Action of SCN^- on Rat Liver Mitochondria[1] (34496)

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Thiocyanate has been shown to inhibit the following functions in liver mitochondria: Succinate and β-hydroxybutyrate oxidation, P/O ratio, ATP binding, P_i–ATP exchange, and valinomycin-induced K^+ uptake. This inhibition can be overcome by increased ADP or substrate concentrations, and is presumably due to interaction of SCN^- with anion-binding sites in the mitochondrial membrane.

It has been shown (1) that the action of SCN^- on the gastric mucosa is not limited to inhibition of HCl secretion, but appears to extend to effects on K^+ permeability and on metabolism since there is alteration in O_2 consumption (2) and inhibition of microsomal and mitochondrial ATPase (3).

By measurements of the action of SCN^- on: (1) P/O ratio of mitochondrial preparations with a variety of substrates; (2) oxidation rate of various substrates; (3) ion transport of mitochondria; and (4) binding of nucleotides by mitochondria, it was shown that SCN^- had significant effects on various aspects of mitochondrial metabolism. These results have been presented in preliminary form (4).

Methods. Rat liver mitochondria were prepared by differential centrifugation using standard techniques with three additional washings (5), and the purity of the preparation was routinely checked by phase-contrast microscopy. The medium was 0.25 *M* sucrose, 0.001 *M* Tris, pH 7.4, and 0.001 *M* EDTA. The mitochondria were either used fresh, aged for 24 hr at 4°, sonicated for 30 sec at 0°, or treated with digitonin (6). For measurement of oxidative phosphorylation, either the Chance-Williams technique (7), or the glucose-hexokinase method were used. The medium contained 50 m*M* Tris HCl, pH 7.4, 5 m*M* $MgNO_3$, 2.5 m*M* K_2HPO_4, 0.5 m*M* EDTA, 2.5 mg/ml albumin, and 55 m*M* KCl. Rate of oxidation of various substrates at 10 m*M* was measured using either the Clark electrode or the Warburg apparatus. NADH-cytochrome *c* reductase was measured spectrophotometrically (8), as was cytochrome *c* oxidase (9) and succinate:Fe $(CN)_6^{3-}$ oxidoreductase (10). K^+ release and uptake by mitochondria was monitored by a system consisting of three electrodes specific for H^+, Na^+, and K^+. Binding of nucleotides was assayed by adding the labeled di- or triphosphopo-nucleotide (0.2 m*M*) to a medium at 0° containing 2 mg mitochondrial protein, 100 m*M* Tris-Cl, pH 7.4, and 5 m*M* $MgCl_2$, spinning at 37,000*g*, and counting the precipitated mitochondria. Controls were run by comparing counts obtained with tracer only to counts obtained with excess cold nucleotide in addition to the same amount of tracer. Results were corrected by the latter amount. P_i–ATP exchange was measured according to Conover *et al.* (11). Protein was measured by the Lowry method (12).

Results. Mitochondria obtained in this study showed respiratory control ratios of better than 8.0 with β-OH butyrate, malate, or succinate. Addition of SCN^- to mitochondria in state 3 inhibits O_2 consumption by 25% at 10 m*M*, and 45% at 30 m*M* when β-OH butyrate is used as substrate. When the mitochondria are uncoupled by using dinitrophenol or carbonyl cyanide *m*-chlorophenylhydrazone the action of SCN^- is quantitatively similar.

Measurement of P/O ratios, using the O_2 consumption increment obtained upon the addition of ADP to state 4 mitochondria,

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TABLE I. Effect of 10 and 30 m*M* SCN^- on P/O Ratios of Rat Liver Mitochondria (Five Experiments in Each Group) Using the ADP Addition (O_2) or Hexokinase Method.

	P/O ratio					
	Control		10 m*M* SCN^-		30 m*M* SCN^-	
	O_2	Hexokinase	O_2	Hexokinase	O_2	Hexokinase
Succinate	1.8	1.48	1.1	1.11	0.85	0.84
β-OH Butyrate	2.5	2.21	1.9	1.76	1.41	1.14

showed that in the preparations a ratio of 1.6 was obtained with succinate as substrate and 2.5 with β-OH butyrate. Addition of SCN^- resulted in a slower rate of ADP-dependent O_2 consumption, as did addition of atractylate of fluoride. The P/O ratios, however, calculated from the total O_2 uptake declined slightly. Similar results were obtained using the hexokinase method (Table I). Increasing the ADP/SCN ratio gradually restored the oxidation rate, which would suggest competitive effects, particularly since similar data are obtained with atractylate. Since previous work used high ADP/SCN ratios, these effects were not observed (13).

Sonicated mitochondria have a much reduced sensitivity to SCN^-, with succinate as substrate, whereas digitonin-treated mitochondria appear identical to intact mitochondria in terms of their response to SCN^-, data similar to those for atractylate (14).

