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UNIQUE UNCOUPLER-STIMULATION PATTERN OF MITOCHONDRIAL ATPase ACTIVITY OF TUMOR CELLS, BRAIN, AND FETAL LIVER

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SUMMARY: Mitochondria were prepared from various kinds of normal tissues and tumor cells of mice, and their ATPase activities were measured in the presence of an uncoupler. The ATPase activities of all mitochondria were stimulated by the uncoupler when it was added to the mitochondrial suspension just before or after addition of ATP. However, when mitochondria were preincubated with the uncoupler for four minutes or more before the addition of ATP, its stimulating effect on mitochondrial ATPase activities was greatly reduced in all tumor cells tested, but not in normal adult liver. Reduction of the stimulating effect of the uncoupler by preincubation with it was also observed with mitochondrial ATPase of brain and fetal liver. Thus this pattern of change in the effect of uncoupler on preincubation may be common to tumor mitochondria, but it is not specific to tumor mitochondria. The pattern of uncoupler stimulation observed in fetal liver changed rapidly to that of adult liver immediately after birth. Thus the difference between the two uncoupler stimulation patterns is probably not due to a difference in molecular species of mitochondrial ATPase.

INTRODUCTION

Pedersen and Morris (1) reported that the mitochondrial ATPase activities of Morris hepatoma cell lines, including slowly growing tumors, were not stimulated by uncouplers so much as those of normal or regenerating liver. However, Kaschnitz et al. (2) showed that when mitochondria were prepared from various Morris hepatoma cell lines in the presence of a high concentration of BSA (1%) and the uncoupler was added to the mitochondria after addition of ATP, the extent of uncoupler-stimulated ATPase activity was similar to that of normal liver. This observation was confirmed by Pedersen (3) using Morris hepatoma 7800 with an intermediate growth rate. Moreover, Kolarov et al. (4) observed that under assay conditions in which the ATPase activity of Zajdela hepatoma was not stimulated at all by uncouplers, that of Ehrlich ascites tumor cells was greatly stimulated by uncouplers.

Abbreviations used: BSA, bovine serum albumin; DNP, 2,4-dinitrophenol; HEPES, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; FCCP, carbonyl cyanide p-trifluoromethoxyphenylhydrazone

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Thus a low rate of uncoupler stimulation does not seem to be a universal characteristic of all tumor mitochondria.

The present work, however, showed that a universal characteristic of tumor mitochondria not seen with normal liver mitochondria was that when the mitochondria were prepared in the presence of 1% BSA, preincubation with an uncoupler, such as DNP, for four minutes or more reduced the stimulation of ATPase activity by the uncoupler. This characteristic stimulation pattern was also observed with mitochondria from fetal liver and adult brain.

MATERIALS AND METHODS

Preparation of mitochondria: Ascites tumor cells of mouse hepatoma 134 and Sarcoma 180 were isolated from the ascites as described previously (5). Ehrlich ascites cells were isolated by the procedure of Pedersen et al. (6). FM3A cells were grown in suspension culture at 37° in Eagle's minimum essential medium supplemented with 2% fetal calf serum and 0.1% Bactopeptone (7). Mitochondria of ascites tumor cells and FM3A cells were prepared by the procedure of Kaschnitz et al. (2). The procedure used for isolation of mitochondria from adult tissues, fetal liver, and spontaneous leukemia cells of AKR mice was the same as that used with tumor cells, except that protease digestion was omitted. Mitochondria were suspended in the isolation medium consisting of 1% BSA, 220mM mannitol, 70mM sucrose, and 2mM HEPES, pH 7.4, and ATPase activity was assayed immediately.

