Biochimica et Biophysica Acta, 985 (1989) 286–292
Elsevier

BBAMEM 74617

Protective effect of the plasticizer di(2-ethylhexyl) phthalate against damage of the mitochondrial membrane induced by calcium: possible participation of the adenine nucleotide translocator

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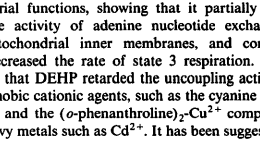
(Received 29 May 1989)

Key words: Plasticizer; Di(2-ethylhexyl) phthalate; Calcium ion transport; Adenine nucleotide translocator; Mitochondria; (Rat liver)

The effect of di(2-ethylhexyl) phthalate (DEHP) on the response of isolated rat liver mitochondria to Ca^{2+} was investigated. DEHP was found to inhibit more than 60% of the auto-accelerating release of respiration induced by 100 μM Ca^{2+}, being maximally inhibitory at 40 μM. Prior addition of DEHP also partially inhibited Ca^{2+}-induced swelling of the mitochondrial matrix. However, DEHP did not change the net rate of Ca^{2+} uptake measured by the steady-state infusion method. DEHP also reduced the rate of adenine nucleotide exchange across the mitochondrial membrane. Another alkyl phthalate and alkyl citrates had similar effects on Ca^{2+}-induced membrane damage, but their potencies depended on the lengths of their alkyl chains. These results suggest that the effects of DEHP and other alkyl esters on mitochondrial functions are mainly based on their actions on membrane lipids surrounding adenine nucleotide translocator (AdNT), resulting in alteration of the interaction between these phospholipids and AdNT, and consequent modification of the state of the protein.

Introduction

DEHP (see Scheme I for chemical structure) is a plasticizer commonly added to poly(vinyl chloride) (PVC) plastics. As it is used in many kinds of medical equipment, its effect on biological functions is important. The protective effect of DEHP on biomembrane systems, such as those of erythrocytes and mitochondria has recently been pointed out [1–4]. In a preceding paper [4], we reported the effect of DEHP on mitochondrial functions, showing that it partially inhibited the activity of adenine nucleotide exchange across mitochondrial inner membranes, and consequently decreased the rate of state 3 respiration. We also found that DEHP retarded the uncoupling actions of hydrophobic cationic agents, such as the cyanine dye tri-S-C_4(5) and the (*o*-phenanthroline)$_2$-Cu^{2+} complex, and of heavy metals such as Cd^{2+}. It has been suggested that these uncouplings are based on the modification of the states of sulfhydryl groups in a 29000 dalton protein in mitochondrial membranes [5], and possibly, under extreme conditions, on damage(s) of mitochondrial



Scheme I. Chemical structures of esters used in the present study.

Abbreviations: DEHP, di(2-ethylhexyl) phthalate; DLP, di-*n*-dodecyl phthalate; ATEC, acetyltriethyl citrate; ATBC, acetyltributyl citrate; ATHC, acetyltrihexyl citrate; AT(OD)C, acetyltri(octyl/decyl) citrate (mixture); AdNT, adenine nucleotide translocator; tri-S-C_4(5), 2,2′-[3-(2-[3-butyl-4-methyl-2-thiazolin-2-ylidene]ethylidene) propenylene]bis(3-butyl-4-methylthiazolinium iodide); P_i, inorganic phosphate; PVC, poly(vinyl chloride); SDS, sodium dodecylsulfate; CATR, carboxyatractyloside; SF 6847, 3,5-di-*tert*-butyl-4-hydroxybenzylidenemalononitrile.

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membrane systems. The 29000 dalton protein modified by the (*o*-phenanthroline)$_2$-Cu^{2+} complex is supposed to be the adenine nucleotide translocator (AdNT) [6]. Therefore, examination of the relationship between the activity of adenine nucleotide exchange and the protective effect of DEHP should be interesting.

