RECIPROCAL RELATIONSHIP BETWEEN UPTAKE OF Ca^{++} AND BIOSYNTHESIS OF GLYCEROLIPIDS FROM sn-GLYCEROL-3-PHOSPHATE BY RAT LIVER MICROSOMES

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SUMMARY: The relationship between uptake of Ca^{++} and incorporation of sn-[^{14}C]-glycerol-3-phosphate into phosphatidate, diglyceride, and triglyceride was evaluated in microsomes isolated from livers of normal fed male rats. Uptake of Ca^{++} was dependent on concentration of Ca^{++} (0.1 - 2.5 mM), and accompanied by a decrease in the rate of glycerolipid synthesis. The quantity of Ca^{++} ion taken up at 20 μM $CaCl_2$ in the presence of ATP was equivalent to that observed with 2.5 mM $CaCl_2$ in the absence of ATP. The ATP dependent uptake of Ca^{++}, like the passive uptake at higher concentrations of Ca^{++}, was correlated with inhibition of incorporation of sn-glycerol-3-phosphate into phosphatidate. Accumulation of Ca^{++} in hepatic microsomes, therefore, appears to result in a calcium-dependent decrease in biosynthesis of phosphatidate and other glycerolipids.

INTRODUCTION

Considerable evidence has been obtained to support the conclusion that calcium ion is of importance in the regulation of hepatic carbohydrate metabolism (1-6). However, no similar information has been reported establishing a relationship between cellular homeostasis of calcium and hepatic biosynthesis of glycerolipids. The existence of such a relationship would be of particular interest since certain factors (e.g., cyclic nucleotides, glucagon, sex) which have been implicated in the regulation of hepatic synthesis and secretion of triglyceride (7-13) may also participate in the homeostasis of calcium in the hepatocyte (3,14,15). The studies reported here are the results of an attempt to evaluate the possibility of a relationship between accumulation of Ca^{++} in microsomes, either by passive or MgATP-dependent active uptake of calcium by rat liver microsomes, and the microsomal biosynthesis of glycerolipids. Evidence in support of a reciprocal relationship between uptake of Ca^{++} and synthesis of glycerolipids is presented in this manuscript.

MATERIALS AND METHODS

Chemicals. Palmitic acid (>99% purity) was purchased from Nu-Chek-Prep, Inc.,

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Elysian, Minn. Bovine serum albumin (fraction V powder), obtained from Pentex, Inc., Kankakee, IL, was purified by a modification (16) of the Goodman method (17). Phosphatidic acid was purchased from Supelco Inc., Bellefonte, PA. Other chemicals were obtained from Sigma Chemical Co., St. Louis, MO. $^{45}CaCl_2$ (sp. act. 20.84 mCi/mg) and sn-[U-^{14}C]-glycerol-3-phosphate (sp. act. 117.4 mCi/mmole) were purchased from New England Nuclear Corp., Boston, MA. Filters (0.45 μm) were purchased from the Millipore Corp., Bedford, MA. Silica gel G and HR plates, 250 μ thick, were purchased from Analtech, Inc., Newark, DE.

Animals. Male Sprague-Dawley rats obtained from the Charles River Breeding Laboratories, Wilmington, MA, weighed 200-300 g at time of sacrifice. All animals had free access to water and Purina Lab Chow (Ralston Purina Co., St. Louis, MO) until used.

Preparation of microsomes. Livers were removed from the rats under light ether anesthesia, and were chilled immediately in cold buffer (0-4°) containing 0.25 M sucrose, 2.5 mM EDTA and 2.25 mM Tris HCl, pH 7.4. The livers were cut into small pieces with scissors and then homogenized with 4 ml buffer/g liver (18). Microsomes were isolated from the homogenates as described by Fallon and Lamb (19) and were used within 30 min of preparation. Microsomal protein was determined by the method of Lowry et al. (20) using bovine serum albumin as a standard.

Measurement of Ca^{++} uptake. Calcium uptake was measured with $^{45}CaCl_2$ using the millipore filtration technique (15). Measurement of the passive accumulation of Ca^{++} by microsomes was carried out in 0.05 M Tris-HCl buffer, pH 7.4, containing 0.25 M sucrose. The suspension of microsomes (1 mg protein/ml incubation medium) was incubated for 10 minutes at 0° in presence of 0.1, 0.25, 0.5, 1.0, and 2.5 mM $^{45}CaCl_2$ (0.3 μCi) prior to filtration of the samples through the millipore filters. Oxalate-facilitated calcium uptake was determined as described by Moore et al. (15) in 30 mM imidazole-histidine buffer (pH 6.8) containing 100 mM KCl, 5 mM NH_4 oxalate, 5 mM NaN_3, 5 mM $MgCl_2$ and appropriate concentrations of $^{45}CaCl_2$. Incubations were carried out with or without 5 mM ATP at 37°. Radioactivity remaining on the filters was determined in a liquid scintillation spectrometer (Beckman LS3155T) using a mixture of 2.5 diphenyloxazole (5 g), napthalene (100 g), dioxane (1 liter) as scintillation fluid.

