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Inhibition of mitochondrial translation by calmodulin antagonist *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide

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The possible role of calmodulin in mitochondrial functions was investigated in Ehrlich ascites tumor cell and mouse liver mitochondria employing sulfonamide compounds as calmodulin indicators. *N*-[6-Aminohexyl)-5-chloro-1-naphthalenesulfonamide (W7), the most potent of the sulfonamide compounds, inhibited mitochondrial protein synthesis and oxidative phosphorylation. The inhibitors had no significant effect on mitochondrial cytochrome *c* oxidase, oligomycin-sensitive ATPase and NADH dehydrogenase activities. Depletion of endogenous ATP pool seemed to be the main mechanism of inhibition of mitochondrial translation by sulfonamides. The results also show that mitochondria from hepatic tissues are relatively less sensitive to sulfonamide drugs as compared to the Ehrlich ascites tumor cell mitochondria. Results of Ca^{2+} autoradiography revealed 2–3-fold higher levels of calmodulin-like Ca^{2+} binding protein in extracts from Ehrlich ascites tumor cell mitoplasts as compared to mitoplasts from mouse liver. These results suggest cell and tissue specific variations in Ca^{2+}-dependent processes in the mitochondrial compartment.

Introduction

The importance of Ca^{2+} as a second message for the regulation of metabolic and physiological activities in different intracellular compartments has been well documented [1]. Recent experiments from various laboratories suggest a direct role for Ca^{2+} in the regulation of some of the key mitochondrial enzymes involved in the energy metabolism [19]. In higher animals, Ca^{2+} has been shown to enhance the activity of three mitochondrial specific dehydrogenases, namely, pyruvate dehydrogenase, αketoglutarate dehydrogenase and isocitrate dehydrogenase [9]. Calmodulin, a multifunctional Ca^{2+} binding protein is known to modulate the activity of several Ca^{2+}-dependent enzymes in different cell compartments including the cytoplasm, rough endoplasmic reticulum, and the nuclear compartment [5,17]. It has also been suggested [13] that rat liver mitochondria contain a low level of calmodulin. Use of calmodulin antagonists as indicators of calcium-calmodulin-mediated processes has revealed that the mitochondrial Ca^{2+} pool may be regulated by both calmodulin-dependent and -independent pathways [22,27]. Sulfonamide compounds such as W5, W7, W12 and W13 are specific calmodulin antagonists which have been used as important discriminating agents for investigations of the physiologic functions of calmodulin at cellular level [15]. In this paper we show that calmodulin antagonists inhibit mitochondrial specific translation possibly by depleting the mitochondrial ATP pool. Our results also show a causal relationship between the mitochondrial content of calmodulin-type Ca^{2+} binding proteins and sensitivity to the inhibitory effects of antagonists.

Abbreviations used: SDS, sodium dodecyl sulfate; PMSF, Phenylmethylsulfonyl fluoride; W5, *N*-(6-aminohexyl)-1-naphthalenesulfonamide hydrochloride; W7, *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide hydrochloride; W12, *N*-(4-aminobutyl)-2-naphthalenesulfonamide hydrochloride; W13, *N*-(4-aminobutyl)-5-chloro-2-naphthalenesulfonamide hydrochloride; R24571, [1-bis(4-chlorophenylmethyl-3(2-[2-4-dichlorophenyl)-2-[2,4-dichlorophenyl)-methoxyl-ethyl]1-*H*-imidazolium.

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Materials and Methods

Isolation of mitochondria

Mitochondria were isolated from Ehrlich ascites tumor cells grown for 6 days in the peritoneal cavity of Swiss colony mice as described before. Briefly, cells from 10–15 tumor-bearing mice were removed, washed with ice-cold Tris-buffered saline, homogenized in the

sucrose-mannitol buffer, and mitochondria were isolated by differential centrifugation as described before [2]. Mitochondria were treated with digitonin (75 μg/mg protein) and washed twice with mitochondrial isolation buffer as described [2]. Freshly isolated mitochondria or mitoplasts were used for various in vitro incubations.

