Vol. 28, No. 2, 1967 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS

OXIDATIVE PHOSPHORYLATION AND THE EFFECTS OF SCN^- ON MITOCHONDRIA ISOLATED FROM RABBIT STOMACH

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Received June 14, 1967

One of the proposed theories to account for H^+ transport by gastric mucosa involves a redox mechanism whereby proton secretion is directly linked to electron flow through an Fe^{++}-Fe^{+++} system with molecular oxygen serving as the final electron acceptor (1,2,3). Simultaneous measurements of the H^+ secreted (qH^+) and O_2 consumed (qO_2) by gastric mucosa have produced qH^+/qO_2 ratios less than 4.0, the theoretical limit of a simple redox scheme (3,4,5). These results have supported the possibility of such a mechanism for gastric H^+ production. Experimental manipulations involving the use of stimulants and inhibitors of gastric H^+ secretion have shown that the $\Delta qH^+/\Delta qO_2$ induced by the particular treatment may be considerably higher than 4.0, but there has been controversy with respect to interpretation of these results and the evaluation of a "resting" oxygen consumption (3,6,7).

A study of oxidative phosphorylation by mitochondria isolated from gastric mucosa was undertaken. If tight coupling between electron transport and high energy phosphate production were demonstrated it would be unlikely that a simple redox scheme would drive the secretion of gastric H^+. The following study was designed to evaluate the biochemical function of stomach

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This work was supported by a grant from USPHS, AM 10141, and in part by a grant from the Hartford Foundation. Dr. Saltman is a Research Career Development Awardee of the USPHS.

mitochondria, and to test the effects of SCN^-, a well-known inhibitor of gastric H^+ secretion, on mitochondrial reactions.

METHODS

Rabbits were killed with an air embolism, the stomach removed and perfused with cold 0.15 M NaCl. The gastric mucosa from the main body of the stomach was isolated from the muscle coats,then minced and homogenized in a medium containing 225 mM mannitol, 75 mM sucrose, 0.2 mM EDTA and 400 mg% bovine serum albumin. The mitochondrial fraction was isolated by differential centrifugation as the pellet which sedimented between 5000 x g for 5 min and 12,000 x g for 10 min. The pellet was washed in the medium and recentrifuged once. It was essential to retain bovine serum albumin in all subsequent experimental solutions in order to provide optimal values for oxidative phosphorylation.

Esterification of $^{32}PO_4$ into ATP was measured using the glucose-hexokinase trap method. The 2.6 ml reaction volume contained 63 mM histidine-HCl buffer (pH 7.5), 11 mM Na glutamate, 0.4 mM ADP, 4 mM $MgCl_2$, 3 mM glucose, 0.15 mg hexokinase (Calbiochem), 6 mg bovine serum albumin, 85 mM sucrose, and mitochondrial suspension (0.2-0.5 mg protein). The reaction vessels were brought to 35°C and the reaction was started by adding 0.1 ml of 0.1 M PO_4 buffer (K^+ salt at pH 7.5) containing 2-5 μC $^{32}PO_4$. After 5 min the reaction was stopped with 0.5 ml 20% trichloracetic acid and the tubes were immediately placed into an ice bath. Subsequently the method of Avron (8) was used to extract inorganic phosphate as the phosphomolybdate complex into an organic phase (isobutanol-benzene) and the counts remaining as organic phosphate esters in the aqueous phase were assayed. Chromatograms showed that 95% of the total radioactivity remaining in the aqueous phase was glucose-6-PO_4.

Mitochondrial respiration was measured polarographically using a Clark oxygen electrode which was mounted in a glass chamber (2.7 ml volume). For the ATPase assay the rate of liberation of inorganic phosphate from ATP was measured by the method of Fiske and Subbarow (9).

RESULTS

Repiratory control in rabbit gastric mitochondria is shown in Fig. 1. Calculated ADP/O values for various gastric mitochondrial preparations were between 2.2 and 2.7 using glutamate as substrate. Rabbit kidney cortex mitochondria prepared by similar procedures gave ADP/O ratios of 3.0 for the same substrate. The coupling ratios between fully supplemented mitochondria and those limited by ADP acceptor were lower in gastric (range 4.0-6.0) than in kidney (7.0-9.0) mitochondria. Addition of SCN^- to the chamber reduced the respiratory rate of fully supplemented mitochondria, but relatively high concentrations were required; 40-50 mM SCN^- produced 50% inhibition.

