Vol. 83, No. 3, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 14, 1978 Pages 869-873

POSSIBLE RELATIONSHIP OF THE HEPATIC MICROSOMAL ATP-DEPENDENT CALCIUM PUMP TO SEX DIFFERENCES IN TRIACYLGLYCEROL SYNTHESIS [1]

Carlos Soler-Argilaga, Robert L. Russell and Murray Heimberg
Department of Pharmacology
University of Missouri, School of Medicine, Columbia, Missouri 65212

Received June 15,1978

SUMMARY: Microsomes were isolated from livers of fed male and female rats and the rates of incorporation of sn-[^{14}C]-glycerol-3-phosphate into phosphatidate, diacylglycerol and triacylglycerol by the microsomes were measured. Simultaneously, microsomal ATP-dependent uptake of calcium was evaluated and correlated with synthesis of phosphatidate from sn-glycerol-3-phosphate. The rate of glycerolipid synthesis by hepatic microsomes from female rats was greater than that of microsomes from male rats. By contrast, the active accumulation of calcium and subsequent inhibition of synthesis of phosphatidate from glycerol-3-phosphate was lower in microsomes from livers of female rats than from male animals. This reciprocal relationship between uptake of calcium and incorporation of sn-glycerol-3-phosphate into phosphatidate as reported earlier (Biochem. Biophys. Res. Commun. 78, 1053-1059 (1977)) may, in part, be responsible for the differences in the rates of hepatic triacylglycerol synthesis between livers from male and female rats.

INTRODUCTION: Perfused livers from female rats esterify a larger fraction of oleate into triacylglycerol and secrete more in the VLDL than do livers from male rats (1,2). Furthermore, the uptake of calcium by hepatic microsomes from male rats is associated with a decrease in the biosynthesis of phosphatidate and other glycerolipids (3). Since the ATP-dependent uptake of calcium by hepatic microsomes from adult female rats is lower than that of microsomes from adult male animals (4), it was of interest to determine whether the sex differences in hepatic synthesis of triacylglycerol was related to the accumulation of calcium in microsomes.

METHODS: Male and female Sprague-Dawley rats (200-250 g), obtained from the Charles River Breeding Laboratories, Wilmington, Mass., were fed Purina Laboratory Chow and water ad libitum. The livers were removed under light anesthesia with ethyl ether, were weighed and homogenized. The methods used for homogenization of the liver, preparation of the microsomes, measurement of ATP-dependent uptake of calcium by the microsomes, estimation of incorporation of sn-[^{14}C]-glycerol-3-phosphate into glycerolipids and sources of materials were described previously (3).

[1] This work was supported by Grant AM-18125 from the National Institutes of Health, U.S. Public Health Service.

0006-291X/78/0833-0869$01.00/0

Table I

INCORPORATION OF sn-GLYCEROL-3-PHOSPHATE INTO GLYCEROLIPIDS BY HEPATIC MICROSOMES FROM NORMAL FED MALE AND FEMALE RATS

METABOLITE ISOLATED	sn-GLYCEROL-3-PHOSPHATE INCORPORATED, nmol/mg MICROSOMAL PROTEIN	
	MALE	FEMALE
A. Phosphatidate	42.3±1.0	49.8±1.3* (17.7)
B. Diacylglycerol	12.8±0.3	14.9±0.7* (16.4)
C. Triacylglycerol	5.9±0.3	7.9±0.7* (33.9)

The assay system (0.45 ml) included 15 mM Tris-HCl (pH 7.5), 35 mM ATP, 50 mM CoA, 0.75 mM dithiothreitol, 35 mM $MgCl_2$, 0.5 mM sn-[U-^{14}C]-glycerol-3-phosphate (0.1 μCi), 25 mM sucrose, 1.5 mM palmitic acid, 4.5 mg bovine serum albumin and ∿ 0.5 mg of microsomal protein. The incubations were performed for 30 minutes at 37°C. The reaction was stopped by addition of chloroform:methanol (2:1 v/v) and lipids were extracted. Lipids were separated into individual classes by thin layer chromatography. Values given are means ± SEM from 18 determinations from 6 microsomal preparations per group. Significance of statistical differences between male and female groups are indicated by an asterisk ($p < 0.05$); significance was evaluated by student's t test. Figures in parentheses are percentage increases above the values for the male.

