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INTERACTION OF MAMMALIAN KIDNEY MEMBRANE AND MITOCHONDRIA *IN VITRO*

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SUMMARY

1. In many parts of the kidney tubule, there exists a close physical association of membrane and mitochondria apparently related to salt transport. The present study examines the influence of adding rat kidney membrane fragments on mitochondrial respiration *in vitro*. The results are compared with previous findings on the effect of rat kidney membrane fragments on cytoplasmic glycolysis.

2. The membrane fraction stimulates mitochondrial respiration. The stimulus decreases with higher levels of ADP and is abolished by dinitrophenol. Stimulus of glycolysis by kidney membranes is related to a specific coupling of membrane ATPase activity and phosphoglycerate kinase. The stimulus of respiration appears to be due solely to the enhanced availability of ADP in presence of an ATPase system.

INTRODUCTION

Kidney mitochondria are concentrated and closely aligned to the deeply infolded membranes of the basal interstitial surface of renal tubule cells in both the proximal and distal tubules[1,2]. According to electrophysiologists, this interstitial membrane surface is the site of the Na^+ pump involved in the active reabsorption of Na^+ from the tubular fluid[3,4]. High levels of ATPase activity appear to be localized at this membrane surface[5]. The association of membrane and mitochondria at the peritubular surface suggests a relation between this feature of cell structure and renal transport of Na^+.

(Na^+-K^+)-dependent ATPase activity is believed to be a manifestation of the membrane system for active Na^+ extrusion coupled to K^+ uptake[6]. It has been proposed that membrane ATPase activity associated with active cation transport serves as a regulating pacemaker for cellular respiration[7]. This control of respiration would be mediated by ADP liberated as a result of the ATPase activity associated with membrane transport.

The present study examines the effect of isolated membrane fragments on respiration of kidney mitochondria *in vitro*. The membranes are found to stimulate respiration of mitochondria in a manner which indicates interaction between mitochondrial ATP production and membrane ATPase activity. The membrane stimulus of respiration, however, differs in some ways from a previously reported membrane ATPase stimulation of cytoplasmic glycolysis[8].

METHODS

Male albino Sprague–Dawley rats 3 months old were employed in this study. The preparation of a rat kidney membrane fraction and the preparation of the soluble cytoplasmic fraction (S_3) of rat kidney has been described previously[9]. The membrane fraction is essentially a fraction of light microsomes and consists exclusively of membrane fragments when examined with the electron microscope[9]. The fraction manifests Mg^{2+}-dependent and (Mg^{2+} + Na^+-K^+)-dependent ATPase activity and is devoid of biochemical activity associated with intact mitochondria[9]. Prior to dialysis or deoxycholate addition, Mg^{2+}-ATPase activity is very high. About half of this activity is associated with Na^+ and K^+ bound to the membrane preparation[9]. When frozen at −60° and stored at −20°, biological activity of the fraction appears to be stable for a considerable period of time. The soluble S_3 fraction contains enzymes of glycolysis.

Mitochondria were prepared in the following manner. Kidneys from 2 rats were homogenized with a teflon pestle Potter homogenizer in 16 ml of 0.35 M sucrose containing 1 mM Na_2 EDTA. The homogenate was centrifuged at 480 × *g* for 10 min in a Servall RC-2 refrigerated centrifuge. The supernatant of this spin was recentrifuged at 1935 × *g* for 10 min. The sediment was resuspended in 0.88 M sucrose and centrifuged at 7700 × *g* for 10 min. This mitochondrial sediment was resuspended in 8 ml of 0.25 M sucrose with 1 mM Na_2EDTA. The mitochondrial preparation was used immediately. All preparative operations were carried out at 0–2°.

Ethacrynic acid [2,3-dichloro-4-(-2 methylenebutyryl) phenoxylacetic acid] was a gift of Dr. HAROLD WILLIAMSON of Merck Sharpe and Dohme, West Point, Pa.

RESULTS

The membrane fraction added to incubating rat kidney mitochondria stimulates the mitochondrial O_2 uptake to a high level (Table I). The stimulus was observed consistently with all substrates that were examined. The increase in respiration is roughly proportional (between 0 and 2 mg membrane protein) to the amount of the membrane fraction added.