Protein synthesis

Protein synthesis with intact mitochondria or digitonin-treated mitoplasts was carried out as described before [2] in presence of 200 μCi/ml of [^{35}S]methionine (600 Ci/mmol, purchased from Amersham Corp.). The labeled proteins (100 to 200 μg) were dissociated in a sample buffer containing 125 mM Tris-HCl (pH 6.8), 5% SDS and 5% 2-mercaptoethanol by boiling in a water-bath for 3 min, and electrophoresed on a gradient SDS-polyacrylamide gel as described before [18]. Immunoprecipitation of ^{35}S-labeled proteins was carried out using the modification of the procedure of Kessler [16] described in a previous paper [3]. The polyclonal antibody to mouse cytochrome oxidase holoenzyme was raised in New Zealand rabbits. This antibody reacted more strongly with cytochrome oxidase Subunit II and weakly with Subunits I and III (Mittal, B. and Avadhani, N.G., unpublished data). The polyclonal antibody to mouse F_0F_1-ATPase subunit A6L raised in rabbits was a gift from Dr. David A. Clayton [20]. The extent of labeling of mitochondria under different experimental conditions or the amount of immunoprecipitable bands were quantitated by scanning the X-ray films through an LKB scanner equipped with an intergrator system.

Measurement of respiratory controls

The rates of respiration and oxidative phosphorylation were measured polarographically at 25°C using a Clark-type oxygen electrode (Yellow Springs Instrument Company) essentially as described by Estabrook [10]. The oxygen electrode was calibrated using phenylhydrazine according to the method of Misra and Fridovich [21]. 0.2 to 0.4 mg of freshly prepared mitochondria were incubated at 25°C in 3 ml of 10 mM potasium phosphate buffer containing 0.25 M sucrose, 5 mM $MgCl_2$, 20 mM KCl, 20 mM Tris-HCl (pH 7.4) for 2 to 4 min before adding the substrates. The addition of 5 mM succinate or malate + glutamate as substrates caused a slow O_2 uptake (State 4). Addition of 0.3 mM ADP stimulated the respiration to the active state 3. After the exhaustion of ADP, the respiration returned to state 4. The respiratory control ratio (RCR) was measured as the quotient of the respiration rate of State 3 to that of State 4. The oxidative phosphorylation efficiency was expressed as the ADP/O ratio, which is the quotient between the amount of ADP added and the oxygen consumed during state 3.

Partial purification of calmodulin-like proteins

Mitochondria were washed with mitochondrial isolation buffer and the pellet was suspended in a hypotonic buffer (10 mM Tris-HCl (pH 7.5), 1 mM β-mercaptoethanol 1 mM $CaCl_2$, 0.2 mM PMSF) at a protein concentration of 10 mg/ml and sonicated for 2 min. The pH of the sonicate was adjusted to 4.3 with 6 M acetic acid and left on ice for 1 h. Following centrifugation at 14000 rpm for 20 min, the pellet was collected and suspended in buffer A (0.05 M Tris-HCl (pH 7.5), 1 mM β mercaptoethanol, 0.1 mM $CaCl_2$ and 0.2 mM PMSF). The pH was adjusted to 7.5 with 1 M Tris base and heated at 100°C for 5 min, cooled and centrifuged at 2000 rpm for 10 min. The supernatant was adjusted to 5 mM with $CaCl_2$ and loaded on a Phenyl-Sepharose-CL4 column (column volume 3 ml) equilibrated with buffer A. The column was washed successively with 30 ml buffer A, 30 ml buffer A containing 0.5 M NaCl and finally with 20 ml buffer A containing 1 mM EGTA and no $CaCl_2$. The EGTA wash was collected, dialyzed and concentrated [12,13]. The level of calcium-binding proteins in the mixture was determined by ^{45}Ca autoradiography essentially as described by Mariyama et al. [24]. The protein samples were subjected to SDS polyacrylamide gel electrophoresis and subsequently transferred to a nitrocellulose membrane by electrophoresis. The membrane was rinsed with a buffer containing 60 mM KCl, 5 mM $MgCl_2$ and 10 mM imidazole (pH 6.8) and incubated with 50 ml of the same buffer containing 50 μCi of [^{45}Ca]calcium chloride for 20 min at room temperature. The membrane was rinsed several times with distilled water to remove unbound ^{45}Ca, dried and exposed to X-ray film for 14–18 h.