ATPase assays: Mitochondrial ATPase was assayed by the procedure of Pedersen et al. (8) with slight modifications. Two assays (assay I and II) were used to measure the extent of the stimulation of ATPase activity by DNP (Table I): in

Table I Mitochondrial ATPase assays

	Assay I (ml)	Assay II (ml)
Mitochondrial suspension (about 10mg protein/ml)	0.075	0.075
0.11M Imidasole buffer, pH 6.9	0.05	0.05
Deionized and distilled water	0.275	0.225
2mM DNP, pH 7.4	—	0.05
Preincubation for 4 minutes or longer at 30°		
Deionized and distilled water	—	0.05
2mM DNP, pH 7.4	0.05	—
0.1M ATP, pH 7.4	0.05	0.05
Incubation for 10 minutes at 30°		
50% trichloroacetic acid	0.1	0.1
Estimation of inorganic phosphate		

assay I, DNP was added to the mitochondrial suspension just before ATP; in assay II, the mitochondrial suspension was preincubated with DNP for 4 minutes or more at 30° before addition of ATP. Incubation was carried out for 10 minutes at 30°, and the reaction was stopped by adding 50% trichloroacetic acid. Activity was determined by measuring release of inorganic phosphate by the procedure of Horstman and Racker (9).

RESULTS

Effect of DNP on activities of mitochondrial ATPases of various normal tissues and tumor cells of mice

Mitochondria were prepared from the liver, four kinds of transplantable tumor cell lines, and spontaneous leukemia cells of AKR mice, and stimulation of ATPase activities by DNP was measured by two assays (assay I and II) as described under MATERIALS AND METHODS.

When the preincubation with DNP was omitted, i.e., when mitochondria were preincubated without DNP and DNP was added to the mitochondrial suspension just before ATP (assay I), the mitochondrial ATPase activities of tumor cells and liver were both significantly stimulated by DNP (Fig. 1). The order of additions of DNP and ATP to the mitochondrial suspension did not influence the rate of DNP stimulation. However, when mitochondria were preincubated with DNP for 4 minutes before addition of ATP (assay II), stimulation of ATPase activity by DNP was much less in tumor cell mitochondria than in normal liver mitochondria; the extent of stimulation of ATPase activity of tumor cell mitochondria by DNP was much less in assay II than in assay I (Fig. 1b and c), whereas stimulation of ATPase activity of liver mitochondria was similar in assay I and II (Fig. 1a').

The mitochondria of Ehrlich ascites showed somewhat different characteristics from those of other tumor cells; their ATPase activity was stimulated more than that of normal liver even when they were preincubated with DNP for 4 minutes (assay II), although the stimulation was not so great as in assay I (Fig. 1c). However, in contrast to liver mitochondria (Fig. 1a), when they were preincubated with DNP for longer (30 min.), the extent of stimulation of ATPase activity by DNP decreased markedly and the pattern of stimulation became similar to that of other tumor cells (Fig. 1b and c).

