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Duramycin effects on the structure and function of heart mitochondria. I. Structural alterations and changes in membrane permeability

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The polypeptide antibiotic duramycin has been reported to interact specifically with two lipids: phosphatidylethanolamine (PE) and monogalactosyldiacylglycerol (Navarro et al. (1985) Biochemistry 24, 4645–4650). PE is a major component of mitochondrial membranes. Duramycin was used to examine the role of PE in maintenance of mitochondrial structure and membrane permeability properties with the following results: (1) Duramycin addition to isolated rat heart mitochondria produced abrupt organelle contraction which was followed, depending on composition of the suspending medium, by pronounced swelling. The most notable morphological effect of the antibiotic was ruffling or crenelation of the outer membrane, which resulted ultimately in its separation from the inner membrane. (2) Low concentrations (< 5 μM) of the antibiotic selectively increased the permeability of the mitochondrial inner membrane to cations and small solutes. This effect was blocked by atractyloside, a highly specific inhibitor of the adenine nucleotide translocator, by palmitoyl coenzyme A, by *N*-ethylmaleimide, and by AMP, ADP and ATP but not GDP or GTP, implicating the adenine nucleotide translocator in the selective permeability increase. (3) Higher concentrations of duramycin induced a more generalized permeability increase which was not subject to inhibition by compounds capable of interacting with the adenine nucleotide translocator.

Introduction

Duramycin is a polypeptide antibiotic (M_r 2012) isolated from cultures of *Streptoverticillium cinnamomeus*, NRRL B-1699 (formerly *Streptomyces cinnamomeus* forma azacoluta) [1]. Recently, duramycin has been reported to interfere with the function of several membrane systems. The chloride transporter of clathrin-coated vesicles [2], ATP-dependent Ca^{2+} uptake by rabbit skeletal muscle sarcoplasmic reticulum vesicles [3], and proton secretion by *Bacillus subtilis* [3] are inhibited 50% at < 25 nmol/mg protein. The proton pump of clathrin-coated vesicles is inhibited 50% at 100 nmol/mg protein [2] and at higher concentrations (500 nmol/mg protein), duramycin inhibits the Na^+/K^+-ATPase purified from dog kidney [4]. Duramycin can also permeabilize intact cells [5].

In model systems the effects of the antibiotic appear to result from its interaction with lipids which, when hydrated under physiological conditions, adopt the hexagonal$_{II}$ phase, a reflection of the low intrinsic radius of curvature (R_{II}) of the lipid monolayer which contains them [6]. Duramycin induces an increase in the turbidity (measured as apparent absorbance at 400 nm) of suspensions of lipid vesicles prepared from phospha-

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Abbreviations: EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; FCCP, carbonylcyanide *p*-trifluoromethoxyphenylhydrazone; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; i.m., inner membrane; MGDG, monogalactosyldiacylglycerol; Mops, 4-morpholinepropanesulfonic acid; o.m., outer membrane; PC, phosphatidylcholine; PCMB, *p*-chloromercuribenzoic acid; PE, phosphatidylethanolamine; Tris, 2-amino-2-hydroxymethylpropane-1,3-diol.

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tidylcholine (PC) in combination with phosphatidylethanolamine (PE) or monogalactosyldiacylglycerol (MGDG), both of which are capable of forming inverted structures [7,8]. It has no effect on suspensions of vesicles made from PC alone or PC plus phosphatidic acid, cardiolipin or phosphatidylserine [3]. Choung et al. [9,10] have recently demonstrated that a related antibiotic Ro09-0198 distinguishes PE from all other common phospholipids as evidenced by the exclusive ability of PE-containing liposomes to protect erythrocytes from Ro09-0198-induced hemolysis. Furthermore, they present chromatographic evidence for a PE-peptide complex [9].

Several observations suggest that the effects of duramycin on biological membranes can also be attributed to specific drug–lipid, rather than drug–protein, interactions. Duramycin inhibits generation of a proton gradient by bacteriorhodopsin reconstituted into PC/PE, but not PC-only, vesicles [3]. Duramycin inhibits proton secretion by wild type *B. subtilis* but not by a mutant strain lacking PE [3]. Finally, the Ca^{2+} uptake function of the sarcoplasmic reticulum ATPase, which has been shown to depend upon the presence of PE [11,12], is inhibited by duramycin.

Lipids with the potential for forming inverted structures are major components of the energy-transducing membranes of mitochondria and chloroplasts. PE represents > 35% of the phospholipid phosphorus of the inner mitochondrial membrane, and cardiolipin, which can be induced by Ca^{2+} to form inverted structures [13,14], constitutes an additional 25% [15,16]. (The outer mitochondrial membrane also contains significant amounts (28%) of PE). Similarly, the glycolipid MGDG accounts for > 50% of the polar lipid in the thylakoids of higher plant chloroplasts [17]. It can be hypothesized that non-bilayer lipids play a central role in the structure and/or function of energy-transducing organelles by virtue either of their ability to form actual non-bilayer structures in situ or their more subtle effects on R_0.

In these studies, duramycin has been used to probe the role of PE in isolated rat heart mitochondria, with particular attention being paid to antibiotic effects on mitochondrial structure and inner membrane permeability. Preliminary reports of these data have appeared [18,19].

