THE RELATIONSHIP BETWEEN THE MONOGLYCERIDE AND GLYCEROL-3-PHOSPHATE PATHWAYS IN ADIPOSE TISSUE*

F. Michael Schultz, Mary Bob Wylie and John M. Johnston

The University of Texas Southwestern Medical School
Department of Biochemistry
Dallas, Texas 75235

Received August 2, 1971

In addition to the glycerol-3-phosphate pathway the biosynthesis of triglycerides by the monoglyceride pathway has been described in the hamster adipose tissue. The interrelationship of the two pathways was investigated. When microsomes were utilized, the 2-monooleyl ether (0-1 mM) progressively inhibited the incorporation of 5 mM ^{14}C-glycerol-3-phosphate into phosphatidic acid. When whole cells were employed, the synthesis of higher glycerides by the glycerol-3-phosphate pathway was similarly inhibited. The reported results provide a possible control mechanism for the biosynthesis of glyceride and, theoretically, a mechanism for the amplification of fatty acid release by this tissue.

Until recently, the only mechanism known for glyceride biosynthesis in adipose tissue was the glycerol-3-phosphate pathway (1,2,3). A second pathway has now been described by our laboratory in which monoglycerides are utilized as the acyl acceptor (4). The presence of these two pathways in which the final product is triglyceride lends itself to an investigation of a possible interrelationship between two enzymatic sequences.

MATERIALS AND METHODS:

Racemic disodium glycerophosphate, ^{14}C-glycerol-3-phosphate, palmityl-CoA, ATP, CoA, glucose-U-^{14}C, albumin (Fraction V), and collagenase were all obtained from commercial sources. The 2-^{3}H-9-10-octadecenyl glycerol ether referred to as 2-monooleyl ether was synthesized via the procedure of Wood and Snyder (5) as previously reported (4). The advantages of employing the ether rather than the corresponding ester when adipose tissue was employed

* Supported by Grant AM-3108 from the U.S. Public Health Service and Grant I-080 from The Robert A. Welch Foundation, Houston, Texas.

as the enzyme source was previously discussed (4). The microsomes of epididymal adipose tissue from male hamsters were prepared as previously described (4). The incubation mixtures contained 5 mM ^{14}C-glycerol-3-phosphate, 25 mM $MgCl_2$, 75 mM KCl, 0.5 mM palmityl-CoA, 0.1 ml 10% Tween 80, 0.5 ml of microsomes in 154 mM KCl containing approximately 1 mg microsomal protein and 0.5 M Tris-maleate buffer, pH 7.0, in a final volume of 2.0 ml. The concentration of 2-monooleyl ether was varied from 0 to 1 mM. When the fatty acid activation system was utilized, the concentration of ATP and CoA were 15 mM and 0.25 mM, respectively. The incubation was carried out at 20°C for 30 minutes.

For the whole cell preparations (6), 3% albumin in Krebs-Ringer-Bicarbonate buffer, pH 7.4, containing 3.0 mM glucose-U-^{14}C, was used. The 2-monooleyl ether concentration varied from 0 to 0.8 mM. The final volume was 2.5 ml and the incubation time was one hour at 37°C in a 95% O_2-5% CO_2 atmosphere.

The incorporation of the radioactive substrates into products was determined via the procedure previously described (4).

RESULTS:

In Figure 1 are given the results obtained on the incorporation of ^{14}C-

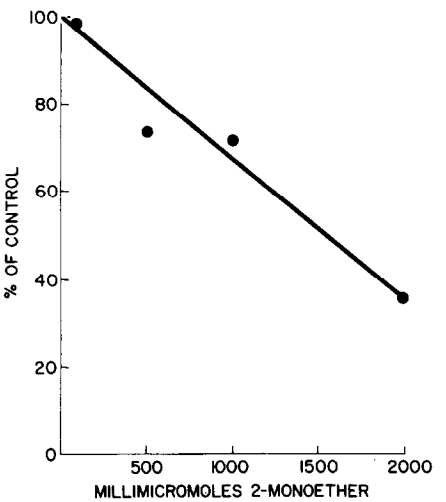

FIGURE 1: The effect of increasing concentrations of 2-monooleyl ether on the incorporation of ^{14}C-glycerol-3-phosphate into phosphatidic acid employing adipose tissue microsomes.

glycerol-3-phosphate into phosphatidic acid in the presence of various concentrations of 2-monooleyl ether, employing adipose tissue microsomes as the enzyme source. As was previously reported (4), phosphatidic acid is the major lipid of the glycerol-3-phosphate pathway synthesized when microsomes are used. All values were corrected for the radioactivity incorporated in the absence of palmityl-CoA. When 1 mM 2-monooleyl ether was employed, a 60% inhibition was observed. Similar inhibitions were observed when: (1) albumin was substituted for Tween 80; (2) the palmityl-CoA concentration was varied between 0.25 mM and 1.5 mM; and, (3) substituting the fatty acid activation system for palmityl-CoA. Therefore, it was concluded that the 2-monooleyl ether was responsible for the inhibition.