Results

Inhibition of protein synthesis

Previous studies from this laboratory [2] showed that mitoplasts from Ehrlich ascites cells incorporate [^{35}S]methionine efficiently under in vitro conditions and that the electrophoretic patterns exhibit distinct protein bands characteristic of mitochondrial translation products. It was therefore decided to use this system in the present study. The pattern of translation products formed in mitoplasts without added inhibitor is presented in Fig. 1 (lane 1). It is seen that the calmodulin antagonist sulfonamide compounds W5, W13 and W7, and also a calmidazolium compound R24571 inhibit mitochondrial translation at varying levels (lanes 3 to 12 and Table I). As quantitated by scanning through a densitometric scanner, the relatively less potent compounds, W5 and W13, inhibit the translation marginally, even at both 400 and 600 μM concentrations. At the same concentration, however, the most potent sulfonamide compound, W7 inhibits

the translation by about 50% (see lane 7 and Table I). Furthermore, R24571, a calmidazolium agent and also the Ca^{2+} ionophore inhibited the activity at relatively higher levels of 45 to 95%. Results presented in Fig. 1 also show that the inhibitory effects of W7 (lanes 7 and 8) and R24571 (lanes 9 to 12) are completely reversible by externally added 5 mM ATP, suggesting that the observed inhibition might be due to the depletion of mitochondrial ATP pool.

The extent of inhibition by various calmodulin antagonists was further ascertained by quantitation of specific tranlation products like cytochrome oxidase subunit II and ATPaseA6L. In this experiment, The total in vitro translation products formed in the presence or absence of various inhibitors were immunoprecipitated with specific antibodies to cytochrome oxidase II and ATPaseA6L, analyzed by electrophoresis on a SDS-polyacrylamide gel, and quantitated by scanning through an LKB scanner. In support of results obtained with the total translation pattern (Fig. 2), it is seen that W5 and W13 do not significantly reduce immunoprecipitable COX II and ATPaseA6L, whereas W7 and R 24571 inhibit the levels by 40 to 100%. These results together show that calmodulin antagonists inhibit mitochondrial translation under in vitro

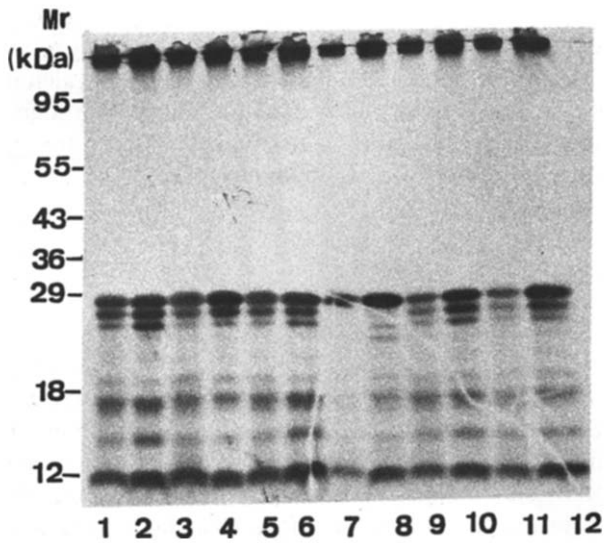

Fig. 1. Inhibition of Ehrlich ascites mitochondrial translation by sulfonamides. Ehrlich ascites tumor cell mitochondria were labelled with [^{35}S]methionine in vitro as described in Table I. After 45 min incubation, mitochondria (1 mg protein) were pelleted at 10000 *g* for 10 min and solubilized in 300 μl Laemmli sample buffer at 100°C for 5 min. Aliquots containing 100 μg of each sample were subjected to electrophoresis on a 12–18% gradients SDS-polyacrylamide gel. Radiolabelled protein bands were detected by fluorography lane 1: control (no ATP), lane 2: control (+ATP); lane 3: 200 μM W5 (no ATP), lane 4: 200 μM W5 (+ATP); lane 5: 100 μM W7 (no ATP), lane 6: 100 μM W7 (+ATP); lane 7: 600 μM W7 (no ATP), lane 8: 600 μM W7 (+ATP); lane 9: 5 μM R24571 (no ATP), lane 10: 5 μM R24571 (+ATP); lane 11: 100 μM R24571 (no ATP), lane 12: 100 μM R24571 (+ATP).

