Rangarajan, Wa Yang, S. Kliewer, L. J. Ransome, J. Bolado, I. M. Verma, and R. M. Evans. 1991. Retinoic acid is a negative regulator of AP-1 responsive genes. *Proc. Natl. Acad. Sci. USA*. **88:** 6092–6096.