When a large amount of Ca^{2+} is added to energized mitochondria in medium containing P_i, spontaneous Ca^{2+} discharge occurs within a few minutes after transient depolarization of the membrane potential of mitochondria due to uptake of Ca^{2+} (see Ref. 7 for a review). Discharge of the accumulated Ca^{2+} is accompanied by collapse of the membrane potential [8], loss of its selective permeability to solutes of less than 1500 dalton [9] and auto-accelerating release of respiration [10]. In other words, the spontaneous Ca^{2+} discharge is due to serious membrane damage caused by the added Ca^{2+}. On the other hand, it is known that inhibitors of AdNT also affect the stability of the mitochondrial membrane against spontaneous Ca^{2+} discharge [8,11–14]. These results prompted us to examine the effect of DEHP on Ca^{2+} transport in relation to AdNT activity.

Materials and Methods

DEHP and DLP were purchased from Kyowa Hakko Kogyo Co., Tokyo (Japan). The amount of contaminating mono(2-ethylhexyl) phthalate in DEHP was less than 0.1 ppm as judged by gas-liquid chromatographic analysis. All citrate esters (ATEC, ATBC, ATHC and AT(OD)C) were gifts from Pfeizer, Tokyo (Japan). Solutions (500 mM) of these alkyl esters in ethanol were used as stock solutions. Other reagents were commercial products of reagent grade.

Rat liver mitochondria were isolated from adult male Wistar rats (about 250 g body weight) by the method of Myers and Slater [15]. Consumption of oxygen due to respiration of mitochondria at 25°C was measured polarographically with a Clark-type oxygen electrode (Yellow Springs, type 5331). The standard incubation medium consisted of 120 mM KCl, 3.3 mM potassium phosphate, and 5 mM Tris-HCl buffer (pH 7.4). Succinate (5 mM) plus rotenone (1 μg/ml) was used as a respiratory substrate. Incubation of mitochondria (0.7 mg protein/ml) was carried out at 25°C in a reaction vessel of 2.53 ml. The amount of protein was determined by the Biuret method [16] in the presence of 1% SDS with bovine serum albumin as a standard.

The concentration of Ca^{2+} in the medium was monitored with a Ca^{2+}-selective electrode (Toko Chemical Laboratories, type 7001). Swelling of the mitochondrial matrix induced by Ca^{2+} was monitored at 25°C as the absorbance change at 520 nm in a Shimadzu recording spectrophotometer, model UV-3000.

The net rate of Ca^{2+} uptake was measured by the steady-state infusion method [17]. In this measurement, 5 mM $MgCl_2$, 0.2 mM ATP and 1 μg/ml oligomycin were added to the standard incubation medium to stabilize mitochondria against Ca^{2+}-induced membrane damage. An infusion pump (INFORS, type 5003) was used to inject a solution of $CaCl_2$ into the incubation mixture at a constant rate.

The effects of DEHP on the swelling of nonrespiring mitochondria due to valinomycin-mediated K^+ entry, in exchange with efflux of H^+ mediated by SF 6847 in medium containing potassium acetate, and that in accordance with the entry of SCN^- in medium containing KSCN, were carried out at 25°C by the methods reported by Henderson et al. [18] and Blok et al. [19], respectively.

The activity of AdNT was assayed by measuring the rate of [^{3}H]ADP uptake by the method of Winkler et al. [20], slightly modified as described previously [21]. The incubation medium consisted of 200 mM sucrose, 2 mM $MgCl_2$, 1 mM Na_2EDTA, 10 mM succinate (sodium salt), 6 μg/ml oligomycin, 2 μg/ml rotenone, and 10 mM Tris-HCl buffer (pH 7.4). As transport of ADP is reported to be very rapid at physiological temperatures [22], the reaction was carried out in test tubes immersed in iced water. 5 min after the addition of DEHP (40 μM) to a suspension of mitochondria (1.0 mg protein/ml) in a total volume of 15 ml, [^{3}H]ADP (spec. radioact., 12.5 mCi/mmol) was added. (The final concentration of ADP was 1 mM.) Volumes of 1 ml of incubation mixture were taken at 30 s intervals and promptly mixed with 50 μM carboxyatractyloside (CATR). The mixtures were centrifuged at 13000 rpm for 1 min in an Eppendorf-type centrifuge (model KM-15000A, Kubota), and the resulting mitochondrial pellet was washed twice with the washing medium (incubation medium containing 50 μM CATR). The pellet was solubilized in 500 μl of 4% SDS, and its radioactivity was measured in an Aloka model LSC-700 liquid scintillation counter.

