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ENHANCEMENT OF ACYL COENZYME A:RETINOL ACYLTRANSFERASE IN RAT LIVER AND MAMMARY TUMOR TISSUE BY RETINYL ACETATE AND ITS COMPETITIVE INHIBITION BY *N*-(4-HYDROXYPHENYL)RETINAMIDE[1]

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Retinol esterification by microsomal acyl coenzyme A:retinol acyltransferase was quantified in rat mammary tumor and liver tissue. Acyltransferase activity in the livers of mammary tumor-bearing rats was 40% of that in normal animals. In response to daily oral doses of 2 mg retinyl acetate for 18-19 days, activity increased 2.8-fold in transplanted rat mammary tumors, 4.1-fold in the livers of tumor-bearing rats, and 1.5-fold in the livers of normal rats. The *in vitro* esterification of retinol was competitively inhibited by all-*trans*-*N*-(4-hydroxyphenyl)retinamide (K_i = 154 μM).

The transacylation of retinol from acyl CoA is catalyzed by a microsomal activity, acyl coenzyme A:retinol acyltransferase (ARAT), in rat liver (1), chick liver (2), rat small intestine (3), human small intestine (4), bovine retina (5,6), human retina (6), ovine kidney (7), and rat mammary gland (8).

ARAT activity in liver microsomes is enhanced by prior short-term treatment of both rats (9) and chicks (2) with vitamin A. ARAT may, therefore, be involved in the regulation of vitamin A storage in the liver and in other tissues. Inhibition of ARAT by compounds structurally similar to retinol has not previously been studied. Since retinoids are used at high doses in the treatment of cancer, their possible influence on ARAT activity may have unwelcome consequences in terms of vitamin A storage.

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Abbreviations ARAT, acyl CoA:retinol acyltransferase; BSA, bovine serum albumin, HPR, all-*trans*-*N*-(4-hydroxyphenyl)retinamide; DMSO, dimethylsulfoxide.

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MATERIALS AND METHODS

Chemicals Retinyl acetate, retinyl palmitate, retinal, palmitoyl CoA, dithiothreitol, and bovine serum albumin (BSA), essentially fatty acid-free, were purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals and solvents were of the highest quality commercially available. All-*trans*-*N*-(4-hydroxyphenyl)retinamide (HPR) was synthesized in our laboratory by Dr. Arun B. Barua (10).

Tumor Transplantable rat mammary tumor cells were acquired from the late Dr. John Balinsky, Dept. of Zoology, Iowa State University. This tumor line, induced by dimethylbenz(a)anthracene, was kindly provided by Dr. Olga Greengard of Mt. Sinai Hospital, New York, NY. The tumor was maintained in Fischer 344/NHSD female rats, being transferred biweekly.

Animals Fischer 344/NHSD female rats (Harlan Sprague-Dawley, Indianapolis, IN) were maintained on the AIN semi-purified diet (11), with free access to tap water. Ten animals received a daily oral dose of 2 mg all-*trans*-retinyl acetate in 0.125 mL corn oil, while ten others received the vehicle only. On day 8 of dosing, tumor cells were transplanted into the napes of five animals from each group according to the following procedure. One gram of tumor was minced in 10 mL Liebovitz' L-15 medium (GIBCO, Grand Island, NY), containing gentamycin sulfate, streptomycin sulfate, and penicillin G; 0.1 mL of the resulting suspension was injected into the loose skin of the nape. The dosed group was sacrificed on day 18 and the control group on day 19. Animals were anesthetized with diethyl ether and their livers and tumors were removed. For the inhibition study rats maintained on the same diet were sacrificed when 200 g in weight. They were anesthetized with 0.06 mL Innovar-Vet (Pitman-Moore, Washington Crossing, NJ) per 100 g body weight, injected *i.p.*, and their livers were subsequently excised.

Microsomes, Substrates, and ARAT Assay Tumor samples were dissected free of necrotic tissue. Liver and tumor microsomes were prepared by differential centrifugation of the tissue homogenate in 0.15 *M* potassium phosphate buffer, *p*H 7.4. The final concentration of microsomal protein (4-20 mg/mL) was determined by absorbance at 280 nm for the enhancement/tumor study and by the Bradford dye-binding method (12) for the inhibition study. In both cases BSA served as standard. Retinol was prepared by reduction of all-*trans*-retinal with sodium borohydride in methanol. ARAT activity was measured according to the following protocol. Microsomes were incubated with retinol and palmitoyl CoA at 37°C. After the addition of 1 mL cold absolute ethanol to stop the reaction, the product, retinyl palmitate, was extracted with hexane and quantified by reversed-phase high-performance liquid chromatography. The detector monitored absorbance at 325 nm, separation was effected on a Whatman Partisil 10 ODS-2 column, and the eluting solvent was methanol:tetrahydrofuran, 90:10 or 80:20. Retinyl palmitate was quantified by integration of its peak.

