Vol. 80, No. 4, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 28, 1978 Pages 1022-1024

INHIBITION OF FATTY ACID SYNTHESIS BY RMI 14,514 (5-TETRADECYLOXY-2-FUROIC ACID)

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Received January 18,1978

Summary: RMI 14,514 strongly inhibited the incorporation of label from [1-^{14}C]acetyl-CoA into fatty acids by rat liver homogenates. No inhibition was observed when [2-^{14}C]malonyl-CoA was used as the labeled fatty acid precursor. These results suggest that the drug inhibits de novo fatty acid biosynthesis at the step mediated by acetyl-CoA carboxylase. The data presented in this communication support earlier reports that RMI 14,514 probably exerts its hypolipidemic effects by inhibition of fatty acid biosynthesis.

Previous reports from this laboratory have shown that administration of RMI 14,514 to rats and monkeys causes significant reductions of plasma cholesterol and triglyceride levels (1,2). The drug inhibits hepatic fatty acid synthesis from [1-^{14}C]acetate (1,2) and [U-^{14}C]alanine (3) in vivo in rats with high rates of lipogenesis induced by meal feeding. Cholesterol synthesis was unaffected under these conditions (2). These studies suggested that inhibition of fatty acid synthesis is the mechanism by which RMI 14,514 produces its hypolipidemic effects. In this communication, evidence is presented which suggests that the drug decreases fatty acid biosynthesis at the step mediated by acetyl-CoA carboxylase (EC 6.4.1.2).

MATERIALS AND METHODS

Homogenates were prepared from livers of untreated male rats in 0.25 M sucrose solution containing EDTA and nicotinamide as described by Iliffe and Myant (4). The rats had been fasted for 2 days and then refed for 2 days to stimulate lipogenesis. The final incubation mixture contained 2.0 ml of homogenate (corresponding to 400 mg of fresh tissue), pH 7.4 Tris buffer (45 mM), $KHCO_3$ (10 mM), $MgCl_2$ (4 mM), KCl (45 mM), K_2HPO_4 (5 mM), potassium succinate (15 mM), NADP (1 mM), ATP (2 mM), glucose-6-phosphate (5 mM), dithiothreitol (5 mM), and CoA (0.4 mM) in a total volume of 4.5 ml. RMI 14,514 was converted to its sodium salt and solubilized by complexing in a 15% solution of albumin (free fatty acid poor (5)) in concentrations such that addition of 0.75 ml to the flask resulted in the desired concentration of drug. Identical volumes of albumin solution without drug were added to control flasks. The samples were preincubated, with gentle shaking, for 1 hour at 37° C under an atmosphere of 95% O_2 and 5% CO_2 prior to addition of drug. After a further preincubation period of 20 minutes, 0.4 μCi (2.55 μmol) of [1-^{14}C]acetyl-CoA was added. The incubations were continued for 10, 20 or 30 minutes. The contents were then subjected to alkaline hydrolysis and unsaponifiable lipids were removed by extraction into petroleum ether. The

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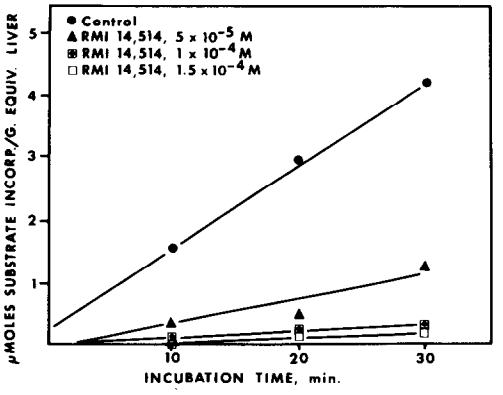

