Biochemical Pharmacology, 1967, Vol. 16, pp. 47–52. Pergamon Press Ltd., Printed in Great Britain.

THE INHIBITION OF THE BIOSYNTHESIS OF LONG-CHAIN FATTY ACIDS BY SALICYLATE AND NICOTINATE

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(*Received* 18 *July* 1966; *accepted* 27 *August* 1966)

Abstract—Salicylate and nicotinate inhibit the biosynthesis of long-chain fatty acids in cell-free enzyme systems from rat liver and adipose tissue. These compounds act by inhibiting the enzyme acetyl-CoA:carbon dioxide ligase (ADP) (E.C. 6.4.1.2). Fifty per cent inhibition of the enzyme occurs at a concentration of 10^{-3} M for salicylate and 6×10^{-3} M for nicotinate.

AMONG the numerous pharmacological actions of salicylates are those concerned with lipid metabolism. They include the depression of acetate incorporation into long-chain fatty acids in rat liver[1] as well as the lowering of serum cholesterol[2] and free fatty acids in man[3] and experimental animals.[4] Such effects are of particular interest since they have been demonstrated at dosage levels which are well tolerated and at blood levels often encountered therapeutically.

The mechanism by which salicylate exerts its effect on the fatty acids of serum is not understood. The depression of biosynthetic reactions to be expected from the uncoupling of oxidative phosphorylation by salicylates has been offered as the explanation for the diminished acetate incorporation into liver fatty acids.[1] Another possible mechanism for the action of salicylates, however, might be a direct one in inhibiting the biosynthesis of long-chain fatty acids. The biosynthesis of long-chain fatty acid proceeds from acetate in three steps: the first, the activation of acetate; the second, the obligate carboxylation of acetyl-CoA;[5] and the third, a complex reaction in which the decarboxylation of malonyl-CoA and the reduction of the condensed product occur successively, catalyzed by a multienzyme system to yield the completed long-chain fatty acid molecule.[6] These reactions are summarized below:

1. Acetate + CoA + ATP $\rightleftarrows$ acetyl-CoA + AMP + pyrophosphate (E.C. 6.2.1.1.).
2. Acetyl-CoA + CO_2 + ATP $\rightleftarrows$ malonyl-CoA + ADP + orthophosphate (E.C. 6.4.1.2).
3. Acetyl-CoA + 7 malonyl-CoA + 14 NADPH $\rightarrow$ palmitate + 8CoA + 14 NADP + $7H_2O$.

In the present work the effect of salicylates on fatty acid synthesis in a cell-free system from rat liver is examined. The results indicate that salicylates at levels often encountered therapeutically strongly inhibit the rate-limiting reaction of fatty acid synthesis, the carboxylation of acetyl-CoA.[7, 8] A similar action has been found for

the drug nicotinic acid, also known to lower serum cholesterol in man[9] and to decrease fatty acid and cholesterol synthesis in experimental animals.[10–12]

MATERIALS AND METHODS

Acetyl-CoA was synthesized by the method of Simon and Shemin[13] and malonyl-CoA by the method of Trams and Brady.[14] Both compounds were purified on DEAE-cellulose (Whatman).[15] Salicylic and nicotinic acids (Eastman Chemicals) were recrystallized from a mixture of ethanol and water, and were used as their sodium salts. Acetic-2-^{14}C anhydride and malonyl-1,3-^{14}C were purchased from New England Nuclear Corp. Glucose 6-phosphate, NAD, NADP, ATP, and glucose 6-phosphate dehydrogenase were purchased from Sigma or Calbiochem, and CoA from P-L Laboratories.

The 100,000-*g* supernatant fraction from livers of young rats of the Sprague-Dawley strain was prepared by the method of Langdon[16] except that the homogenization was carried out in a medium that contained 0·25 M sucrose, 0·001 M 2-mercaptoethanol, 0·001 M disodium EDTA, and 0·01 M potassium phosphate buffer, pH 7·4. The 100,000-*g* supernatant fraction, used in all assays, was either freshly prepared or had been stored at −196° for several weeks without loss of activity.

