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All-*trans*-retinoic acid inhibits retinol esterification by acyl-CoA: retinol acyltransferase (*EC* 2.3.1.76) from rat and human small intestinal mucosa

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1. Formation of retinyl esters catalyzed by acyl-CoA:retinol acyltransferase (ARAT; retinol fatty-acyltransferase; *EC* 2.3.1.76) from intestinal mucosa has been studied in vitro in the presence of all-*trans*-retinoic acid.

2. The incubation system contained microsomal preparations from the tissue tested, radioactive retinol and palmitoyl-CoA. The product formed was separated from the substrates by chromatography on alumina columns.

3. All-*trans*-retinoic acid inhibited ARAT both from rat and human intestinal mucosa.

4. Inhibition occurred instantly. At a concentration of retinol of 80 μM, a 50% inhibition was obtained with 50 μM-retinoic acid.

5. The inhibition of ARAT by retinoic acid may be of importance for normal retinol absorption in patients receiving retinoid therapy.

Natural sources for vitamin A include retinyl esters of animal origin and β-carotenes of vegetable origin. Retinyl esters are hydrolysed in the gut yielding retinol, and β-carotene absorbed into the mucosal cell may be cleaved and reduced to retinol (Huang & Goodman, 1965).

Retinol in the mucosal cell is esterified with a long-chain fatty acid before it is packed into the core of the chylomicrons (Goodman, 1984). Esterification is catalyzed by an acyl-CoA:retinol acyltransferase (ARAT; retinol fatty-acyltransferase; *EC* 2.3.1.76), a microsomal enzyme which has been found in rat and human small intestines (Helgerud *et al.* 1982, 1983). Since essentially all the retinol leaving the mucosal cell is esterified, compounds which inhibit esterification may inhibit the absorption of retinol. Several different retinoids have been used in recent years in the treatment of some skin disorders (Peck, 1984) and some carcinomas (Moon & Itri, 1984).

In the present study we found that all-*trans*-retinoic acid is a potent inhibitor of retinol esterification by intestinal ARAT in vitro.

MATERIALS AND METHODS

Chemicals

[15(n)−^{3}H]retinol (all-*trans*), with a specific activity of 13·6 Ci/mmol, was obtained from New England Nuclear, Boston, MA, USA. Retinol (all-*trans*), retinoic acid (all-*trans*), palmitoyl CoA, bovine serum albumin, dithiothreitol, dimethylsulphoxide and butylated hydroxytoluene were from Sigma Chemical Co., St Louis, MO, USA. All other chemicals were of standard commercial high purity.

Animals

Male rats (200–300 g weight) of Wistar strain were supplied from Dyrlæge Møllegaard Hansen Avlslab, Ejby, Denmark. They were given a pelleted rat diet: 5% of the energy came from fat, 21% from protein and 74% from carbohydrates. The diet contained about 10 μmol retinol equivalents/kg, and adequate amounts of other vitamins and minerals. The animals

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had free access to pellets and water. They were fasted for 24 h before they were killed at 09.00 hours by a blow on the neck. The small intestines were perfused with saline (9 g sodium chloride/l) containing 1 mM-dithiothreitol, the mucosa was scraped and the microsomal fraction isolated as described by Norum *et al.* (1981).

Human material

A small piece of apparently healthy jejunum was taken from a segment of intestine removed from a patient with terminal ileitis. The microsomal fraction was prepared as described by Helgerud *et al.* (1981).

Assay for testing ARAT activity

The enzyme assay was essentially that described by Helgerud *et al.* (1982), except that retinol was dissolved in dimethylsulphoxide. Microsomes (about 75 μg protein) were incubated in the presence of 20 nmol radioactive retinol and 5 nmol palmitoyl-CoA at 37° for 2 min. Potassium phosphate buffer (0·2 M, pH 7·4), bovine serum albumin, *NN'*-diphenyl-*p*-phenylenediamine and dithiothreitol were included to give optimal conditions. When retinoic acid was tested as an inhibitor it was dissolved in dimethylsulphoxide, usually together with retinol. The final volume was 0·25 ml.

The reaction was stopped by the addition of ethanol and the retinoids were extracted with hexane. Retinylpalmitate was separated from retinol by alumina oxide column chromatography (Harrison *et al.* 1979). Radioactivity was measured in a Packard Tri-Carb liquid scintillation spectrometer using Insta-Gel II as scintillator. The enzyme activity was calculated based on the percentage of retinol esterified and expressed as nmol ester formed/mg protein per min; in inhibition experiments the activities were presented as a percentage of non-inhibited control incubation.

Protein

Protein was determined according to Lowry *et al.* (1951) using bovine serum albumin as a standard.