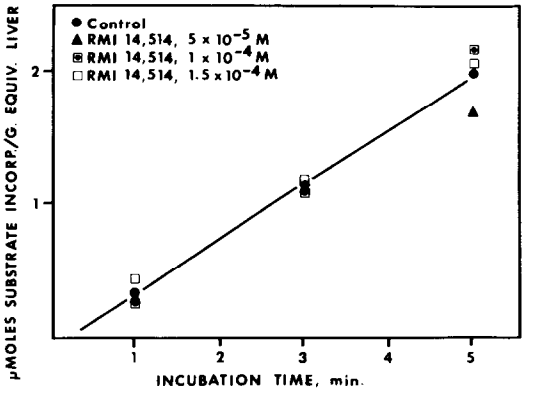

Figure 1. Effect of RMI 14,514 on incorporation of label from [1-^{14}C]acetyl-CoA into fatty acids *in vitro*. Drug was added to incubation flasks containing rat liver homogenates prepared from untreated rats. Conditions are described in text.

Figure 2. Effect of RMI 14,514 on incorporation of label from [2-^{14}C]malonyl-CoA into fatty acids *in vitro*. Conditions were the same as described in Figure 1. The line represents the calculated regression line for the control samples.

remaining alkaline phase was acidified and fatty acids were extracted into petroleum ether. After thorough washing of the extract, aliquots were taken for determination of radioactivity in a liquid scintillation spectrometer.

The same conditions were used when labeled malonyl-CoA was used as the substrate, except that 0.2 μCi (2.30 μmol) of [2-^{14}C]malonyl-CoA and 1.4 μmoles of unlabeled acetyl-CoA were added as substrates and the incubation periods were 1, 3 and 5 minutes.

The radioactive substrates were obtained from New England Nuclear.

RESULTS AND DISCUSSION

These experiments were done to locate the site in the pathway of *de novo* synthesis of fatty acids that is inhibited by RMI 14,514. When labeled acetyl-CoA was the substrate, the rate of fatty acid synthesis was decreased by 79% by 0.05 mM RMI 14,514 and by over 95% by concentrations of 0.1 and 0.15 mM of drug (Fig. 1). Incorporation of label from malonyl-CoA was not affected by these same concentrations of drug, as is shown in Fig. 2.

Taken together, these *in vitro* experiments strongly suggest that RMI 14,514 inhibits fatty acid biosynthesis at the step mediated by acetyl-CoA carboxylase, the rate limiting step of the overall process, and that no inhibition of fatty acid synthetase occurs. It is not possible to determine from these experiments whether RMI 14,514 itself or its CoA ester caused the

inhibition of acetyl-CoA carboxylase. The drug resembles a long chain fatty acid in structure, and the conditions of the experiments were such that activation of long chain fatty acids could occur. It is possible that the CoA ester of RMI 14,514 was formed, and that this is the active form of the drug. If so, the inhibition of fatty acid synthesis by RMI 14,514 probably occurs by the mechanism generally regarded as the physiological regulatory mechanism for fatty acid biosynthesis (6-8). Interference of acetate activation, such as produced by a number of acyl-aromatic acids (9), could not be responsible for the reduction of fatty acid synthesis since acetyl-CoA was used as the substrate.

It has been shown that RMI 14,514 also inhibits the translocation of tricarboxylate anions across mitochondrial membranes in an isolated mitochondrial system (3). A decrease in the rate of transfer would result in a decreased supply of acetyl-CoA for lipogenesis and long-chain acyl CoA derivatives are known to inhibit citrate transporter (10). However, recent work using intact hepatocytes has provided indirect evidence that the observed inhibition of fatty acid synthesis by RMI 14,514 cannot be entirely explained by inhibition of citrate transporter, and that the drug acts more strongly on some other process involved in de novo fatty acid biosynthesis (11). This is supported by our earlier study (2) in which fatty acid synthesis in vivo was strongly inhibited in rats pretreated with this drug when acetate was used as the labeled fatty acid precursor.

The data presented in this report indicate that RMI 14,514 is a potent inhibitor of fatty acid biosynthesis in vitro, and that the inhibition occurs at the step mediated by acetyl-CoA carboxylase. These data are consistent with earlier proposals that RMI 14,514 produces its hypolipidemic effects by inhibition of fatty acid biosynthesis.

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