TABLE I

Inhibition of Ehrlich ascites tumor cell mitochondrial protein synthesis by calmodulin antagonists

Mitochondria were isolated from Ehrlich ascites tumor cells and incubated with [^{35}S]methionine (200 μCi/ml) in the presence of energy generating system (ADP mix) plus cycloheximide (300 μg/ml) at a final protein concentration of 10 mg/ml for 45 min at 32°C. At intervals of 0 and 45 min aliquots were spotted on 2.5 cm filter disks, air dried and boiled for 5 min in 10% trichloroacetic acid containing 50 mM methionine. The filter discs were washed with ethanol and ether-dried and ^{35}S radioactivity was determined in a liquid scintillation counter. The radioactivity in the control tube without antagonists ($4.13 \cdot 10^6$ dpm) was taken as 100%.

Antagonist	Concentration (μM)	[^{35}S]Methionine incoporation (dpm/mg protein)	Percent inhibition
W5	200	$4.12 \cdot 10^6$	–
	400	$3.54 \cdot 10^6$	14
	600	$3.38 \cdot 10^6$	18
W13	200	$4.10 \cdot 10^6$	–
	400	$4.34 \cdot 10^6$	–
	600	$3.62 \cdot 10^6$	12
W7	200	$3.71 \cdot 10^6$	10
	400	$2.26 \cdot 10^6$	45
	600	$1.23 \cdot 10^6$	70
R24571	100	$2.27 \cdot 10^6$	45
	200	$1.85 \cdot 10^6$	55
Calcium Ionophore	10	$0.20 \cdot 10^6$	95

conditions and that the extent of inhibition directly correlates with the physiological potencies of the compounds. To understand the possible mechanism of inhibition by W7 and calmidazolium, the effects of externally added Ca^{2+} and ATP were studied. Although not shown, addition of Ca^{2+} to control mitoplasts or in the presence of calmodulin antagonists did not affect the in vitro translation. Addition of 5 mM ATP, however, almost completely reversed the inhibitory effects of sulfonamide compounds, including that of W7 and calmidazolium compound (see Fig. 1, lanes 7–12). These results suggest that the inhibitory effects of sulfonamide may somehow be associated with the intramitochondrial ATP pool.

Effects of respiration

In order to gain further insight into the mode of inhibition of mitochondrial translation by sulfonamide and imidazolium compounds, the effects of these agents on the mitochondrial respiration and oxidative phosphorylation were studied. As shown in Table II the mitochondrial respiration was affected by calmidazolium and sulfonamide compounds. It is seen that W5 and W13 caused only a marginal inhibition of 15 to 25% even at 200 μM concentrations. W7, on the other hand, inhibited the O_2 uptake by about 50% at about