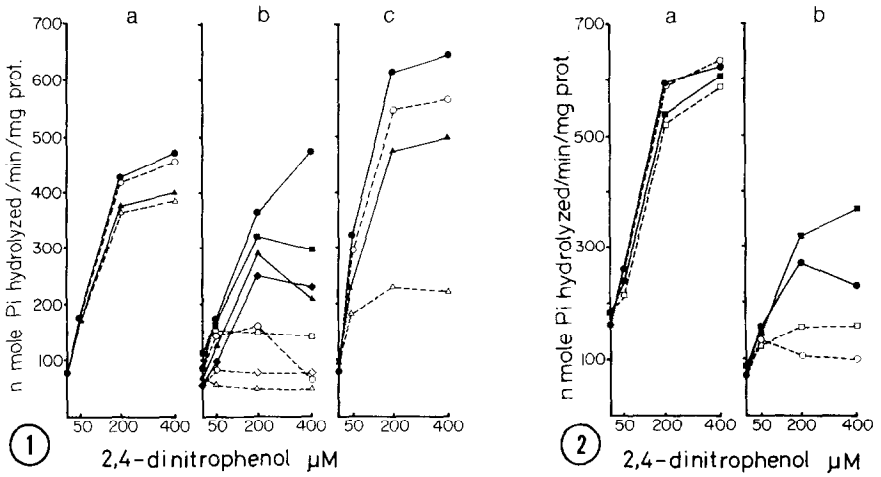


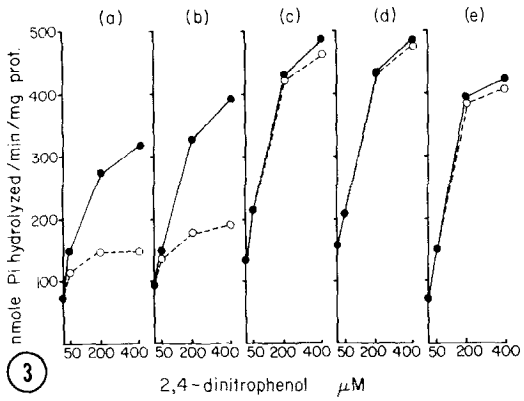
Figure 1. Effect of DNP on mitochondrial ATPase activities of mouse liver and tumor cells. In assay I, DNP was added to the mitochondrial suspension just before ATP. In assay II, mitochondria were preincubated with DNP for 4 minutes or longer before addition of ATP. Closed symbols and solid lines, assay I; open symbols and broken lines, assay II. (a) ●○, liver mitochondria; ▲△, liver mitochondria preincubated for longer (30 min.) (b) ●○, mitochondria of mouse hepatoma 134; ■□, Sarcoma 180; ▲△, FM3A; ◆◇, spontaneous leukemia (c) ●○, mitochondria of Ehrlich ascites tumor; ▲△, mitochondria of Ehrlich ascites tumor preincubated for longer (30 min.)

Figure 2. Effect of DNP on mitochondrial ATPase activities of normal tissues of mouse. Closed symbols and solid lines, assay I; open symbols and broken lines, assay II. (a) ●○, heart mitochondria; ■□, kidney mitochondria (b) ●○, brain mitochondria; ■□, fetal liver mitochondria

To determine whether this change in the pattern of DNP stimulation was a specific characteristic of tumor cell mitochondria, we prepared mitochondria from other normal tissues, such as heart, kidney, brain, and fetal liver, and measured the stimulation of ATPase activity by DNP by the two assays (assay I and II). The mitochondrial ATPase activities of heart and kidney were stimulated by DNP similarly in assays I and II (Fig. 2a), but those of brain and fetal liver, like those of tumor cells, were stimulated less in assay II than in assay I (Fig. 2b). The stimulation patterns of all ATPase activities tested were similar using other potent uncouplers, such as FCCP, instead of DNP (not shown).

Change in sensitivity to DNP of liver mitochondria ATPase activity during development

As shown above, the mitochondrial ATPase activity of fetal liver showed the same pattern of stimulation by DNP as that of tumor cells. We examined at what stage of development the fetal form of liver ATPase activity changed to the adult



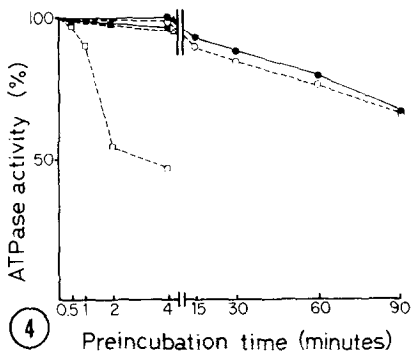


Figure 3. Change in sensitivity to DNP of liver mitochondrial ATPase activity during development. Closed symbols and solid lines, assay I; open symbols and broken lines, assay II. Liver mitochondria of (a) 15-day fetus, (b) 19-day fetus (just before birth) (c) neonate (just after birth) (d) neonate (2 days old), (e) adult (5 weeks old)

Figure 4. Effect of time of preincubation with or without DNP on mitochondrial ATPase activity. Closed symbols and solid lines, assay I; open symbols and broken lines, assay II. ●○, mitochondria of neonatal liver preincubated with (○) or without 0.2mM DNP (●), ▲△, with (△) or without a high concentration of DNP (1.2mM) (▲); ■□, mitochondria of fetal liver preincubated with (□) or without 0.2mM DNP (■)

form. Fig. 3 (a and b) shows that throughout fetal life, liver mitochondrial ATPase activity was stimulated by DNP less when the mitochondria were preincubated with DNP (assay II). This pattern persisted until just before the birth. However, within about half an hour after birth the pattern changed; liver ATPase activity of new born mice was stimulated similarly in assays I and II (Fig. 3 c-e). This DNP-stimulation pattern of liver ATPase activity of new born mice was not affected by increase in the concentration of DNP to 1.2mM, or increase in the preincubation time to 90 minutes in assay II (Fig. 4).