RESULTS

The esterification of retinol by the microsomal fraction from mucosal cells of rats was linear for only 2 min when a concentration of 80 μM-retinol was used (Fig. 1). When retinoic acid was added (40 μM) the esterification rate decreased considerably, but the shape of the time-curve was the same as that obtained without retinoic acid (Fig. 1).

With a constant retinol concentration an increasing concentration of retinoic acid led to substantial inhibition of retinol esterification (Fig. 2(*a*)). Inhibition was about 50% when 50 μM-retinoic acid was used in the presence of 80 μM-retinol. The activity of ARAT varied somewhat in the intestinal mucosa of the rat. Mucosal preparations with a specific ARAT activity from 3·4 to 0·7 nmol ester formed/mg protein per min were tested for inhibition with retinoic acid, and the same relative inhibition was obtained in all the different enzyme preparations (values not shown).

The inhibition of ARAT by retinoic acid occurred instantly and the same degree of inhibition was observed whether retinoic acid and retinol were added together or retinoic acid was preincubated with the microsomes for up to 20 min (Fig. 2(*b*)).

When a constant concentration of retinoic acid and an increasing concentration of retinol were used, it was observed that there was a pronounced inhibition at low retinol concentrations. However, at high retinol concentrations, no inhibition by retinoic acid could

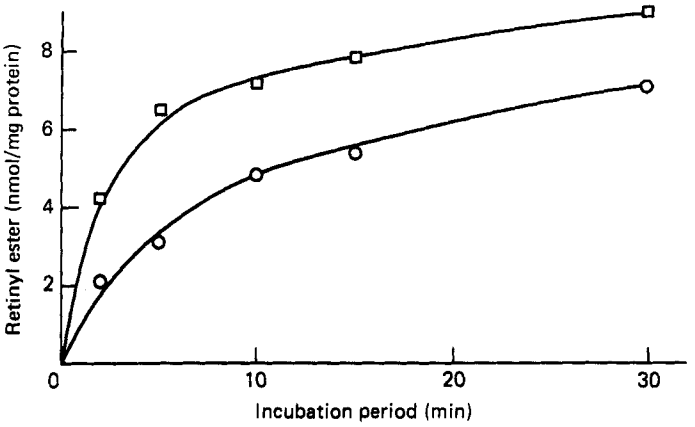

Fig. 1. Formation of retinyl ester catalyzed by acyl-CoA:retinol acyltransferase (retinol fatty-acyltransferase; *EC* 2.3.1.76) in microsomes from rat intestinal mucosa. The incubation mixture containing the standard mixture is described on p. 38. (□), Without retinoic acid; (○), with the addition of retinoic acid (40 μM).

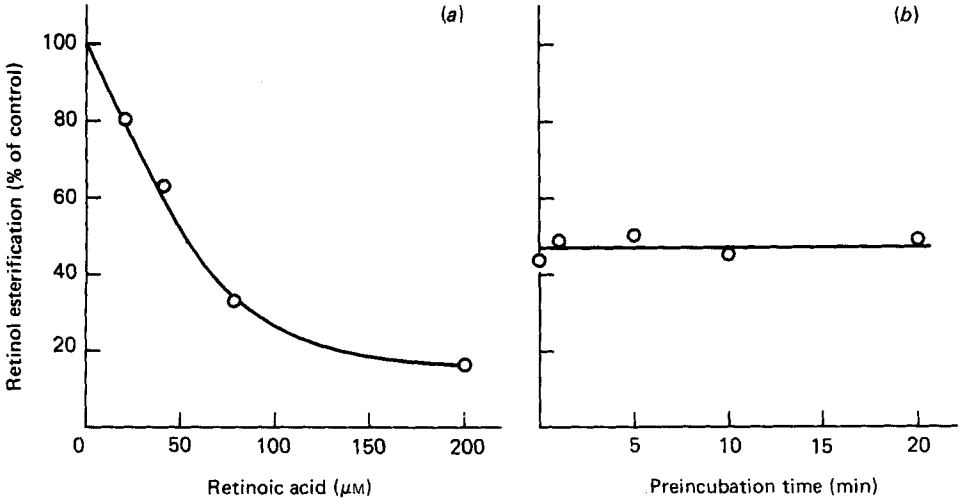

Fig. 2. Inhibition by retinoic acid of the formation of retinyl ester by microsomes from rat intestinal mucosa. (*a*) The amounts of retinoic acid were varied and inhibitor and retinol were added at the same time. (*b*) Retinoic acid (40 μM) was preincubated with the microsomes for a period of 0–20 min. The microsomal preparation used had a specific acyl-CoA : retinol acyltransferase (retinol fatty-acyltransferase; *EC* 2.3.1.76) activity corresponding to 2·6 nmol retinyl ester/mg protein per min. Incubation conditions are described on p. 38.

be detected (Fig. 3). Thus, retinoic acid may be a competitive inhibitor for retinol esterification in vitro. However, the interpretation of these findings is somewhat uncertain as retinol at concentrations higher than 80 μM also inhibited the formation of retinyl ester (Fig. 3).