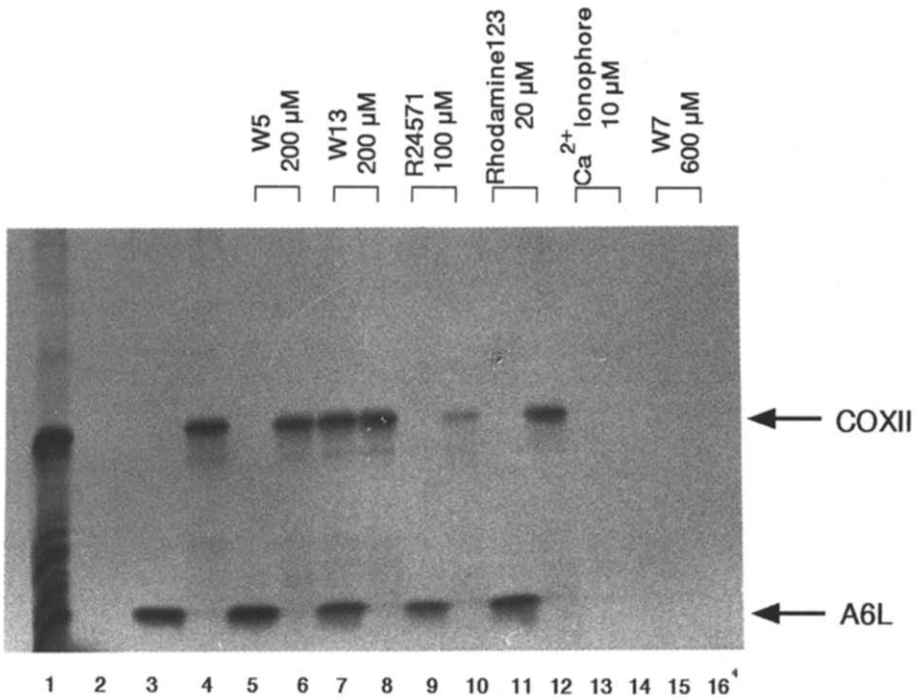

Fig. 2. Effect of calmodulin antagonists on the synthesis of mitochondrially coded cytochrome oxidase and ATPase subunits. Mitochondria isolated from Ehrlich ascites tumor cells were incubated with [^{35}S]methionine with or without added calcium antagonists as described in Table II. After 45 min of incubation the translation products were immunoprecipitated either with preimmune serum (Pre-imm. 1:50 dilution; lane 2) or polyclonal antibody to mouse cytochrome oxidase holoenzyme (Cox-ab, 1:500 dilution; lanes 4, 6, 7, 8, 10, 12, 14), or antibody to mouse ATPase subunit A6L (A6L-ab, 1:500 dilution; lanes 3, 5, 7, 9, 11, 13, 15). Lane 1: Total translation products, lanes 2–4: immunoprecipitates of control mitochondrial translation products, and lanes 5–16: immunoprecipitates of mitochondrial proteins labeled in presence of inhibitors. The inhibitors and concentrations used are indicated at the top.

TABLE II

Inhibition of oxygen uptake by calmodulin antagonists in Ehrlich ascites mitochondria

The rates of respiration and oxidative phosphorylation were measured by using a Clark-type oxygen electrode. Freshly prepared mitochondria from Ehrlich ascites cells were incubated at 25°C in a reaction chamber of 3 ml volume containing 0.25 M sucrose, 5 mM $MgCl_2$ 20 mM KCl, 20 mM Tris and 10 mM PO_4 (pH 7.4). Substrate (State 4) and ADP (State 3) stimulated respiration rates were measured by the addition of 5 mM succinate and 0.3 mM ADP. The ratio of State 3 to State 4 respiration rates is the respiratory control ratio (RCR).

Antagonist	Concentration (µM)	Respiration (µmol O_2/min mg protein)		RCR	ADP/O
		State 3	State 4		
Control	0	0.081	0.007	11.6	2.2
W7	3	0.071	0.006	11.8	2.4
	6	0.067	0.006	11.2	2.4
	33	0.054	0.012	4.5	3.1
	66	0.042	0.012	3.5	4.0
	200	–	–	0.0	–
W5	100	0.080	0.007	11.4	2.4
	200	0.070	0.006	11.6	2.2
W13	100	0.080	0.006	13.3	1.8
	200	0.064	0.006	10.6	2.2
R24571	1	0.073	0.006	12.1	2.4
	2	0.058	0.013	4.5	4.0
	10	0.000	–	0.0	–

70 μM concentration. As seen from Table II, calmidazolium was the most potent inhibitor and even at the 10 μM concentration it reduced the O_2 uptake to near zero level.

To determine the generality of the inhibition of mitochondrial translation and respiratory processes by calmodulin antagonists, parallel experiments were carried out using mouse liver mitochondria. As shown in Table III, W5 and W13 did not inhibit liver mitochondrial translation upto a dose level of 400 μM tested. Surprisingly, W7, the most effective sulfonamide compound, caused only a marginal inhibition of protein synthesis in liver mitochondria. Furthermore, as shown in Table III, these sulfonamide compounds had limited effects on the oxidative phosphorylation coupled respiration in liver mitochondria, as opposed to a significant inhibition in Ehrlich ascites mitochondria. These results suggest the possibility that mitochondria from different cells and tissues might respond differently to the calmodulin antagonist sulfonamides.