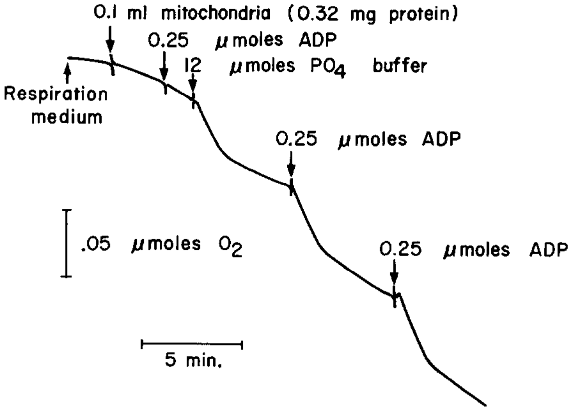

Fig. 1. Respiratory control in rabbit stomach mitochondria. Respiration was polarographically measured at 24°C. The respiration medium contained 63 mM histidine-HCl buffer (pH 7.5), 4 mM $MgCl_2$, 85 mM sucrose, 2 mg/ml bovine serum albumin, and 11 mM Na glutamate as substrate. Average ADP/O values of 2.5 were obtained for the above preparation.

The incorporation of inorganic phosphate into ATP by stomach mitochondria and its inhibition by SCN^- is demonstrated in Table 1. The experiments using KCl were included to show the effect of ionic strength on $^{32}PO_4$ incorporation. High concentrations of SCN^- are required to significantly reduce phosphate esterification (40% inhibition with 83 mM SCN^-) in contrast to the almost complete oligomycin sensitivity (98% inhibition with 2 µg/ml).

Table 1

EFFECT OF SCN^- ON THE ESTERIFICATION OF $^{32}PO_4$
INTO ATP BY RABBIT STOMACH MITOCHONDRIA

Concentration of salt	KSCN	KCl
0	6.7 (6)	
0.8 mM	6.7 (3)	6.3 (2)
8.3 mM	5.8 (6)	6.6 (2)
83.0 mM	4.1 (6)	6.0 (2)
166.0 mM	1.0 (2)	3.4 (2)

Values are given as µmoles PO_4 esterified/mg protein/5 min. The number of mitochondrial preparations tested is given in the parentheses.

Oxidative phosphorylation and respiratory activity of rabbit stomach mitochondria are uncoupled by 2,4 dinitrophenol (Fig. 2). Maximum coupling occurs at about 10^{-4}M DNP, whereas respiratory activity is decreased by higher concentrations. The effects of DNP on mitochondrial respiration observed here are consistent with those resported by Davies (10) and by LeFevre and Rehm (11) using intact frog gastric mucosa.

SCN^- is an effective inhibitor of frog gastric microsomal ATPase (12). We have previously reported its influence on gastric microsomal ATPase (13). A summary of the effects of this anion on the Mg^{++}-activated ATPase of intact rabbit stomach mitochondria is given in Table 2. Again it should be noted that relatively high concentrations of SCN^- are required to reduce mitochondrial ATPase activity.