For examination of the concentration-dependent effects of DEHP and ATEC on the activity of AdNT, the mitochondria were suspended in 1 ml of the medium described above, and known amounts of the esters were added. Then, after preincubation for 2 min, 1 μmol [^{3}H]ADP (spec. act. 12.5 mCi/mmol) was added. The reaction was terminated after 30 s by addition of CATR, and the radioactivity was measured as described above.

Results

Fig. 1 shows the effect of the plasticizer DEHP on the release of succinate-supported respiration induced by exogenous Ca^{2+}. In the absence of DEHP, the initial rapid release of respiration just after addition of 100 μM Ca^{2+} corresponds to the transient depolarization of

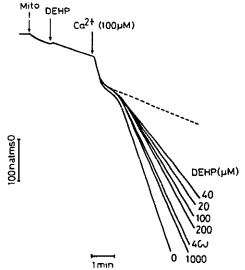

Fig. 1. Effect of DEHP on the rate of oxygen consumption stimulated by addition of Ca^{2+}. Isolated rat liver mitochondria (1 mg protein/ml) were incubated in the medium described in Materials and Methods. 2 min after the addition of DEHP, Ca^{2+} (final concentration 100 μM) was added. Numbers adjacent to the traces are the DEHP concentrations in μM. The broken line represents oxygen consumption of mitochondria in the presence of 5 mM $MgCl_2$, 0.2 mM ATP and 1 μg/ml oligomycin. In this case, mitochondria were stabilized against spontaneous Ca^{2+} discharge and the second stage of increase in the rate of oxygen consumption did not occur.

the membrane potential due to uptake of Ca^{2+} by mitochondria, and the later stage of release of respiration corresponds to the spontaneous Ca^{2+} discharge. This Ca^{2+} movement was confirmed by monitoring the Ca^{2+} concentration in the medium with a Ca^{2+}-selective electrode, as shown in Fig. 2. As expected, when mitochondria were stabilized against the spontaneous Ca^{2+} discharge by previous addition of ATP, Mg^{2+} and oligomycin to the incubation medium, the second stage of release of respiration did not occur, as indicated by the broken line in Fig. 1.

Figs. 1 and 2 also show that DEHP had an inhibitory effect on the spontaneous Ca^{2+} discharge. The concentration dependence of the effect of DEHP is depicted in Fig. 3 (closed circles). DEHP exhibited maximal

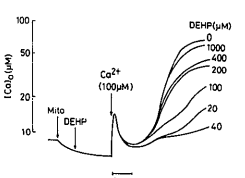

Fig. 2. Effect of DEHP on uptake and discharge of Ca^{2+}. Traces of Ca^{2+} concentrations in the medium monitored with a Ca^{2+}-selective electrode are shown. Experimental conditions were as for Fig. 1. Numbers adjacent to the traces are DEHP concentrations in μM.

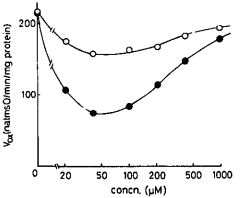

Fig. 3. Concentration dependence of the inhibitory effect of the phthalate esters DEHP (closed circles) and DLP (open circles) on oxygen consumption stimulated by spontaneous Ca^{2+} discharge. V_{ox} values calculated from the traces of the O_2-electrode at 2 min after the addition of Ca^{2+} are plotted.

inhibition of more than 60% at about 40 μM, and was less effective below and above this concentration.