The nature of mitochondrial calmodulin-like Ca^{2+}-binding proteins

To understand the observed differences between the liver and Ehrlich ascites mitochondria with respect to their relative sensitivities to W7, the levels of calmodulin-like Ca^{2+}-binding proteins in the mitochondrial isolates were investigated. In this experiment, heat-denatured supernatant fraction from mitochondrial extracts and protein fractions partially purified by chromatography on Phenyl-Sepharose were assayed for the levels of Ca^{2+}-binding proteins using the ^{45}Ca autoradiography of proteins transblotted to nitrocellulose membrane. As shown in Fig. 3, heat-denatured supernatant from 100 mg of mouse liver mitochondria contains very low to negligible levels of Ca^{2+}-binding protein. The comparable extract from Ehrlich ascites mitochondria, on the other hand, shows three distinct ^{45}Ca bands of 29, 18 and 12 kDa. The 18 kDa band comigrates with purified bovine calmodulin, and may therefore represent mitochondrial calmodulin protein. This possibility was further verified by partial purification, which involves the affinity binding of Ca^{2+}-bound calmodulin proteins to a Phenyl-Sepharose column and elution with the Ca^{2+}-chelator, EGTA. As shown in lanes 2 and 4 (Fig. 3) the 18 kDa ^{45}Ca-binding protein preferentially purifies by this method, further support-

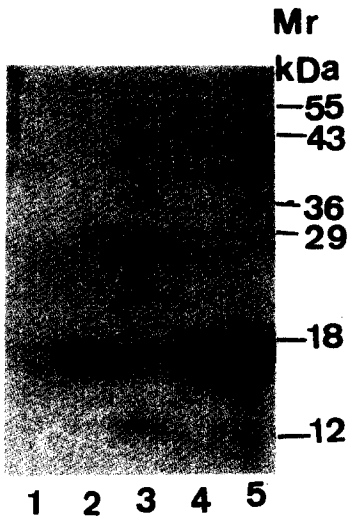

Fig. 3. Detection of calmodulin like calcium binding proteins in Ehrlich ascites cell and mouse liver mitochondrial fractions by ^{45}Ca autoradiography. Heat-denatured supernatant fractions from mitochondrial extracts and protein fractions partially purified by chromatography on Phenyl-Sepharose were subjected to SDS polyacrylamide gel electrophoresis and electrophoretically transferred to a nitrocellulose membrane. The membrane was incubated with 50 ml buffer containing 60 mM KCl, 5 mM $MgCl_2$ 10 mM imidazole (pH 6.8) and 50 μCi [^{45}Ca]calcium chloride for 20 min at room temperature. The membrane was rinsed several times with distilled water to remove unbound ^{45}Ca, dried, and exposed to X-ray film for 14–18 h. Lanes 1 and 3: heat denatured supernatants from mouse liver and Ehrlich ascites mitoplasts, respectively. Lanes 2 and 4: Phenyl-Sepharose bound fractions from mouse liver and Ehrlich ascites mitoplast supernatants. Lane 5: Bovine brain calmodulin (20 μg).

TABLE III

Effect of sulfonamide compounds on hepatic mitochondrial translation and respiration

Mitochondria were isolated from mouse liver and used for in vitro protein synthesis and respiration rate measurements as described in Materials and Methods and in Tables I and II. 100% value for [^{35}S]methonine incorporation is 80862 dpm/mg protein and O_2 uptake is 0.061 μmol O_2/min per mg mitochondrial protein.

Compound	μM	[^{35}S]Methionine incorporation dpm/mg Protein	% inhibition	Respiration μmoles O_2/min/mg Protein		RCR
				State 3	State 4	
None	–	80862	0.0	0.061	0.0110	5.5
W5	200	78840	2.6	0.055	0.0110	5.0
	400	77870	3.8	0.048	0.0110	4.4
W13	200	80862	0.0	0.055	0.0110	5.0
	400	79245	2.0	0.044	0.0096	4.4
W7	200	78060	3.5	0.041	0.0110	4.1
	400	63477	21.5	0.033	0.0096	3.3

ing the concept that it has properties similar to other calmodulin proteins. It should be noted that sample in lane 2 represents heat-denatured extract from 100 mg mouse liver mitochondria, while that in lane 4 is from 35 mg Ehrlich ascites mitochondria. The results of the Ca^{2+}-binding with heat-denatured mitochondrial extracts and partially purified protein together suggest that Ehrlich ascites mitochondria contain nearly 3–5-fold higher levels of calmodulin-like proteins than mouse liver mitochondria.

