PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 155, 48-50 (1977)

Effect of Salicylate on Ureagenesis in Rat Liver (39742)

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Salicylate is one of the most frequently used drugs and is also the most common cause of poisoning in young children. Thus, the mechanisms of salicylate toxicity are of importance. A recent report described two children with salicylate toxicity and elevated blood ammonia levels (1). Salicylate interferes with mitochondrial energy metabolism by uncoupling oxidative phosphorylation (2). The results reported here indicate that salicylate also inhibits ureagenesis in rat liver.

Materials and methods. Urea production from 10 m*M* NH_4Cl was measured in approximately 0.3-mm thick liver slices from adult male Sprague–Dawley rats incubated 2 hr at 37° as previously described (3) except that Krebs Ringer bicarbonate buffer (4) was used instead of phosphate buffer. The activities of carbamyl phosphate synthetase (EC 2.7.2.5) and ornithine transcarbamylase (EC 2.1.3.3.) were measured in liver homogenates as previously described (3). The methods for measurement of mitochondrial citrulline synthesis, carbamyl phosphate levels, and mitochondrial ornithine uptake have been reported elsewhere (5).

Results. Net urea production by liver slices from four rats was 79.1 ± 8.5 (SEM) μmole of urea/g wet wt/2 hr with no salicylate and 44.8 ± 7.9 μmole/g wet wt/2 hr ($P < 0.01$ paired t test) by slices from the same rats in the presence of 5 m*M* (69 mg/100 ml) salicylate.

The effect of salicylate on mitochondrial citrulline synthesis was also examined. There was a progressive increase in the inhibition of citrulline production as the salicylate concentration was increased from 1 to 5 m*M* (Fig. 1) resulting in 85% inhibition at the higher concentration. Salicylate inhibited citrulline synthesis with all oxidizable substrates tested (experiment 1, Table I).

The activities of carbamyl phosphate synthetase and ornithine transcarbamylase were measured in liver homogenates with and without 5 m*M* salicylate. For carbamyl phosphate synthetase, the mean activities of duplicates with and without salicylate were 602 and 574 μmole/g wet wt/hr, respectively. For ornithine transcarbamylase, the mean activities were 13,900 and 13,100 mole/g wet wt/hr with and without salicylate, respectively. Thus, there was no evidence of a direct inhibition *in vitro* of either of the enzymes required to synthesize citrulline.

In the experiments on citrulline synthesis exogenous ATP was added. Previous studies have shown that although exogenous ATP is necessary for optimal citrulline synthesis it will not directly support citrulline synthesis (6). However, if oligomycin and dinitrophenol are present exogenous ATP will directly support citrulline synthesis (7). Salicylate had little if any effect on citrulline synthesis in the presence of these two compounds (experiment 2, Table I).

The concentrations of carbamyl phosphate in duplicate samples of mitochondria incubated for 15 min as described in the legend to Fig. 1 but without ornithine to minimize citrulline synthesis were 0.038 and 0.042 μmole/mg of protein. Addition of 5 m*M* salicylate reduced the carbamyl phosphate concentrations in duplicate samples to 0.015 and 0.014 μmole/mg of protein. Salicylate at 5 m*M* stimulated mitochondrial ornithine uptake (Table II) when either glutamate or succinate was the oxidizable substrate. This was unexpected as the process is energy requiring and is inhibited by many respiratory inhibitors and uncoupling agents (8). The reason for it is unknown.

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ISSN 0037-9727

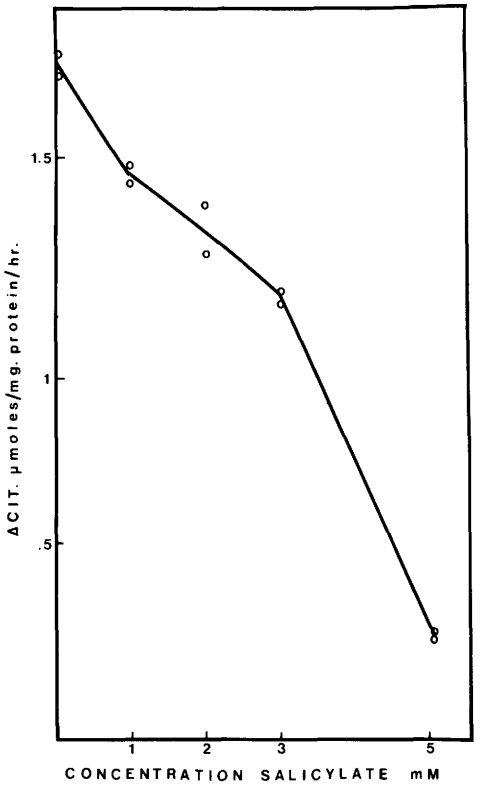

FIG. 1. Mitochondria (2.8 mg of protein) were incubated as described by Charles *et al.* (6) for the measurement of citrulline synthesis for 1 hr at 25° in a medium containing 15 m*M* KCl, 2 m*M* EDTA, 5 m*M* $MgCl_2$, 50 m*M* Tris-HCl buffer, 10 m*M* ornithine, 16.6 m*M* $KHCO_3$, 3 m*M* ATP, 5 m*M* KH_2PO_4–K_2HPO_4 buffer, 25 m*M* sucrose, 10 m*M* NH_4Cl, and 10 m*M* glutamate. The pH was 7.4 and the gas phase was 95% O_2/5% CO_2.