For all enzyme assays the reaction mixture contained potassium phosphate buffer, pH 7·4, 20 μmoles; NAD, 2 μmoles; 2-mercaptoethanol, 3 μmoles; and an NADPH-generating system (glucose 6-phosphate, 2 μmoles; glucose 6-phosphate dehydrogenase, 0·2 Kornberg units; and NADP, 0·5 μmole). When malonyl-1,3-^{14}C-CoA was the substrate no further additions were made. When acetate-2-^{14}C or acetyl-2-^{14}C-CoA was the substrate, the following additions were also made: $MgCl_2$, 10 μmoles; $KHCO_3$, 15 μmoles; and potassium citrate, 20 μmoles. Additional components of the reaction mixtures are specified for each experiment. Incubations in a volume of 1 ml at 37° were terminated by the addition of 10 volumes of isopropanol:isooctane:1 N H_2SO_4 (40:10:1). The extraction of radioactive long-chain fatty acids and their assay by liquid scintillation spectrometry has been described.[17]

The assay of fatty acid synthesis in the Spinco supernatant of rat adipose tissue followed the procedure of Martin and Vagelos[8, 18] except that the radioactive product was extracted as above.

RESULTS

Since the supernatant fraction of rat liver can catalyze the synthesis of long-chain fatty acids from acetate, acetyl-CoA, or malonyl-CoA, the effect of salicylate on synthesis from each of these substrates was examined. As seen in Fig. 1, synthesis of long-chain fatty acids from either acetate or acetyl-CoA is similarly inhibited by salicylate, while synthesis of fatty acids from malonyl-CoA is not inhibited. These results suggested that the salicylate inhibits the reaction catalyzing the carboxylation of acetyl-CoA.

A comparable inhibition of the synthesis of long-chain fatty acids from acetyl-CoA but not from malonyl-CoA is found when sodium nicotinate is examined under conditions of Fig. 1. However, whereas salicylate causes 50 per cent inhibition at a concentration of 10^{-3}M, a 50 per cent inhibition with nicotinate occurs at 6×10^{-3}M.

A possible explanation for the action of these compounds at this step is the removal of a substrate required in the carboxylation of acetyl-CoA. Acetylation of salicylate comparable to the acetylation of a sulfanilamide[19] would remove acetyl-CoA, while an activation of salicylate comparable to that of benzoate[20] would remove ATP. Under these circumstances salicylate would cause little inhibition in the initial rate of

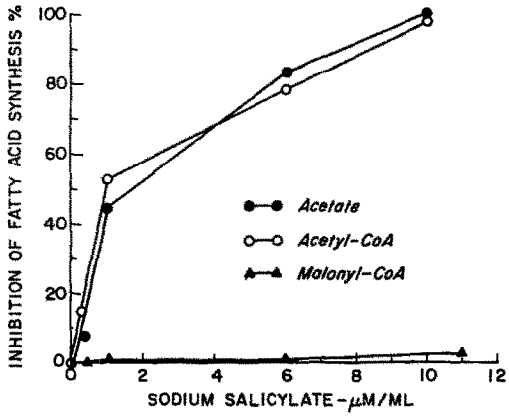

FIG. 1. The effect of salicylate on fatty acid synthesis from acetate, acetyl-CoA, and malonyl-CoA. Incubation mixtures were as described in Methods and contained either 1·0 μmole sodium acetate-2-^{14}C, 0·05 μmole acetyl-2-^{14}C'-CoA, or 0·05 μmole malonyl-1,3-^{14}C-CoA. Incubation with 20 mg protein was for 30 min.

fatty acid synthesis, and its inhibitory action would be more noticeable under conditions of longer incubation. The results of an experiment in which the length of incubation is varied (Fig. 2) indicate that at a concentration of salicylate of 1 μmole/ml, inhibition is 50–60 per cent, regardless of the time of incubation.

The possibility of substrate exhaustion as an explanation of the effect of salicylate was further examined by experiments in which an excess of ATP or acetyl-CoA was added to see if the additional substrate would overcome the inhibitory effect. As seen in Table 1, the addition of ATP does not overcome the inhibition caused by salicylate. In these experiments, 0·10 μmole of acetyl-CoA was saturating for the acetyl-CoA carboxylase and so at least a fivefold excess of acetyl-CoA does not remove the salicylate effect. However, the tendency for higher concentrations of acetyl-CoA to reduce the salicylate inhibition suggests that salicylate may act competitively with acetyl-CoA. At the end of the incubation an aliquot of the reaction mixture was subjected to DEAE chromatography[15] in conditions under which acetyl-CoA would be separated from acetate and free fatty acids. The amount of radioactivity remaining in the acetyl-CoA fraction was 20 per cent in the presence of salicylate and 19 per cent in its absence. Thus there was no evidence that the inhibitor removed acetyl-CoA.