Discussion

Earlier attempts to elucidate the role of calmodulin in mitochondria employed phenothiazines and calmidazolium compounds as indicators of a calmodulin-mediated process. These compounds, however, were found to be nonspecific, as they affected mitochondrial functions independent of calmodulin. The sulfonamide compounds such as W5, W7, W12 and W13 found to be specific indicators of calmodulin-mediated functions. W5 and W12 lack chlorine in the molecule and are weaker antagonists of calmodulin. They are less effective as calmodulin antagonists than W7 and W13. The difference in the levels of inhibition by these compounds is often considered as evidence for calmodulin dependence of the biological process.

Following this generality, we observed that W7 inhibited mitochondrial translation as well as mitochondrial respiration and oxidative phosphorylation. Although not shown, the drug was without any effect on mitochondrial respiratory enzyme complexes: NADH-ferricyanide reductase, cytochrome *c* oxidase and oligomycin-sensitive ATPase. The compound R24571 (calmidazolium) inhibited all mitochondrial functions at much lower concentrations than the sulfonamide compounds. It was earlier suggested that calmidazolium may be affecting mitochondrial functions in a mechanism independent of drug-calmodulin interaction [11]. Further the degree of inhibitions of mitochondrial respiration and translation observed for sulfonamide compounds is in the order of W7 > W13 > W5, which is identical to the calmodulin antagonist activity.

In support of other studies [6–8], our results suggest the need for energized mitochondrial membrane for protein synthesis. Agents which inhibit electron transport, oxidative phosphorylation and membrane potential have been shown to inhibit mitochondrial protein synthesis, too. The inhibition of protein synthesis by W7 under energy-generating conditions (ADP + isocitrate/succinate) and the rescue of inhibition by ATP in energy regenerating system (ADP + ATP) demonstrate the essential role of oxidative phosphorylation in mitochondrial protein synthesis. The disruption of membrane potential by W7 may be affecting the mitochondrial electron transport and oxidative phosphorylation, thus depleting mitochondria of endogenously generated ATP pool. This view lends support to the possibility of drug-calmodulin interaction at the mitochondrial inner-membrane level suggested in a previous study [13].

The evidence on the presence of calmodulin in mitochondria are not rigorous. Carafoli et al. [4] failed to detect any calmodulin like 17 kDa protein band in the boiled liver mitochondrial extracts; Ruben et al. [26], on the other hand, assumed that the heat-stable Ca^{2+}- and chloropromazine-mediated stimulation of phosphodiesterase in the 0.4 M NaCl eluted fractions of mitochondrial extract to be calmodulin.In this report we demonstrate the presence of calmodulin in purified mitochondria as well as in digitonin-stripped mitoplasts as indicated by their binding to ^{45}Ca. Although the precise physiological role of calmodulin in mitochondrial function remains unknown, it is possible that mitochondria, like the cytosol, may use calmodulin as a coupling agent in an as yet unidentified Ca^{2+} response. Recent studies of Miernyk and Randall [23] suggest that calmodulin might be a regulatory subunit of plant mitochondrial pyruvate dehydrogenase complex. In support of this, it was recently demonstrated by Miernyk et al. [22] that calmodulin antagonists inhibit pyruvate oxidation.

The increased sensitivity of Ehrlich ascites mitochondria to calmodulin antagonists and also the presence of additional Ca^{2+}-binding proteins as compared to normal hepatic mitochondria suggest additional Ca^{2+}-dependent processes in this tumor cell mitochondria [14]. Additionally, the increased mitochondrial sensitivity to sultonamide compounds with increased mitochondrial Ca^{2+}-binding calmodulin-like proteins suggests that the observed inhibition may involve an altered mitochondrial free Ca^{2+} pool.

It is becoming increasingly clear that mitochondria employ Ca^{2+}-activated ATP generation. Our own observations that calmodulin antagonists were more inhibitory to ascites mitochondria than liver mitochondria are consistent with the findings that normal and tumor cell mitochondria employ different modes of Ca^{2+}-activated ATP generation [25]. Thus the inhibitory effects of calmodulin antagonists on mitochondrial respiration and translation lends credence to a possible calmodulin role in primary mitochondrial events.

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