As the spontaneous Ca^{2+} discharge is known to be associated with swelling of the mitochondrial matrix space [23,24], the effect of DEHP on Ca^{2+}-induced swelling was examined. Addition of Ca^{2+} at 100 μM to mitochondria energized with succinate (plus rotenone) induced a decrease in absorbance at 520 nm corresponding to swelling of the mitochondrial matrix space. Prior addition of DEHP to the mitochondria reduced the degree of swelling depending on the concentration of DEHP, as shown in Fig. 4. Interestingly, the protective effects of DEHP were similar to its effects on the release of respiration induced by exogenous Ca^{2+} and on the spontaneous discharge of Ca^{2+} (cf. Figs. 1–3). At lower concentrations of up to 100 μM of DEHP, the

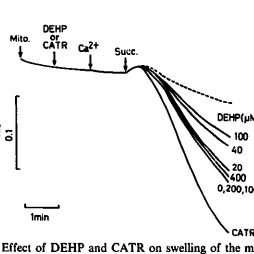

Fig. 4. Effect of DEHP and CATR on swelling of the mitochondrial matrix space induced by Ca^{2+}. Mitochondria were suspended in medium consisting of 200 mM sucrose, 2 mM $MgCl_2$ and 10 mM potassium phosphate buffer (pH 7.4). First, DEHP or CATR was added. After 1 min, $CaCl_2$ (200 μM) was added, and 1 min later, Ca^{2+} uptake was started by addition of a respiratory substrate, 5 mM succinate (plus 1 μg/ml rotenone). Swelling was monitored as the decrease in the absorbance at 520 nm. The broken line represents swelling of the mitochondrial matrix space in the presence of 5 mM $MgCl_2$, 0.2 mM ATP and 1 μg/ml oligomycin.

inhibitory effect increased with increases in DEHP concentration, but the inhibition was reversed to the original level at DEHP concentrations above 100 μM. In this case also, the addition of ATP, Mg^{2+} and oligomycin to the incubation medium inhibited the swelling, as shown by the broken line in Fig. 4, and the addition of CATR, an inhibitor of ADP transport via the AdNT, caused an increase of the swelling due to the induction of AdNT to the c-state, as reported by Lê Quôc and Lê Quôc [14].

To determine whether the effect of DEHP on the swelling mitochondria is specific to that associated with the transport of Ca^{2+}, we next examined the effect of DEHP on the swelling of nonrespiring mitochondria induced by valinomycin-mediated K^+ transport coupled with either H^+ efflux mediated by the weakly acidic uncoupler SF 6847 or with the entry of SCN^- [18,19]. In both cases, DEHP at concentrations up to 200 μM had little effect on the swelling of mitochondria (data not shown), indicating that DEHP has a specific effect on the transport of Ca^{2+}.

We next examined the effect of DEHP on the first transient depolarization of mitochondrial membranes by the uptake of Ca^{2+}. Determination of the kinetics of the first transient uptake of Ca^{2+} is difficult because of the low time resolution of the Ca^{2+}-selective electrode. Therefore, we adopted the steady-state infusion method developed by Zoccarato and Nicholls [17]. The distribution of calcium between the suspension medium and mitochondrial matrix is believed to be maintained by the simultaneous operations of the Ca^{2+} uniporter that introduces Ca^{2+} into mitochondria and the independent efflux pathway [7]. In other words, Ca^{2+} transport is the result of steady-state recycling of Ca^{2+} across the mitochondrial membrane. When Ca^{2+} is infused at a constant rate into a mitochondrial incubation medium containing ATP, Mg^{2+} and oligomycin to stabilize mitochondria against spontaneous Ca^{2+} discharge, the free Ca^{2+} concentration in the incubation medium ($[Ca]_o$) will rise until a steady state is attained. At this $[Ca]_o$, the net rate of Ca^{2+} accumulation by the mitochondria balances the rate of Ca^{2+} infusion into the medium. Therefore, the net rate of uptake of Ca^{2+} at the $[Ca]_o$, monitored with the Ca^{2+}-selective electrode, should be equal to the rate of Ca^{2+} infusion.