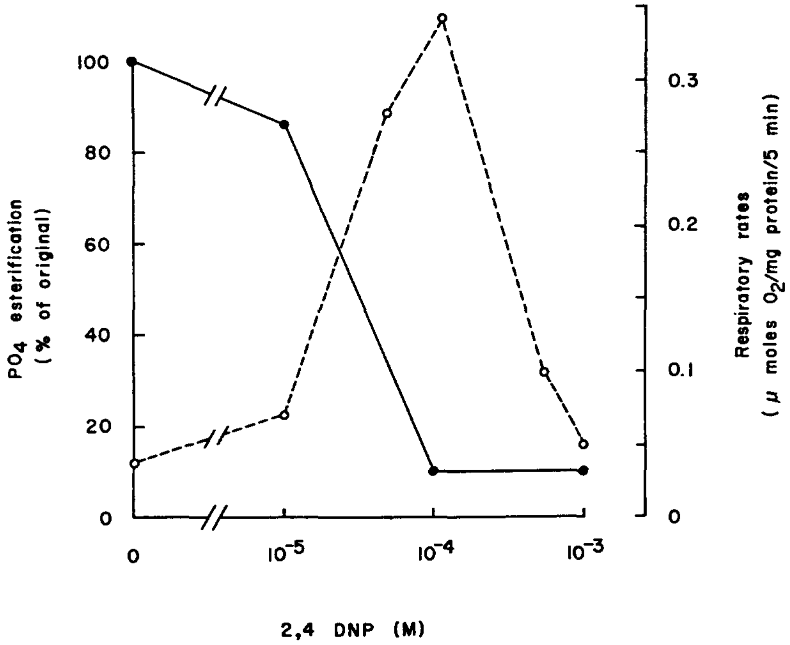

Fig. 2. Effect of 2,4 DNP on PO_4 esterification and respiratory rate of rabbit stomach mitochondria. Conditions for the measurement of PO_4 esterification ( ) were as described in the text. Glutamate was used as substrate for mitochondrial respiration ( ) and experimental conditions were as described in the legend to Fig. 1.

Table 2

EFFECT OF SCN^- ON MG^{++}-ACTIVATED ATPase of RABBIT STOMACH MITOCHONDRIA

	μmoles P_i liberated/mg protein/hr
Control (no additions)	12.3 ± 0.9
0.8 mM KSCN	11.1 ± 1.2
8.3 mM KSCN	8.2 ± 1.2
16.6 mM KSCN	7.6 ± 1.2
33.3 mM KSCN	5.2 ± 0.3
83.0 mM KSCN	3.6 ± 0.5
oligomycin (3.3 μg/ml)	2.4

Values are given as the mean ±S.E. of four individual mitochondrial preparations. Reactions were carried out at 35°C in 1.5 ml volume containing: 43 μmoles histidine-HCl buffer (pH 7.5); 4.5 μmoles $MgCl_2$; 4.5 μmoles ATP (Na salt); and 0.1 ml mitochondrial suspension containing 0.5-1.0 mg protein. Control reaction tubes contained 220 μmoles sucrose which was removed progressively as the concentration of KSCN was increased in order to maintain the same osmolarity.

DISCUSSION

These experiments demonstrate that mitochondria isolated from rabbit gastric mucosa have mechanisms of oxidative phosphorylation and respiratory control typical of mitochondria from many tissues. Undoubtedly these preparations contain mitochondria from cell types of the stomach other than the acid-secreting cells. However, because of the large number of parietal cells in rabbit fundic mucosa and the great abundance of large mitochondria within these cells, it is likely that the predominate population of mitochondria in these preparations is from the acid-secreting cells.

The chemiosmotic hypothesis, proposed by Mitchell for mitochondrial oxidative phosphorylation, has recently stimulated a great deal of interest (14). The present experiments do not represent an unequivocal test of the chemiosmotic hypothesis. However, if such a mechanism were operative in a physiological system, the mitochondria of acid-secreting cells might represent a specially evolved case whereby the proton gradient created by electron transport would be directly available for secretion, and not be used for the synthesis of ATP. That gastric mitochondria do readily synthesize ATP makes it unlikely that the H^+ of the secretory product are provided by substrate H atoms directly linked to electron transfer, through the mitochondrial cytochrome chain, to molecular oxygen. With numerous, large, actively phosphorylating mitochondria competing for molecular oxygen in order to synthesize ATP, a simple redox hypothesis to account for gastric H^+ secretion is untenable.

Alternative mechanisms have been proposed where ATP produced by mitochondria is utilized at the apical secretory membrane or the smooth-surfaced endoplasmic reticulum of the acid-secreting cell to provide gastric H^+. A detailed mechanism for such a scheme may be provided by an ATPase or phosphatase in the secretory membrane whose active site is assymetrically oriented with respect to H^+ and OH^- accessibility (14) or by mechanisms which include a redox scheme driven by a phosphorylated high energy carrier system (15).

The results also show that SCN^- does inhibit mitochondrial function, i.e. oxidative phosphorylation, respiration and ATPase. However, the concentration of SCN^- required to produce significant decrements in these parameters is considerably higher than that required to inhibit H^+ secretion by gastric mucosa (7). Thus it is unlikely that SCN^- manifests its primary effect on H^+ secretion by inhibiting mitochondrial reactions. On the other hand, inbibition of frog gastric microsomal ATPase by SCN^- more closely parallels the observed effects of this anion on the secretion of acid by gastric mucosa.

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