RESULTS AND DISCUSSION: The rate of incorporation of sn-[^{14}C]-glycerol-3-phosphate into phosphatidate, diacylglycerol and triacylglycerol was higher in hepatic microsomes from female rats than in microsomes from male animals (Table 1). The sex differences in the synthesis of triacylglycerol are seen at all steps in the pathway and suggest that sex affects a step(s) prior to the formation of phosphatidate. Sex differences in the enzymes involved in the formation of triacylglycerol from phosphatidate, however, cannot be excluded from these data.

The relationship between the ATP-dependent active uptake of calcium and biosynthesis of phosphatidate from sn-glycerol-3-phosphate by hepatic microsomes from male and female rats is shown in figure 1. The uptake of calcium by microsomes from the female was lower than that of the male (Fig. 1, inset),

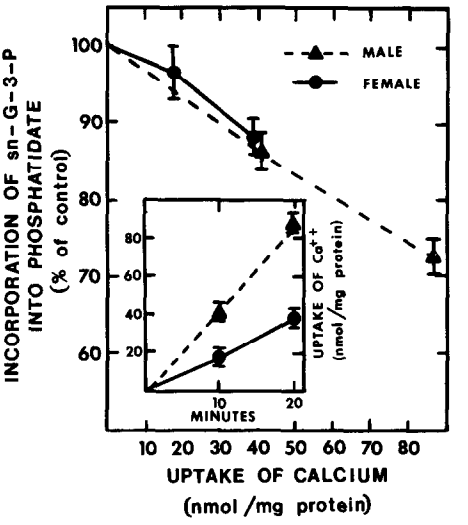

FIGURE 1: Reciprocal relationship between ATP dependent active uptake of Ca^{++} and biosynthesis of phosphatidate from glycerol-3-phosphate by liver microsomes from male and female rats.

Microsomes (~1 mg protein/ml) were incubated in a medium containing 5 mM ATP, 30 mM imidazole-histidine buffer (pH 6.8), 100 mM KCl, 5 mM NH_4 oxalate, 5 mM NaN_3, 5 mM $MgCl_2$ and 50 μM $^{45}CaCl_2$ (0.1 μCi/ml). Incubations were performed in a total volume of 3.0 ml at 37°. Samples were removed at 0, 10, and 20 minutes. At each time period, aliquots were taken (in duplicate) for measurement of Ca^{++} uptake (4). The amounts of Ca^{++} bound at each time period are presented in the inset. Other aliquots were removed simultaneously in duplicate and used as the source of the enzyme for measurement of incorporation of sn-[U-^{14}C]-glycerol-3-phosphate. Incubations were carried out with 0.2 mg microsomal protein in a final volume of 1.0 ml at 37° for 30 minutes. The rates of incorporation of sn-[U-^{14}C]-glycerol-3-phosphate into phosphatidate were 30.8±0.7 (male) and 35.5±0.9 (female) nmol/mg microsomal protein/30 minutes, respectively. Data are means ± SE from six separate preparations of microsomes per group.

in agreement with observations reported by Moore et al. (4). In agreement with previous studies from this laboratory using male animals only (3), the incorporation of sn-glycerol-3-phosphate into phosphatidate was inhibited by active accumulation of calcium by the microsomes. The reduction in phosphatidate synthesis was observed in both sexes, albeit quantitatively different. This reciprocal relationship between uptake of calcium and synthesis of phosphatidate from sn-glycerol-3-phosphate may modulate, at least in part, the observed differences in rate of triacylglycerol synthesis and secretion

between the two sexes (1,2,5-9). Again, in these experiments, the basal rate of glycerolipid synthesis was sex dependent. It is of interest also that, even though uptake of Ca^{++} by hepatic microsomes from the female was about half that from male animals, the synthesis of phosphatidate, as per cent of control, was inversely proportional to uptake of calcium, suggestive of a linear relationship.