SCN^- had no effect on ferrocytochrome *c* oxidation but succinate:$Fe(CN)^3_6$ oxidoreductase was inhibited 45%.

Table II summarizes data on the effect of SCN^- and atractylate on binding of ADP and ATP to intact mitrochondria. Whereas atractylate reduces binding of ADP by about 90%, SCN^- reduces nucleotide binding, ATP more than ADP, by about 40%. Additionally P_i–ATP exchange is inhibited by 30 m*M* SCN^- as effectively as by oligomycin.

With the demonstration that K^+ flux is inhibited in the gastric mucosa, measurements of K^+ movement in intact mitochondria using cation-specific electrodes showed that SCN^- significantly slows the uptake of K^+ induced by valinomycin, but is without effect on subsequent nigericin-induced K^+ efflux (Fig. 1).

Discussion. Thiocyanate ion (SCN^-) appears to inhibit coupled or uncoupled mitochondrial respiration. Although no effect was observed distal to cytochrome *c*, inhibition of NADH-cytochrome *c* oxidoreductase and succinate:$Fe(CN)^3_6$ oxidoreductase was demonstrated, and the degree of inhibition could account for the respiratory inhibition observed in uncoupled mitochondria. A slight reduction in P/O ratios was observed, and the respiration rate was slowed, which could be overcome by increasing the ADP/SCN^- ratio, similar data being observed for atractylate (15).

TABLE II. Binding and Exchange Activity of Rat Liver Mitochondria.

	Binding (mμ*M* mg^{-1} protein)		ATP-P_i exchange (mμ*M* P_i incorporated mg^{-1} protein)
	H^3ATP	H^3ADP	
Control	2.4	2.5	23
Atractylate (10^{-6} *M*)	.7	0.8	—
Oligomycin (0.05 mg/ml)	2.3	2.4	2.8 (0.1 mg/ml)
SCN^- (30 m*M*)	1.4	2.0	1.7
Oligomycin + atractylate	1.3	0.5	—
Oligomycin + SCN^-	1.3	1.4	—

Means of 3 experiments.

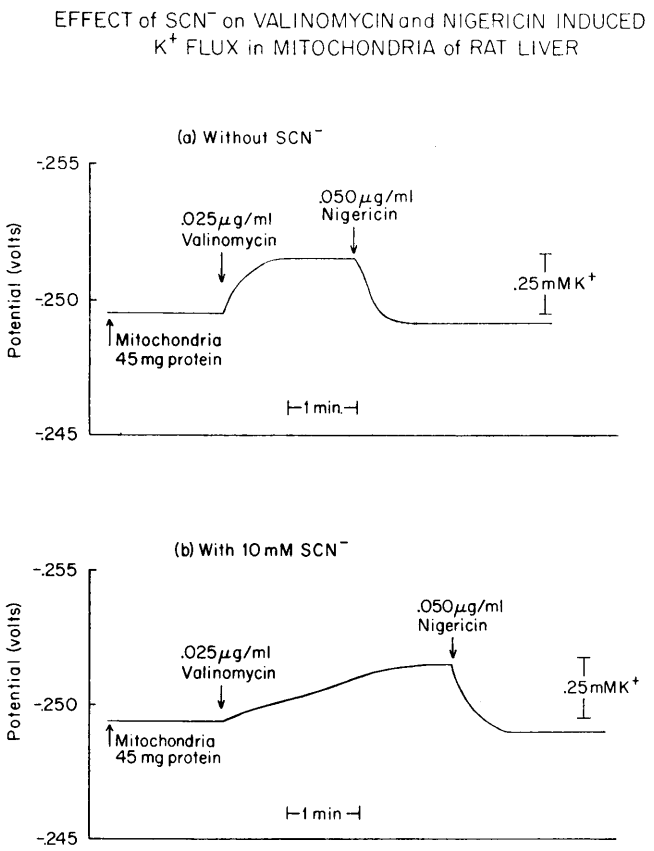

FIG. 1. Effect of SCN^- on K uptake and efflux in rat liver mitochondria induced by valinomycin and nigericin.

Since sonication, but not digitonin treatment, reduced SCN^- effectiveness, there would appear also to be a structural requirement for the action of SCN^--inhibited binding of labeled ATP or ADP by mitochondria. These effects were less than those observed with atractylate and were greater for ATP than for ADP. Since nucleotide binding is required for P_i–ATP exchange, the inhibitory action of SCN^- on this reaction confirmed the action of SCN^- on nucleotide binding.

From these data, and those reported for the gastric mucosa (1), it would appear that SCN^- interferes with both cation- and anion-transport processes across membranes, presumably by binding at anion sites and altering the electrostatic properties of those membranes.

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