This experiment was carried out both in the presence and absence of DEHP. Typical traces with the Ca^{2+}-selective electrode in the absence of DEHP are shown in Fig. 5. At all injection rates, a steady state was obtained 2–3 min after the start of infusion. Fig. 6 (closed circles) depicts the relationship between the rate of Ca^{2+} uptake into mitochondria and $[Ca]_o$. These results are consistent with those of Zoccarato and Nicholls [17]. Addition of 40 μM DEHP had no effect on the net rate of steady-state Ca^{2+} accumulation in mitochondria stabilized by Mg^{2+}, ATP and oligomycin (Fig. 6, open

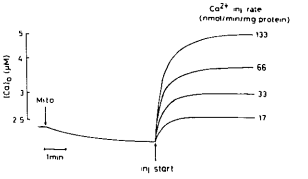

Fig. 5. Typical traces of the net rate of Ca^{2+} uptake by the steady-state infusion method monitored with a Ca^{2+}-selective electrode in the absence of DEHP. Numbers adjacent to the traces are infusion rates of Ca^{2+} in nmol/min per mg of mitochondrial protein. Similar results were obtained in the presence of 40 μM DEHP (see Fig. 6).

circles). As these results indicate that DEHP does not affect the rate of Ca^{2+} accumulation via the Ca^{2+} uniporter and the independent efflux pathway, the amount of calcium accumulated via the transport system during the first transient depolarization phase seems to be almost identical in the absence and presence of DEHP. Therefore, DEHP does not seem to reduce the amount of calcium accumulated in mitochondria, but to inhibit only the progress of membrane damage resulting in spontaneous discharge of Ca^{2+}.

A close relationship between spontaneous Ca^{2+} discharge from mitochondria and the activity of AdNT has been suggested [8,11–14], therefore we next investigated the effect of DEHP on the ADP uptake rate. As shown in Fig. 7a, DEHP at 40 μM reduced the rate of ADP uptake into mitochondria. The first-order rate constant for the exchange process was calculated using the following equation

$$A_t = A_\infty(1 - e^{-kt})$$

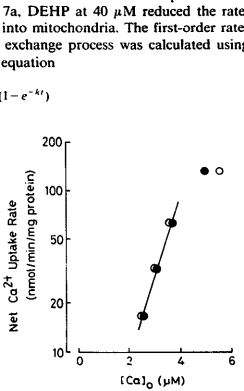

Fig. 6. Effect of DEHP on the net rate of Ca^{2+} uptake measured by the steady-state infusion method. The net rate of Ca^{2+} uptake is plotted as a function of $[Ca]_o$ in the absence (closed circles) and presence (open circles) of 40 μM DEHP.

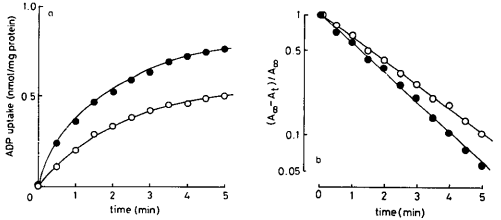

Fig. 7. Effect of DEHP on the rate of ADP uptake into mitochondria in the absence (closed circles) and presence (open circles) of 40 μM DEHP. Experimental procedures are described in the Materials and Methods. (a) Time course of ADP uptake. (b) Relationship between $(A_\infty - A_t)/A_\infty$ and time. A_t, amount of ADP taken up at time t; A_∞, amount of ADP taken up at infinite time, obtained by least-squares analysis.

where A_t and A_∞ represent the amounts of ADP taken up by mitochondria at time t and at infinite time, respectively, and k is the first order rate constant. By least-squares analysis, the results in Fig. 7a were in good agreement with this equation, and values of $(A_\infty - A_t)/A_\infty$ are plotted against time t in Fig. 7b. The first-order rate constants (k) in the absence and presence of 40 μM DEHP were calculated to be 0.56 min^{-1} and 0.40 min^{-1}, respectively; that is, the value of k in the presence of DEHP was approx. 70% that in the absence of DEHP.

Next we examined the effects of various concentrations of DEHP on the uptake of ADP into mitochondria in 30 s. As shown in Fig. 8 (closed circles), the effect of DEHP was biphasic: with increases in its concentration, DEHP initially inhibited ADP transport progressively, and showed a maximum of 45% inhibition at concentrations between 40 μM and 100 μM, but at concentrations of more than 100 μM, its inhibitory effect decreased, reaching zero at 400 μM. This profile of inhibition is very similar to that of its effect on spontaneous Ca^{2+} discharge shown in Fig. 3. DEHP was found to cause slight inhibition of the uncoupler (SF 6847)-stimulated respiration of mitochondria with succinate (plus rotenone) as the respiratory substrate (less than 20% inhibition at 40 μM and 400 μM). These results suggest that the activity of AdNT is closely related with the process of Ca^{2+} discharge.