It was anticipated that the increase in respiration would be due to membrane ATPase activity enhancing the available ADP for respiration. Several observations support this concept. Increasing levels of ADP in the incubation medium stimulates mitochondrial respiration and also reduces stimulation by the membrane. The incubations in Table I were carried out with 1 mM ADP. When 2 mM ADP is present, the membrane stimulation is reduced about 40 %. The stimulation is largely abolished with 4 mM ADP. Similarly 20 μM dinitrophenol, which uncouples oxidative phosphorylation and eliminates the requirement for ADP in these preparations, abolishes the membrane effect. Hexokinase (0.02 mg crystalline enzyme) and glucose, when added to the mitochondrial incubations enhanced respiration to a considerably lesser extent than the addition of membrane. This hexokinase stimulus is presumably due to the ADP generated. The small stimulus of hexokinase and the larger stimulus of the membrane appear to be additive.

Ethacrynic acid, a potent diuretic compound in some species, inhibits mitochondrial respiration[10]. The effect of ethacrynic acid on mitochondrial respiration

TABLE I

THE EFFECT OF ADDING A KIDNEY MEMBRANE FRACTION ON THE RESPIRATION OF RAT KIDNEY MITOCHONDRIA

Mitochondria (about 2.5 mg protein) were incubated in Warburg flasks containing 3 ml medium and 0.1 ml KOH in center well. Flasks were shaken at 37°. O_2 uptake was measured manometrically. Medium contained 77 mM KCl, 17 mM $NaPO_4$ (pH 7.4), 1 mM ADP, 3 mM $MgCl_2$, 75 mM sucrose and 10 mM of each designated substrate. Approx. 2 mg of membrane fraction protein (0.4 ml of a standard membrane preparation) was added in each incubation where this is indicated. Pyruvate oxidation in these incubations is negligible in absence of malate. Results are tabulated ± S.E. Values between parentheses indicate the range of values.

Substrate	*Number of experiments*	*μl O_2/110 min/2.5 mg mitochondrial protein*		
		No membrane addition	*Membrane fraction added*	*Increment due to membrane addition*
α-Ketoglutarate	25	405 ± 6.2 (346 to 456)	560 ± 8.7 (465 to 648)	155 ± 9.6 (86 to 256)
Pyruvate + malate	14	415 ± 10 (341 to 454)	622 ± 17.5 (525 to 715)	207 ± 17.4 (118 to 355)
Glutamate	6	335 ± 13.4 (282 to 369)	533 ± 13.0 (498 to 562)	198 ± 21.7 (136 to 268)
Succinate	7	417 ± 12 (370 to 468)	559 ± 27 (460 to 692)	142 ± 18 (82 to 224)

was compared in presence and absence of membrane. 50 μM ethacrynic acid was found to have no effect on ATPase activity of the membrane fraction employed even with prolonged preincubation of drug and membrane. This level of the compound depresses respiration when tested with the four substrates in Table I. Moderate enhancement of the percentage inhibition of mitochondrial respiration consistently occurs when the respiration was examined in presence of the membrane fraction. Possibly of greater importance, the ethacrynic acid largely abolishes the membrane stimulated component of the respiration.

The enhanced inhibition by ethacrynic acid in presence of membrane could very simply be a result of rate-limiting reaction blocked by the metabolic inhibitor which greatly limits the faster system. Another possibility is that energy transfer from the mitochondria to membrane is blocked by ethacrynic acid.

DISCUSSION

Physiological studies indicate that renal tubular re-absorption of Na^+ *in vivo* is linked to mitochondrial oxidative metabolism[11–13]. Studies of respiration in kidney slices have suggested a coupling of Na^+ transport and respiration[7,14]. Respiration associated with cation transport in kidney slices is depressed by ouabain which apparently acts solely as an inhibitor of ion transport[7]. Respiration in homogenates of rabbit kidney cortex is also depressed by ouabain inhibition of ATPase activity[15].

In previous studies, it was found that isolated kidney membrane fragments stimulate cytoplasmic glycolysis *in vitro*[8]. This stimulation was due to a coupling of membrane ATPase activity and ATP production catalyzed by phosphoglycerate kinase activity of the cytoplasm[8]. The effect was due to ATP removal from the kinase enzyme and not due to ADP generation. Unlike the stimulus of respiration, increases

of ADP or ATP did not abolish the stimulus. Specific coupling of cellular ATP production and ATPase activity of the membrane associated with Na^+ transport was postulated.

The present data suggest that mitochondria closely associated with membranes take up O_2 at a considerably faster rate, at least, *in vitro*. The effect here is apparently due to ADP generation and, unlike stimulated glycolysis, membrane stimulation of respiration disappears at higher ADP levels. There is no evidence in these experiments for a direct coupling between the membrane and electron-transport intermediates of the mitochondria as proposed by CHANCE[16], SLATER[17] and FUJIMOTO, NASH AND KESSLER[13].

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