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Department of Microbiology, New York Medical College, Valhalla, NY 10595, U.S.A.

HERBERT S. ROSENKRANZ

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The effect of salicylate on the activity of acetyl-CoA carboxylase in rat liver

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One of the characteristics of the widely used pharmacological drug—salicylate—is its effect on lipid metabolism, particularly, on lipid synthesis. It has been established that salicylate administered intraperitoneally to rats in doses of 200–500 mg/kg inhibits the incorporation of the labelled acetate into fatty acids of the liver [1]. Goldman[2] has shown that salicylate in concentrations of 10^{-4} and 10^{-3} M inhibits by 30 and 90 per cent respectively the synthesis of fatty acids in the soluble fraction of rat liver.

In our laboratory it has been shown that salicylate inhibits the incorporation of [1-^{14}C]acetate into the total unsaponifiable lipids and fatty acids but does not affect the incorporation of [2-^{14}C]mevalonate into cholesterol by the supernatant fraction (700 *g*) of rat liver homogenate [3]. Later it was shown that salicylate in concentration of 10^{-2} M almost completely inhibits the incorporation of [1-^{14}C]acetyl-CoA into unsaponifiable lipids and decreases markedly (by 65–79 per cent) incorporation of this substrate into fatty acids. However, the incorporation of [2-^{14}C]malonyl-CoA into unsaponifiable lipids and fatty acids is not inhibited by salicylate [4, 5]. It was assumed that salicylate acts upon the stage of carboxylation of acetyl-CoA, inhibiting the key enzyme of fatty acid synthesis—acetyl-CoA carboxylase (Acetyl-CoA: CO_2 ligase (ADP), E.C.6.4.1.2).

The present research concerns the effect of salicylate on the activity of acetyl-CoA carboxylase in the partially purified soluble fraction of rat liver. In the experiments *in vitro* salicylate was added directly to the soluble fraction; in the experiments *in vivo* salicylate was administered intraperitoneally.

METHODS

Wistar male rats (150–200 g) were used and kept on the usual laboratory ration. To study the possible inhibition of carboxylase activity by salicylate *in vivo* potassium salicylate was administered intraperitoneally twice, 14 and 2 hr prior to decapitation, in a dose of 250 mg per 1 kg of body wt. The control animals received saline twice. After decapitation of the animals the blood in the liver was washed out with physiological saline through *V. portae* and the pooled 4–8 livers passed through a tissue press to remove the connective tissue and then homogenized in phosphate buffer solution, pH 7.4, containing EDTA and 2-mercaptoethanol [6]. All procedures were conducted at 0°. The homogenate was centrifuged at 700 *g* for 10 min. The resulting supernatant was centrifuged twice at 12,000 *g* for 15 min and once at 140,000 *g* for 60 min, and then subjected to gel filtration in a Sephadex G-25 column to remove

Table 1. Inhibition by salicylate of acetyl-CoA carboxylase in the partially purified soluble rat liver fraction

Index	Control	Salicylate 5×10^{-3} M	2.5×10^{-3} M	10^{-3} M	10^{-4} M
Activity of enzyme* (M ± m)	8110 ± 1146	3046 ± 479	4556 ± 596	5680 ± 1574	6035 ± 1229
n	6	6	6	6	9
P	—	< 0.01	< 0.05	< 0.2	< 0.4
% of decrease	—	62	44	30	25

* The activity of enzyme is expressed in cpm of $H^{14}CO_3^-$ fixed by acetyl-CoA in the liver fraction per mg of protein for 10 min of incubation.

endogenic low-molecular substrates. The purified fraction was used as a crude enzyme preparation. The activity of acetyl-CoA carboxylase in this fraction was determined by fixation of $H^{14}CO_3^-$ after activation of enzyme by citrate according to the method of Chang *et al.* [7]. $NaH^{14}CO_3$ was added in concentration of 10 μM per sample ($6–8 \times 10^5$ cpm). Radioactivity was measured in the liquid scintillation counter Mark II (Nuclear Chicago). The background radioactivity was measured in all instances without acetyl-CoA in reaction mixture. Protein determination was made by the biuret method [8]. Acetyl-CoA was received from Sigma.

RESULTS AND DISCUSSION

The data obtained in the experiments *in vitro* (Table 1) show that salicylate does inhibit the activity of acetyl-CoA carboxylase in partially purified rat liver supernatant. At a salicylate concentration of 5×10^{-3} and 2.5×10^{-3} M, the inhibition of $H^{14}CO_3^-$ incorporation was in average 62 and 44 per cent respectively. At a concentration of 10^{-3}–10^{-4} M the salicylate inhibited reaction by 30–25 per cent, although this inhibition was not statistically significant. Approximately the same degree of inhibition was observed in earlier studies on the inhibiting effect of salicylate on [^{14}C]acetate and [^{14}C]acetyl-CoA incorporation in fatty acids and unsaponifiable fraction [4, 5]. This allows the suggestion that the latter is due to the inhibition of acetyl-CoA carboxylase activity.

The *in vivo* experiments show that i.p. injection of salicylate (250 mg per kg body wt) in rats produces in average 63 per cent inhibition of acetyl-CoA carboxylase in liver (Table 2).

Table 2. Effect of intraperitoneal administration of 250 mg/kg body wt of salicylate on acetyl-CoA carboxylase activity in liver (cpm/mg protein)

Number of experiment*	Control	Experiments
1	3080	1520
2	2570	1250
3	3000	394
M ± m	2880 ± 171	1050 ± 314
P	—	< 0.01
% of decrease	—	63.5

* Each experiment represents the data obtained by investigation of homogenate prepared from 4 rat livers.

Thus salicylate inhibits the activity of acetyl-CoA carboxylase both *in vitro* and *in vivo*.

This appears to account for the inhibition by salicylate of fatty acid synthesis observed by several workers [1–5]. The doses used in the present study (250 mg per 1 kg of body wt) were also employed by other researchers to study the effect of salicylate on the synthesis of fatty acids [1].

The molar salicylate concentration of the order of 10^{-3} and 10^{-4} M used in the *in vitro* experiments correspond to the wt concentrations of about 0.1 and 0.01 mg/ml. Considering that in some diseases salicylate is taken in large doses, one may assume that the above concentrations can also be attained in the liver. Note that many workers concerned with salicylate action on various types of metabolism used salicylate in similar concentrations [9–11].

The results obtained permit the conclusion that the hypolipidemic action of salicylate observed by many workers is related to the inhibition of acetyl-CoA carboxylase activity.

Laboratory of Lipid Metabolism,
Institute of Experimental Medicine,
Leningrad, USSR

A. N. KLIMOV
T. V. DENISENKO
E. D. POLIAKOVA

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