Conceivably, as proposed by Moore et al. (4), differences in lipid composition of the microsomal membranes may in some way determine the differences in uptake of calcium between microsomes from male and female rats. These investigators observed that hepatic microsomal phospholipid from male rats contained a higher percentage of palmitic and linoleic acids than the females, while females contained a higher percentage of stearic acid than did males. Membrane phospholipids have been shown to be essential to the calcium pump activity in microsomes isolated from skeletal muscle (10-13). The influence of sex hormones on the ATP-dependent calcium pump in hepatic microsomes was amplified recently by the observation that calcium uptake by microsomes from male rats was decreased on castration and restored on treatment with testosterone, whereas treatment of male rats with estradiol resulted in a reduction of calcium uptake (14). Since ovariectomy in the rat reduces triacylglycerol output by the liver (6), while it is stimulated by estrogens (15-18), it is possible that the effects of estrogens on hepatic biosynthesis of triacylglycerol are mediated, in part, by estrogen induced changes in the active sequestration of calcium by hepatic microsomes. The mechanism by which calcium reduces the microsomal biosynthesis of phosphatidate from sn-glycerol-3-phosphate remains to be determined.

The data reported here further support the hypothesis that the Mg-ATP-dependent uptake of calcium by rat liver microsomes, which appears to be modulated by sex, may also be affected by various endocrine agents (glucagon, cAMP and Bt_2cAMP) which participate in the regulation of hepatic cellular homeostasis of calcium (19-21) and the metabolism of free fatty acids and the

synthesis of triacylglycerol* (22-24). It is quite possible, therefore, that hormonally regulated uptake of Ca^{++} by hepatic microsomes may be a critical factor in the regulation of glycerolipid biosynthesis.

REFERENCES

1. Soler-Argilaga, C. and Heimberg, M. (1976) J. Lipid Res. 17, 605-615.
2. Soler-Argilaga, C., Wilcox, H.G. and Heimberg, M. (1976) 17, 139-145.
3. Soler-Argilaga, C., Russell, R.L. and Heimberg, M. (1977) Biochem. Biophys. Res. Commun. 78, 1053-1059.
4. Moore, L., Chen, T., Knapp, H.R., Jr. and Landon, E.J. (1975) J. Biol. Chem. 250, 4562-4568.
5. Otway, S. and Robinson, D.S. (1967) J. Physiol. 190, 321-332.
6. Watkins, M.L., Fizette, N. and Heimberg, M. (1972) Biochim. Biophys. Acta 280, 82-85.
7. Wilcox, H.G., Woodside, W.F., Breen, K.J., Knapp, H.R., Jr. and Heimberg M. (1974) Biochem. Biophys. Res. Commun. 58, 919-926.
8. Hernell, O. and Johnson, O. (1973) Lipids 8, 503-516.
9. Soler-Argilaga, C., Danon, A., Wilcox, H.G. and Heimberg, M. (1976) Lipids 11, 517-525.
10. Martonosi, A., Donley, J. and Halpin, R.A. (1968) J. Biol. Chem. 243, 61-70.
11. Fiehn, W. and Hasselbach, W. (1970) Eur. J. Biochem. 13, 510-518.
12. Meissner, G. and Fleischer, S. (1972) Biochim. Biophys. Acta 255, 19-33.
13. Seiler, D. and Hasselbach, W. (1971) Eur. J. Biochem. 21, 385-387.
14. Moore, L., Knapp, H.R., Jr. and Landon, E.J. (1977) Endocrinology 100, 1516-1520.
15. Weinstein, I., Seedman, S. and Veldhuis, M. (1975) Proc. Soc. Exp. Biol. Med. 149, 181-184.
16. Kim, H.R. and Kalkhoff, R.K. (1975) J. Clin. Invest. 56, 888-896.
17. Weinstein, I., Soler-Argilaga, C. and Heimberg, M. (1977) Biochem. Pharmacol 26, 77-80.
18. Weinstein, I., Turner, F.C., Soler-Argilaga, C. and Heimberg, M. (1978) Biochim. Biophys. Acta, in press.
19. Pilkis, S.J., Claus, T.H., Johnson, R.A. and Park, C.R. (1975) J. Biol. Chem. 250, 6328-6336.
20. Berridge, M.J. (1976) Adv. Cyclic Nucleotide Res. 6, 1-98.
21. Keppens, S., Vandenheede, J.R. and DeWulf, H. (1977) Biochim. Biophys. Acta 496, 448-457.
22. Heimberg, M., Weinstein, I. and Kohout, M. (1969) J. Biol. Chem. 244, 5131-5139.
23. Klausner, H.A., Soler-Argilaga, C. and Heimberg, M. (1978) Metabolism 27, 13-25.
24. Soler-Argilaga, C., Russell, R.L. and Heimberg, M. (1978) Arch. Biochem. Biophys., in press.

*C. Soler-Argilaga, R.L. Russell, and M. Heimberg, submitted for publication.