Incorporation of sn-glycerol-3-phosphate into glycerolipids. Incorporation of sn-[^{14}C]-glycerol-3-phosphate into glycerolipids by hepatic microsomal preparations was measured using the assay system described by Fallon et al. (18) except that palmitate, as the complex with bovine serum albumin (21), was added to the medium. The reactions were started by adding microsomes, which had been preincubated under different conditions with varying quantities of $CaCl_2$, to the medium. Details of each type of experiment are given in legends to the figures or table. After 30 minutes incubation at 37°, the reactions were stopped by addition of chloroform-methanol (2:1 v/v). Lipids were extracted (22) and separated into individual classes by thin layer chromatography (23,24). The bands of lipid were visualized with iodine and then transferred to counting vials with diluted Permafluor for measurement of radioactivity (11).

RESULTS AND DISCUSSION

The inhibitory effect of calcium, passively accumulated, on microsomal synthesis of phosphatidate, diglyceride and triglyceride from sn-glycerol-3-phosphate is shown in Figure 1. This inhibitory effect was dependent on the amount of Ca^{++} accumulated in the microsome, which was, in turn, dependent on

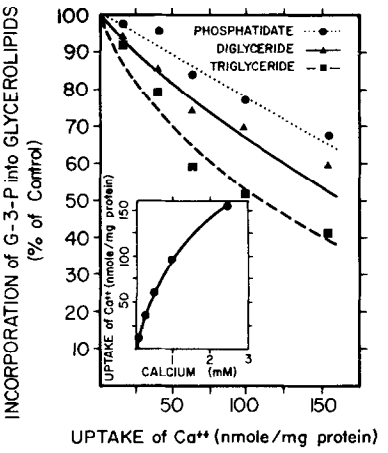

Figure 1: Inhibition of synthesis of glycerolipids from sn-glycerol-3-phosphate by passive accumulation of calcium in rat liver microsomes. Microsomes (1 mg protein/ml), suspended in 0.05 M Tris-HCl buffer, pH 7.4, containing 0.25 M sucrose, were incubated at 0° for 10 minutes with different concentrations of $^{45}CaCl_2$, each containing 0.3 μCi. At the end of the period of incubation, duplicate aliquots were taken for measurement of uptake of Ca^{++}, using the millipore filtration technique (15). The amount of Ca^{++} bound at each concentration is presented in the inset. Simultaneously, other aliquots were removed in triplicate for measurement of microsomal incorporation of sn-[^{14}C]-glycerol-3-phosphate into glycerolipids. Incubations for estimation of biosynthesis of glycerolipids were carried out in triplicate using 0.1 mg microsomal protein in a final volume of 0.45 ml at 37° for 30 minutes. See the text for additional details. The rates of incorporation of sn-[^{14}C]-glycerol-3-phosphate into phosphatidate, diglyceride and triglyceride for the controls (no calcium added) were 24.9, 6.1 and 2.1 nmoles/mg microsomal protein/30 min incubation, respectively.

the concentration of calcium added to the medium (Fig. 1, inset). Inhibition of microsomal glycerolipid synthesis was observed also when the calcium was preincubated with the homogenate (400 g supernatant) prior to isolation of the microsomes. The calcium which accumulated in the microsomes under these conditions was the result of passive transport, and relatively high concentrations of Ca^{++}, in the millimolar range, were required to produce inhibition of glycerolipid synthesis. The physiological meaning of these data is, therefore, open to considerable doubt.

Since it has recently been demonstrated by other workers (15) that rat

Table I. Inhibition of the synthesis of phosphatidate from sn-glycerol-3-phosphate by active ATP-dependent accumulation of calcium in rat liver microsomes

EXPERIMENT	UPTAKE OF Ca^{++} nmoles/mg protein	INCORPORATION OF G3P INTO PHOSPHATIDATE nmoles/mg protein
A. ATP omitted	4.0	18.5 (100)
B. ATP added	145.7	12.2 (65.9)

Microsomes (0.14 mg protein/ml) were incubated with or without 5 mM ATP in a medium containing 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 20 μM $^{45}CaCl_2$ (0.1 μCi/ml). Incubations were performed in a total volume of 6.0 ml at 37° for 30 minutes. At the end of the incubation period, samples were taken (in duplicate) for measurement of Ca^{++} uptake by the millipore filtration technique. Simultaneously, another aliquot was removed and centrifuged at 105,000 g for 30 minutes. The sedimented microsomes were resuspended in the original sucrose-Tris buffer and aliquots were used as source of the enzyme for measurement of incorporation of sn-[^{14}C]-glycerol-3-phosphate (G3P) into phosphatidate. Incubations were carried out with 0.12 mg microsomal protein in a final volume of 0.45 ml at 37° for 30 minutes. See the text for additional details. Results are the average of two separate experiments. The figures in parenthesis represent percent of control.