Salicylate in serum is partially protein bound. The addition of 5% albumin (Experiment 3, Table I) inhibited citrulline synthesis so that it is hard to tell whether it partially reversed the effects of salicylate. It clearly did not prevent the inhibition by salicylate.

Discussion. Salicylate at a concentration of 5 m*M* inhibited ureagenesis in rat liver slices by about 45%. The demonstration that salicylate inhibits citrulline synthesis in isolated mitochondria suggests that the block in ureagenesis is in the mitochondrial part of the urea cycle, however, a block at a more distal site has not been directly excluded.

It is well established that salicylate uncouples oxidative phosphorylation. This effect of salicylate is similar to that of dinitrophenol, although about 100-fold higher concentrations of the former are required. Since dinitrophenol is a potent inhibitor of citrulline synthesis it is not surprising that salicy-

TABLE II. EFFECT OF SALICYLATE ON MITOCHONDRIAL ORNITHINE UPTAKE.[a]

Substrate	No salicylate	5 m*M* salicylate
Glutamate (10 m*M*)	35.1, 37.4	83.5, 99.4
Succinate (10 m*M*)	13.9, 14.1	73.9, 89.1

[a] Ornithine uptake was determined by a modification of the procedure of Gamble and Lehninger (8). Values from duplicate incubations are given in nmole of ornithine uptake/flask. Each flask contained 3.7 mg of protein when glutamate was the substrate and 3.6 mg of protein when succinate was the substrate.

TABLE I. EFFECTS OF SALICYLATE ON MITOCHONDRIAL CITRULLINE SYNTHESIS.[a]

Experiment	Substrate or addition	No salicylate	5 mM Salicylate
1. Substrate variable	Glutamate	1.25, 1.26	0.53, 0.55
	Succinate (10 m*M*)	0.58, 0.60	0.06, 0.11
	Citrate (10 m*M*)	1.06, 1.07	0.09, 0.10
	Fumarate (10 m*M*)	0.50, 0.51	0.06, 0.06
2. Substrate glutamate	None	1.32, 1.34	0.40, 0.50
	Oligomycin (10 μg)	0.10, 0.14	–
	Dinitrophenol (0.04 m*M*)	0.24, 0.24	–
	Oligomycin + dinitrophenol	0.60, 0.60	0.55, 0.55
3. Substrate glutamate	None	1.94, 1.94	0.82, 0.88
	5% albumin	1.15, 1.18	0.70, 0.74

[a] Mitochondria were incubated as described in Fig. 1. Glutamate was the oxidizable substrate in all experiments except where indicated in experiment 1. The albumin was essentially fatty-acid-free bovine serum albumin. The flasks contained 4.0, 3.5, and 3.6 mg of protein from different mitochondrial preparations in experiment 1, 2, and 3, respectively. Incubation was for 1 hr in experiments 1 and 3, and for 30 min in experiments 2. Values for duplicate incubations are given in μmol of citrulline produced/60 min/mg of protein.

late inhibits the same process. The data presented here suggest that salicylate inhibits citrulline synthesis, and therefore ureagenesis, by depleting mitochondrial ATP, which in turn results in impaired carbamyl phosphate synthesis. The depletion of mitochondrial ATP is probably due to the effect of salicylate on oxidative phosphorylation.

The concentrations of salicylate used in these studies are comparable to serum concentrations encountered in clinical medicine. Serum concentrations of salicylate of about 0.5 m*M* are reached in an adult taking two aspirins (5 gr each) and a serum concentration of about 2 m*M* would be considered therapeutic in patients with rheumatoid arthritis. A serum concentration of 5 m*M* 6 hr after salicylate ingestion is in the "moderately" toxic range (9). Thus, if a similar effect of salicylate occurs in man as observed in the rat, inhibition of ureagenesis by salicylates may at times be clinically significant.

Data on blood ammonia levels in salicylate toxicity are limited. As mentioned above two children with salicylate intoxication and elevated blood ammonia levels have recently been reported. It is possible that inhibition of ureagenesis, with a resulting elevated blood ammonia level, may be a factor in some cases of salicylate intoxication. In addition, consideration should be given to the possibility that salicylates even in moderate doses might further inhibit ureagenesis in patients with disorders such as urea cycle defects, Reye's syndrome, or propionic acidemia where the urea cycle is already compromised.

Summary. Salicylate at 5 m*M* inhibited ureagenesis from NH_4Cl by about 45% in rat liver slices. Salicylate also inhibited citrulline synthesis in isolated mitochondria. It had no direct effect on carbamyl phosphate synthetase or ornithine transcarbamylase assayed in liver homogenates. Salicylate did not inhibit citrulline synthesis supported by exogenous ATP. Salicylate lowered the carbamyl phosphate concentration in mitochondria incubated without ornithine to minimize citrulline synthesis. Unexpectedly salicylate stimulated mitochondrial ornithine uptake. The inhibition of ureagenesis is probably due to the uncoupling of oxidative phosphorylation which depletes mitochondrial ATP which in turn impairs carbamyl phosphate synthesis.

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Received July 8, 1976. P.S.E.B.M. 1977, Vol. 155.