RESULTS

The activity of microsomal acyl CoA:retinol acyltransferase has been measured in livers from normal and tumor-bearing rats and in transplanted mammary tumors (Table 1). Relative to normal liver, ARAT activity in the livers of tumor-bearing rats was depressed 60% ($p<0.001$). The activity in tumor tissue, although much lower than in liver, was appreciable. Upon treatment of normal and tumor-bearing rats with retinyl acetate for 18-19 days,

Table 1

ARAT ACTIVITY IN RAT LIVER AND MAMMARY TUMOR BEFORE AND AFTER DOSING WITH RETINYL ACETATE

	TUMOR-BEARING		TUMOR-FREE
	Tumor	*Liver*	*Liver*
Control	12.4 ± 5.3	67.9 ± 17.2	168.8 ± 30.5
Dosed	34.2 ± 23	277.5 ± 111	261.2 ± 74
Dosed/Control	2.8	4.1	1.5
p value	< 0.07	< 0.025	< 0.05

All incubations comprised 20 μ*M* BSA, 5 m*M* dithiothreitol, and 0.15 *M* potassium phosphate buffer, *p*H 7.4, at a final volume of 0.5 mL, including 200 μg microsomal protein, 100 μ*M* palmitoyl CoA, and 100 μ*M* all-*trans*-retinol. Mean values ± S.D. are reported for five animals, expressed as pmol retinyl palmitate formed/min/mg microsomal protein. Each incubation, 30 minutes in length, was conducted in duplicate. Statistical significance was calculated by means of Student's *t* test.

ARAT activity increased 2.8-, 4.1-, and 1.5-fold in transplanted mammary tumors, in the livers of tumor-bearing rats, and in the livers of normal rats, respectively (Table 1). After dosing, activity in livers from tumor-bearing rats was the same as in livers from normal rats, an indication that the depression of ARAT activity in undosed tumor-bearing rats was fully offset by the administration of vitamin A.

ARAT activity of the liver, besides being depressed by an unknown mechanism in tumor-bearing rats, was inhibited *in vitro* by the retinol analog all-*trans*-*N*-(4-hydroxyphenyl)retinamide. The inhibition seems to be competitive, as shown in Figure 1. HPR changed the slope of the line by a factor of 1.9 when present in slight excess over retinol, while K_i = 154 μ*M*. The correlation coefficient for each line in the Lineweaver-Burk plot is greater than 0.99. All-*trans*-retinamide and all-*trans*-retinoic acid also inhibited ARAT competitively; the former was about as active as HPR whereas the latter was much less active.

DISCUSSION

In liver the concentration of retinyl ester increases in response to dosing with vitamin A (13,14). This phenomenon is explained, at least in part, by the observed rise in ARAT activity under the same circumstances. The presence of ARAT in a tissue implies an ability to store vitamin A, and

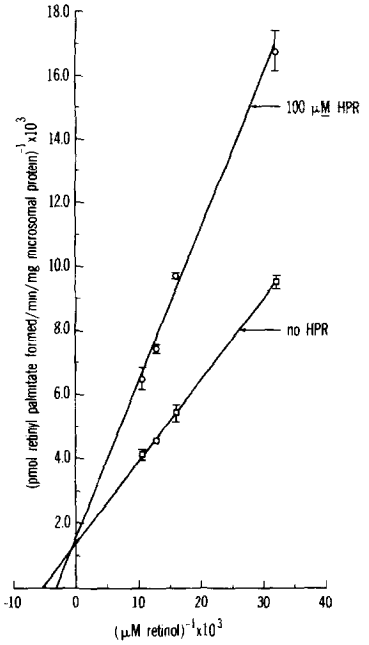

Figure 1: Inhibition of ARAT by hydroxyphenylretinamide. The assay mixture included 150 μg microsomal protein, 100 μ*M* palmitoyl CoA, and all-*trans*-retinol at 30.9, 61.7, 77.2, or 92.6 μ*M*. Incubation was 20 minutes in length. HPR (100 μ*M*) was introduced in DMSO. The concentration of DMSO was constant over the incubations. Data points represent the mean of duplicate incubations and error bars enclose the range. The regression line was defined by the method of least-squares.

this study demonstrates for the first time such an ability in rat mammary tumor. ARAT activity in tumor, albeit low relative to that in liver, should be sufficient to form a detectable amount of retinyl ester in the tumor. Recent work, however, has revealed that rat hepatoma (15) and mammary tumor (16; O. Amédée-Manesme and H. C. Furr, Iowa State University, unpublished data) contain very little vitamin A, either as retinyl ester or retinol. The lack of retinyl ester in tumors clearly is not due to an absence of ARAT activity. Tumor tissue may, therefore, catabolize vitamin A quickly, be supplied with less vitamin A than normal tissue, or not effectively absorb vitamin A from the plasma, all of which possibilities could reduce the concentration of vitamin A in the tumor to very low values.

ARAT activity in liver from undosed tumor-bearing rats was only 40% of that in liver from undosed tumor-free animals. Administration of vitamin A not only enhanced the activity in liver but also equalized liver ARAT activities in tumor-bearing and tumor-free rats. The reduction of ARAT activity in tumor-

bearing rats must result from some influence exerted by the tumor on the liver. The nature of this inhibition is not clear. In contrast, the activities of specific isozymes of hexokinase and pyruvate kinase in liver are higher in animals bearing this tumor (17). Whether the mechanism of *in vivo* ARAT enhancement by vitamin A involves enzyme induction, stimulation, or removal of inhibition is unknown.

ARAT activity, apart from being inhibited in undosed tumor-bearing rats, was inhibited *in vitro* by retinol analogs. HPR competitively inhibited ARAT, and preliminary data from our laboratory suggest that retinamide and retinoic acid inhibit likewise. HPR has antineoplastic activity, particularly against breast cancer, and, although HPR does not accumulate in the liver, it can be found at high concentrations in mammary tissue when administered to rats (18). Because in chemotherapy it is present in large excess over retinol *in vivo*, HPR may inhibit the esterification and storage of retinol, consequently affecting the patient's vitamin A status adversely.

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