Retinoic acid also inhibited the esterification of retinol in a microsomal preparation from human mucosa (Fig. 4), and the degree of inhibition was of the same order of magnitude as that obtained in enzyme preparations from rat mucosa.

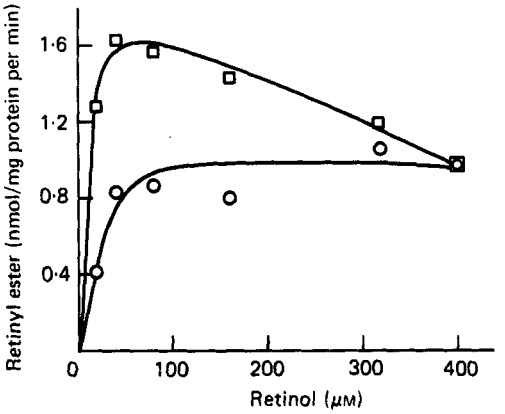

Fig. 3. The relation between the formation of retinyl ester and retinol concentration, with (○) and without (□) 40 μM-retinoic acid during incubation. Microsomes from rat intestinal mucosa were used. Incubation conditions are described on p. 38.

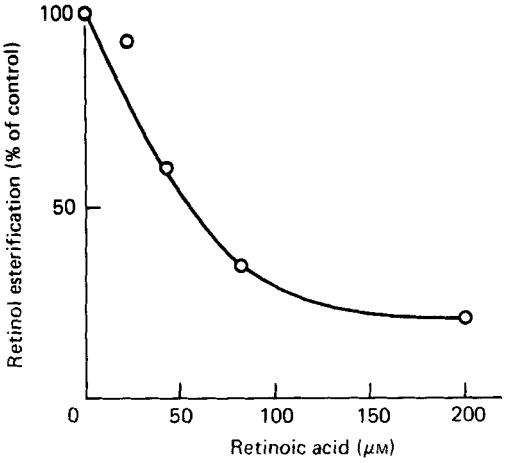

Fig. 4. Inhibition by retinoic acid of the formation of retinyl ester by microsomes from human intestinal mucosa. The concentration of retinol in the incubation medium was 80 μM and the concentration of retinoic was varied. Incubation conditions are described on p. 38. The specific activity of acyl-CoA: retinol acyltransferase (retinol fatty-acyltransferase; *EC* 2.3.1.76) in the microsomal preparation was 1·0 nmol retinyl ester/mg protein per min.

DISCUSSION

The retinoids have gained increased scientific interest in recent years, mainly because of their potent effect on some skin disorders and their possible effect in the treatment of malignant diseases. The field has recently been reviewed (Peck, 1984; Moon & Itri, 1984). Several reports from our laboratory have shown the importance of ARAT in the esterification of retinol in the gut (Helgerud *et al.* 1982, 1983; Norum *et al.* 1983). Mucosal ARAT has its highest activity in the absorbing cells at the top of the villi (Norum *et al.* 1983), and the activity varies with the diet and intake of retinol (Rasmussen *et al.* 1984), suggesting that the enzyme is of physiological importance in the absorption of retinol.

The present report shows that all-*trans*-retinoic acid strongly inhibits ARAT in vitro in enzyme preparations from both rat and human mucosa. The inhibition is probably

competitive with respect to retinol, and occurs without delay, suggesting that retinoic acid does not affect ARAT *per se*. This is in accordance with the report that feeding retinoic acid does not interfere with the activity of ARAT in isolated microsomal fractions from rat intestinal mucosa (Rasmussen *et al.* 1984). The strong inhibition of retinol esterification by retinoic acid in vitro may suggest that this compound may interfere with the true absorption of retinol from the gut. However, this possibility must be tested by an investigation of the transport of retinol and retinyl esters from the intestines with and without retinoic acid in the diet. Such experiments are in progress.

The esterification of retinol in the liver is probably of great importance in the handling and storage of retinol in liver. Preliminary experiments in our laboratory have suggested that liver ARAT is also inhibited by retinoic acid and other retinoids. Thus, the retinoids may have profound effects on retinol metabolism, which should be investigated in greater depth during the administration of different retinoids.

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