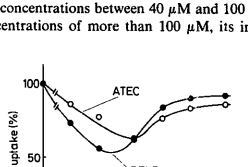

Fig. 8. Effect of DEHP and the citrate ester ATEC on the uptake of ADP into mitochondria. The amount of ADP taken up by mitochondira within 30 s after the addition of ADP was measured at various concentrations of DEHP (closed circles) and ATEC (open circles). In the absence of these compounds, the amount of ADP uptake was approx. 1.7 nmol/mg mitochondrial protein. The amount of ADP transported is expressed as a percentage of that without esters.

We next examined the effect on spontaneous Ca^{2+} discharge of another phthalate ester, DLP, which has *n*-dodecyl chains (see Scheme I for chemical structure). The results in Fig. 3 (open circles) showed that DLP had a similar effect to DEHP, but that its effect was weaker. These results suggested that the lengths of alkyl chains in the esters influence their effects on spontaneous Ca^{2+} discharge. Therefore, we next examined the effects of the tri-alkyl esters of acetyl citrate, ATEC, ATBC, ATHC and AT(OD)C, which have different alkyl chain lengths. The chemical structures of these citrate esters are shown in Scheme I, and their effects at 40 μM are summarized in Table I. All these citrate esters had similar effects to those of phthalate esters, but the magnitudes of their effects depended on their alkyl chain lengths. Of these citrate esters, ATBC

TABLE I

Effect of citrate esters on oxygen consumption stimulated by spontaneous Ca^{2+} discharge

Mitochondria: 0.7 mg protein/ml in a total volume of 2.53 ml.

Reagent (40 μM)	V_{ox} (natoms O/min per mg protein	V_{ox} relative to control (%)
Control	225	100
ATEC	205	91
ATBC	45	20
ATHC	82	36
AT(OD)C	197	88

inhibited state 4 respiration by more than 70% (data not shown), possibly due to its toxic action on the respiratory chain. Like DEHP, these citrate esters also inhibited the uptake of ADP into mitochondria. The results with ATEC are indicated by the open circles in Fig. 8. Details of the effects of citrate esters will be reported subsequently.

Discussion

In this study, we found that 20–1000 μM DEHP partially inhibited the release of respiration induced by Ca^{2+}-dependent membrane damage, followed by the spontaneous discharge of Ca^{2+} (Figs. 1 and 2). It caused maximal inhibition at about 40 μM, and was less effective at below and above this concentration (Fig. 3). DEHP also inhibited swelling of the mitochondrial matrix space induced by Ca^{2+} in a similar manner to its effects on the respiration and the spontaneous discharge of Ca^{2+} (Fig. 4). These results reflected the inhibitory effect of DEHP on spontaneous Ca^{2+} discharge.

We also found that DEHP had no effect on the rate of Ca^{2+} accumulation mediated by the Ca^{2+} uniporter and independent efflux pathway in the presence of P_i, when the mitochondria were stabilized against spontaneous Ca^{2+} discharge by Mg^{2+}, ATP and oligomycin (Figs. 5 and 6). Therefore, the inhibitory effect of DEHP on spontaneous Ca^{2+} discharge was not due to its effect on the rate of Ca^{2+} accumulation mediated by the transport system. In addition, we have already reported that DEHP had no effect on the activity of the mitochondrial P_i carrier [4]. Therefore, DEHP has an inhibitory effect only on the process of spontaneous discharge of Ca^{2+}.