liver microsomes possess MgATP-dependent ability to sequester Ca^{++}, it was of interest to determine whether the ATP-dependent active accumulation of calcium could also be correlated with the rate of incorporation of radiolabeled sn-glycerol-3-phosphate into phosphatidate. Evidence for a reciprocal relationship between accumulation of Ca^{++} and synthesis of phosphatidate is presented in Table 1 and Figure 2. It is of particular interest that the amount of calcium which accumulated in microsomes in the ATP-dependent system in the presence of 20 μM $CaCl_2$ (Table 1) and the subsequent inhibition of incorporation of sn-glycerol-3-phosphate into phosphatidate were similar to those observed with passive accumulation of Ca^{++} at the higher concentration of 2.5 mM $CaCl_2$ (Fig. 1).

The reciprocal relationship between the ATP-dependent active uptake of

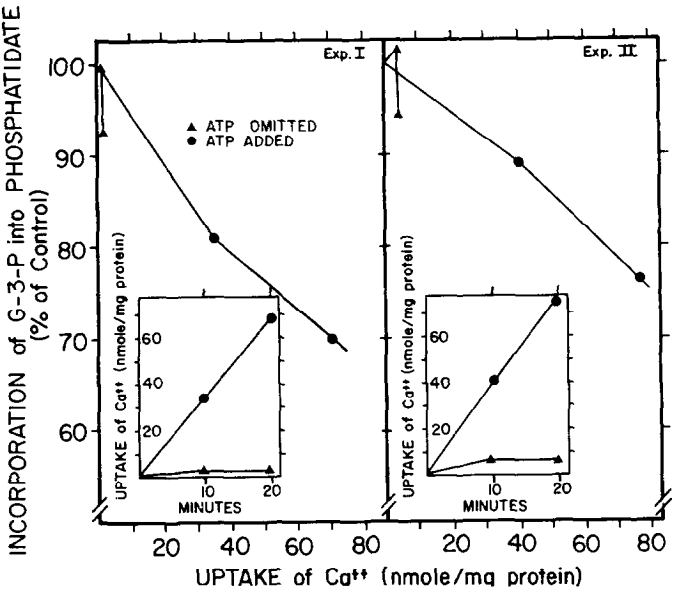

Figure 2: Reciprocal relationship between ATP dependent active uptake of Ca^{++} and biosynthesis of phosphatidate from glycerol-3-phosphate by rat liver microsomes. Microsomes (1.3 and 2.1 mg protein/ml for experiments I and II, respectively) were incubated with or without 5 mM ATP in the medium described in the legend for Table I, except that the final concentration of calcium was 100 and 200 μM for experiments I and II, respectively. 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 by the millipore filtration technique (25). The amounts of Ca^{++} bound at each time period are presented in the insets. Simultaneously, other aliquots were removed (in triplicate) and used as the source of the enzyme for measurement of incorporation of sn-[^{14}C]-glycerol-3-phosphate (G3P) into phosphatidate. Incorporation of G3P was measured as described in Table I. Incubations were carried out with 0.26 mg (Exp I) and 0.42 mg (Exp II) microsomal protein in a final volume of 1.0 ml at 37° for 30 minutes. See the text for additional details. The rates of incorporation of sn-[^{14}C]-glycerol-3-phosphate into phosphatidate for controls were 27.0 (Exp I) and 30.4 (Exp II) nmoles/mg microsomal protein/30 minutes incubation.

Ca^{++} and biosynthesis of phosphatidate from glycerol-3-phosphate by rat liver was also observed in experiments in which the amount of Ca^{++} accumulated per mg of microsomal protein was similar despite differences in concentration of calcium (100 and 200 μM for experiments I and II, respectively) (Fig. 2).

It may be concluded from these observations that the concentration of calcium in microsomal vesicles can modify the biosynthesis of triglyceride by influencing the sequence of biochemical events preceeding the formation of phosphatidate. The mechanism(s) by which calcium reduces the microsomal bio-

synthesis of phosphatidate from sn-glycerol-3-phosphate remain to be determined. The low and high concentrations of calcium in the membrane regulated by active transport may influence certain membrane properties, and directly or indirectly alter the enzymatic mechanisms participating in the microsomal biosynthesis of glycerolipids. Since various factors which have been demonstrated to alter rates of hepatic synthesis and release of triglyceride, such as dibutyryl adenosine-3',5'-monophosphate (7,8), glucagon (7), and sex of the animal (9-13), also have been reported to participate in regulation of hepatic cellular homeostasis of calcium (3,14,15) and, therefore, may affect the microsomal content of Ca^{++}, it is conceivable that MgATP-dependent uptake of calcium by rat liver microsomes may be critical in regulation of hepatic glycerolipid biosynthesis *in vivo* as well as *in vitro*.

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