DISCUSSION

Kaschnitz et al. (2) showed that when mitochondria of various Morris hepatoma cell lines were isolated in the presence of 1% BSA, the ATPase activities of the tumor cell mitochondria could be maximally stimulated by uncouplers such as DNP when they were added after ATP. Barbour and Chan (10) pointed out the necessity of using a high concentration of BSA (1%) during isolation of mitochondria from tumors for maximal stimulation of ATPase activity by DNP. On the other hand, Pedersen (3)

emphasized the influence of the orders of addition of ATP and DNP to the mitochondrial suspension: addition of ATP before DNP was necessary for maximal ATPase activity.

Our results show that when mitochondria were isolated in the presence of 1% BSA, the ATPase activities of mitochondria of all the tumor cells studied were stimulated considerably by DNP, even when the uncoupler was added before ATP (assay I): the extent of stimulation was not affected appreciably by the order of additions of ATP and DNP. However, as the preincubation time with DNP was prolonged, the extent of stimulation by DNP gradually decreased, and when the mitochondria of tumor cells were preincubated with DNP for several minutes or more before addition of ATP (assay II), their ATPase activities were stimulated very little by DNP (Fig. 1b and c). Since this characteristic decrease in the stimulatory effect of DNP during preincubation was also observed with mitochondria from spontaneous leukemia cells of AKR mice, this characteristic does not seem to be restricted to transplantable tumor cell lines induced by various carcinogens. It should be noted that the mitochondrial ATPase activity of Ehrlich ascites tumor cells was stimulated considerably by DNP even when the mitochondria were preincubated with DNP (assay II) (Fig. 1c). This finding is consistent with the report of Kolarov et al. (4) that ATPase activity of Ehrlich ascites cells was greatly stimulated by uncouplers under assay conditions in which the ATPase activity of Zajdela hepatoma was not stimulated at all by uncouplers. However, if the preincubation time with DNP was prolonged from 4 to 30 minutes in assay II, the extent of DNP stimulation of Ehrlich ascites tumor ATPase activity was reduced to the level observed with other tumor ATPase activities (Fig. 1c). Thus, the ATPase activities of tumor mitochondria are stimulated by DNP, but preincubation of tumor mitochondria with DNP for 4 minutes or longer before addition of ATP reduces the extent of this stimulation.

This characteristic is not specific to mitochondria of tumor cells; the ATPase activities of brain and fetal liver mitochondria were also stimulated by DNP more in assay I than in assay II. The finding that the DNP-stimulation patterns of tumor mitochondria resemble those of fetal liver and adult brain are analogous to findings on isozymes: the patterns of many isozymes of tumor cells in general resemble those

of fetal tissue and/or of tissues other than those from which the tumor cells originate (11-13). However, in contrast to these isozymes, it is unlikely that expression of mitochondrial ATPase genes changes during development, differentiation, and/or neoplastic transformation, because the DNP-stimulation pattern of liver ATPase activity changed within about 30 minutes after birth to that of adult liver (Fig. 3b and c). Thus, this rapid and drastic change is probably not caused by change of mitochondrial ATPase molecules themselves, but by some change in the environment of the ATPase molecules. In fact, no difference has been observed between purified F_1ATPase molecules of liver and hepatoma cells (14, 15).

This possible environmental difference of mitochondrial ATPase molecules must be common to tumor cells, fetal liver, and adult brain. Further investigations are necessary on the problem of why preincubation of mitochondria from tumor cells and these normal tissues with uncoupler decreases the stimulatory effect of the uncoupler on ATPase activity.

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