By assaying the synthesis of long-chain fatty acids from acetyl-CoA, the effects of salicylate and nicotinate on acetyl-CoA carboxylase have been demonstrated. In the experiments described in Table 2 it can be seen that salicylate and nicotinate also inhibit the fatty acid synthetase reaction; but inhibition of this enzyme occurs at a

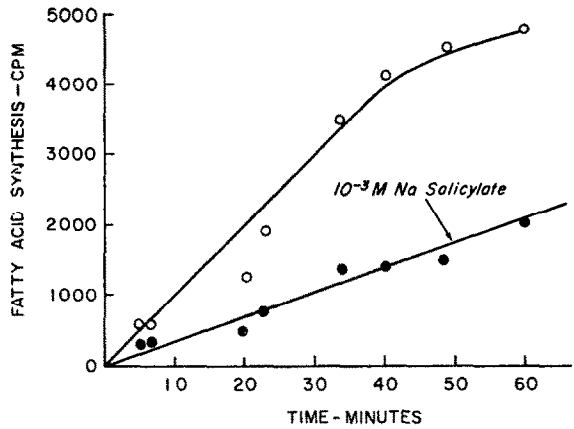

FIG. 2. Time course of fatty acid synthesis under conditions of salicylate inhibition. Incubation mixtures were as described in Methods, with the addition of 0·05 μmole acetyl-2-^{14}C-CoA (220,000 counts/min) and either no salicylate or 10^{-3}M salicylate. Each incubation mixture contained 20 mg enzyme protein and was incubated for the time indicated.

TABLE 1. THE EFFECT OF ATP AND ACETYL–COA ON INHIBITION BY SALICYLATE

Additions to reaction mixture			Fatty acid product	
ATP (μmoles)	Acetyl CoA (μmoles)	Salicylate (μmoles)	(counts/min)	(% inhib.)
	0·025		1100	
	0·025	1·0	540	51
1	0·025		960	
1	0·025	1·0	390	59
	0·5		4050	
	0·5	1·0	3010	26
	0·5		4050	
	0·5	3·0	2500	38

Incubation mixtures were as described in Methods, with the additions noted above. All incubations contained 20 mg protein and were of 30-min duration. Specific activity of acetyl-CoA: 4·4 × 10^6 counts/min/μmole.

TABLE 2. EFFECT OF INHIBITORS ON THE FATTY ACID SYNTHETASE REACTION

Inhibitor	Conc. × 10^3 M	(% inhib.)
Sodium nicotinate	3	0
	10	0
	30	11
	100	40
Sodium salicylate	0·6	0
	2	4
	6	15
	20	44

Assay conditions were as described in Methods, with 0·05 μmole malonyl-1,3-^{14}C-CoA, 8 mg enzyme protein, and the inhibitor additions noted above. Incubation was for 10 min.

concentration at least 20-fold higher than that causing inhibition of the carboxylase reaction. These data and the evidence that the carboxylase is rate limiting in fatty acid synthesis[7, 8] indicate that the significant effect of salicylate and nicotinate is on the carboxylase reaction of fatty acid synthesis.

TABLE 3. FATTY ACID SYNTHESIS BY ENZYMES FROM ADIPOSE TISSUE

Salicylate conc.	% Inhibition of fatty acid synthesis	
(μmoles/ml)	From acetyl-2-^{14}C-CoA	From malonyl-1,3-^{14}C-CoA
1	32	6
10	98	16

In view of the contribution to serum fatty acids made by adipose tissue, the effect of salicylate was also examined in a fatty acid-synthesizing system from rat adipose tissue. The results shown in Table 3 indicate that, as in the liver system, salicylate inhibits fatty acid synthesis from acetyl-CoA, while it exerts no significant inhibition on synthesis from malonyl-CoA.

DISCUSSION

Inhibition of the enzyme acetyl-CoA carboxylase seems to explain the action of salicylate and nicotinate in lowering the incorporation of acetate into long-chain fatty acids of liver.[1, 10, 11] It would be intriguing if the depressed synthesis of fatty acids in adipose tissue were in some way related to the lowering of serum fatty acids by salicylate.

It has been shown that nicotinate reduces the incorporation of acetate into cholesterol in liver[10, 11, 21] and, although some workers do not confirm these findings,[12] this block seems to occur prior to mevalonate.[21] The finding that mevalonate is derived at least in part from malonyl-CoA[22] suggests that such a block in cholesterol biosynthesis may also be due to the inhibition of acetyl-CoA carboxylase. Thus at least some of the derangements in lipid metabolism reported for nicotinate and salicylates may be due to the effects of these drugs on acetyl-CoA carboxylase.

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