The inhibition by 2-monooleyl ether on the synthesis of triglycerides from glycerol-3-phosphate was next examined in intact adipose cells. The concentration of monooleyl ether was varied from 0 to 0.8 mM. The glycerol-3-phosphate was formed from the added glucose-U-^{14}C by glycolysis. The calculations are based on 2 moles of glycerol-3-phosphate being synthesized for each mole of glucose utilized. We had previously reported that at least 90% of the ^{14}C radioactivity found in the di- and triglycerides was present in the glycerol position of the molecule under the conditions described (4). The results are given in Figure 2. As can be seen, a significant decrease in the glycerol-3-phosphate pathway is observed in the presence of various concentrations of 2-monooleyl ether. Although the 2-monooleyl ether was

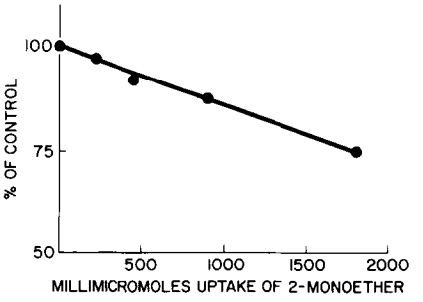

FIGURE 2: The effect of increasing concentrations of 2-monooleyl ether on the incorporation of ^{14}C-glucose into the glycerol portion of di- and triglycerides.

employed as the inhibitor in the reported investigations, for the reasons previously discussed, similar experiments were carried out with ^{3}H-2-monopalmitin and 2-monooleyl ether in which a similar inhibition was observed using microsomes and whole cell preparations.

DISCUSSION:

Previous investigations have examined the concentrations of glycerol-3-phosphate and acyl-CoA (7) as possible controlling systems of the glycerol-3-phosphate pathway. Limited correlations were observed. In the reported experiments the two pathways have been simultaneously monitored and the inhibition by 2-monooleyl ether demonstrated. By increasing the concentration of 2-monooleyl ether, the synthesis of phosphatidic acid progressively decreases. Although the inhibition was somewhat less when intact cells were employed, it is difficult to assess the effective concentration of the 2-monooleyl ether under these conditions. However, it should be emphasized that the concentration of 2-monooleyl ether employed in this study is of the same order of magnitude as that reported for monoglyceride in rat epididymal fat paid (8). Certain hormones, such as epinephrine, are known to facilitate the release of fatty acids by this tissue via the activation of a hormone-sensitive lipase which is mediated by 3'5' cyclic AMP (9,10). In addition to fatty acid, the monoglyceride concentration could theoretically be elevated (11). The resulting monoglycerides could then inhibit the glycerol-3-phosphate pathway and thus decrease the reesterification of fatty acid by this pathway. The net effect of the inhibition would be to further facilitate the release of fatty acids by this tissue. The release of fatty acids would therefore be analogous to that reported by numerous laboratories on the effect of epinephrine on the release of glucose by the liver in which the hormone increases the activity of phosphorylase and decreases glycogen synthetase activity. After the hormone had exerted its effect, the remaining monoglycerides could then theoretically be re-incorporated to triglycerides via the reactions described (4). Thus the inhibition of the glycerol-3-phosphate

pathway would be removed and this pathway would now function as the major pathway for the synthesis of triglycerides by this tissue.

REFERENCES:

1. Rose, G. and Shapiro, B., Bull. Res. Council Israel Sect. A: Chem., 9, 15-22 (1960).

2. Steinberg, D., Vaughan, M., Margolis, S., J. Biol. Chem., 236, 1631-1637 (1961).

3. Roncari, D.A.K. and Hollenberg, C.H., Biochim. Biophys. Acta, 137, 446-463 (1967).

4. Schultz, F.M. and Johnston, J.M., J. Lipid Res., 12, 132-138 (1971).

5. Wood, R. and Snyder, F., Lipids, 2, 161-171 (1967).

6. Rodbell, M., J. Biol. Chem., 239, 375-380 (1964).

7. Denton, R.M. and Halperin, M.L., Biochem. J., 110, 27 (1968).

8. Vaughan, M. and Steinberg, D., J. Lipid Res., 4, 193 (1963).

9. Vaughan, M., Berger, J.E. and Steinberg, D., J. Biol. Chem., 239, 401-409 (1964).

10. Butcher, R.W., Ho, R.J., Meng, H.C. and Sutherland, E.W., J. Biol. Chem., 240, 4515 (1965).

11. Wadstrom, L.B., Nature (London), 179, 259-260 (1957).