The inhibitory effect of DEHP on the respiratory chain was very small as judged by its effect on uncoupler-stimulated respiration. We have reported that DEHP partially inhibits state 3 respiration, being maximally inhibitory at about 40 μM [4]. Furthermore, DEHP had little effect on the swelling of nonrespiring mitochondria induced by K^+ entry mediated by valinomycin either in exchange with the efflux of H^+ mediated by a weakly acidic uncoupler, or in accordance with the entry of the hydrophobic anion SCN^-. As the uncoupler-stimulated respiration and the swelling of mitochondria induced by valinomycin-mediated K^+ transport are dependent on the fluidity of membrane phospholipids [18,19,25], it is possible that DEHP does not change the state of the mitochondrial membrane in a nonspecific way, but specifically affects the membrane portion associated with the process of spontaneous discharge of Ca^{2+}. The similar concentration dependences of DEHP for inhibitory effects on the release of state 3 respiration and on spontaneous discharge of Ca^{2+} suggest that those effects are based on the same mechanism. The inhibitory effect of DEHP on AdNT activity seems to participate in the effects on both state 3 respiration and Ca^{2+} discharge, because inhibitors of AdNT are known to affect the stability of the mitochondrial membrane against Ca^{2+} discharge (Fig. 4 and Refs. 8, 11–14).

We found that DEHP reduces the amount of ADP uptake into mitochondria mediated by AdNT in a similar manner to its inhibition of Ca^{2+} discharge (Figs. 7 and 8). We previously reported that DEHP retarded uncoupling by hydrophobic cations and heavy metals [4]. Uncoupling induced by cationic uncoupling agents and spontaneous Ca^{2+} discharge seem to have some common characteristics. For example, the uncoupling action of the cationic cyanine dye tri-S-C_3(5) results in P_i-dependent auto-accelerating release of respiration. This uncoupling is inhibited by exogenous adenine nucleotides, and is associated with swelling of the mitochondrial matrix space [26]. Spontaneous Ca^{2+}-discharge also shows these features [7]. Shinohara and Terada [5] pointed out the involvement of a 29000 dalton protein, which is suggested to be AdNT, in the action of cationic uncouplers. These findings together with the present results support the possibility that the inhibitory effect of DEHP on spontaneous Ca^{2+} discharge is based on modification of the activity of the AdNT.

In reconstituted liposomes, the activity of AdNT is affected by the lipid composition of the liposomes [27,28]. Further, Krämer [29] reported that AdNT activity was modified by incorporation of cholesterol into the reconstituted liposome system. These results suggest that the activity of the AdNT is sensitive to perturbation of membrane lipids. From this point of view, it is interesting that Pfeiffer et al. [30] found that the local anesthetic nupercaine inhibited spontaneous Ca^{2+} discharge, because the anesthetizing actions of local anesthetics are known to be based on their actions on the lipid-water boundary of membranes [31]. In the present study, DLP and alkyl citrates were also found to inhibit the spontaneous discharge of Ca^{2+}. Furthermore, they affected the uptake of ADP into mitochondria. These results support the possibility that the inhibitory effect of DEHP on adenine nucleotide exchange is due to its interaction with membrane lipids surrounding the AdNT, thus causing modification of the state of the AdNT. Induction of AdNT to the c-state by CATR increases the susceptibility of mitochondria to Ca^{2+} damage (Fig. 4 and Ref. 14).

All these compounds including DEHP are plasticizers for PVC plastics. The plasticizing effect of a plasticizer is attained by weakening the dipole–dipole interaction of C–Cl bonds in PVC molecules. For a plasticizing action, two molecular characteristics are essential: (1) association of the polar parts (ester bonds) of the plasticizer molecules with C–Cl bonds in PVC chains, and (2) shielding of the dipole moments by

hydrophobic alkyl chains of plasticizer molecules. Therefore, the length of alkyl chains is an important factor for the action of plasticizers. These two characteristics of plasticizer molecules may also be essential for their action on biological membranes. Accordingly, the interaction of plasticizers with plastics may be analogous with that of plasticizers with mitochondrial membrane lipids.

Thus, we may conclude that the effects of DEHP on mitochondrial functions are based on its action on membrane lipids, resulting in alteration of lipid–protein interaction in the mitochondrial membrane. The most susceptible protein would be the AdNT. This newly discovered property of DEHP seems to be very important for evaluating the biological effects of plasticizers, including DEHP.

Acknowledgments

The authors thank Mr. Yasuo Shinohara for helpful advice, and Mr. Yosuke Fukui for technical assistance.

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