Materials and Methods

Mitochondria, a mixed population containing both interfibrillar and subsarcolemmal organelles, were isolated by a modification [20] of the procedure described by Sordahl [21] from the hearts of large (> 250 g) male Sprague-Dawley rats. Unless otherwise noted, experiments were carried out at 25°C in a resin (Chelex)-treated reagent (sucrose, 100 mM; KCl, 50 mM; Mops-KOH, pH 7.2, 20 mM; KH_2PO_4, 1.7 mM) supplemented with 0.8 μM rotenone. Mitochondria equivalent to 0.2 mg protein/ml were preincubated in this reagent for 3 min prior to the onset of experimentation. Where indicated, they were energized by the addition of 5 mM succinate. Data shown are representative of multiple (≥ 3) experiments.

Changes in mitochondrial volume were followed qualitatively at 540 nm in an LKB Ultrospec II UV-VIS spectrophotometer, with a decrease in apparent absorbance indicating swelling [22]. Mitochondrial permeability was assessed by measuring passive swelling (no succinate) in the presence of various inorganic salts (150 nm) or neutral solutes (200 mM) buffered with 20 mM Hepes-Tris (pH 7.2) and supplemented with rotenone as above. Protein was determined according to Lowry et al. [23] with bovine serum albumin as standard.

Mitochondria were prepared for electron microscopy by fixation in 5% glutaraldehyde. After centrifugation (15000 × g, 3 min), pellets were rinsed with buffer, postfixed in 1% OsO_4 containing 1.5% $K_3Fe(CN)_6$, stained in block with 5% aqueous uranyl acetate, dehydrated through an ethanol series, and embedded in an Em-bed/araldite mixture. Ultrathin sections were stained with methanolic uranyl acetate followed by lead citrate and examined with a Siemens Elmiskop 101 electron microscope at 80 kV. Average mitochondrial cross-sectional area was determined with a digitizing graphics tablet and morphometry analysis software [24]. A minimum of 30 mitochondria were measured for each of two sections per sample. Statistical analyses used a two-tailed Student's *t*-test to determine the significance of the difference between two independent means.

Duramycin was generously supplied by Dr. O.L. Shotwell (Northern Regional Research Center, United States Department of Agriculture, Peoria, IL). Chelex 100 (100–200 mesh) was purchased from Bio-Rad, Richmond, CA, and FCCP from Aldrich, Milwaukee, WI. Biochemicals and enzymes were from Sigma Chemical Company, St. Louis, MO. All other reagents were of the highest quality available.

Results

Duramycin increases the turbidity of suspensions of small unilamellar liposomes that contain PE or MGDG [3]. The antibiotic also had dramatic effects on the apparent absorbance of suspensions of isolated rat heart mitochondria (Fig. 1). Duramycin addition induced an abrupt increase in apparent absorbance at 540 nm (A_{540}), which was followed by a slower decrease. (Duramycin addition to standard buffer had no effect on A_{540}.) The duramycin-induced decrease in A_{540} could be further resolved into an energization-dependent component, which was inhibited by the uncoupler FCCP or

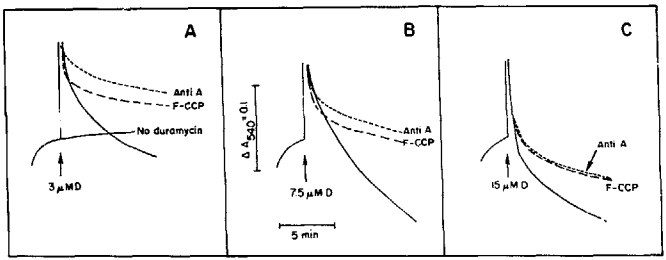

Fig. 1. Effect of duramycin on the apparent absorbance at 540 nm (A_{540}) of isolated rat heart mitochondria. Mitochondria were pre-incubated for 3 min with rotenone and, where indicated, FCCP (100 nM) or antimycin A (0.1 μg/ml). At the arrow, duramycin (3, 7.5 or 15 μM) and succinate (5 mM) were added. Temperature was maintained at 25°C. A_{540} upon mitochondrial addition was 0.4 ± 0.19 (S.D.).

the electron transport inhibitor antimycin A (Fig. 1) or by omission of succinate (data not shown), and an energization-independent component. Entirely analogous results were obtained when the mitochondria were energized with glutamate plus malate, with the exception that the decrease in A_{540} could now be partially blocked by rotenone (not shown). Thus, three components of the mitochondrial response to duramycin addition can be identified: (1) a rapid increase in A_{540}, (2) a slow energization-dependent decrease in A_{540} which is supported by both NADH-linked and flavoprotein-linked substrates, and (3) an energization-independent decrease in A_{540}.

When the osmolarity of the suspending solution is manipulated, A_{540} is qualitatively proportional to the inverse of mitochondrial volume [22,25]. However, other factors, e.g., aggregation, lysis, alterations in the absorption spectrum of mitochondrial components, can also affect suspension turbidity [22]. Transmission electron microscopy indicated that the changes in A_{540} induced by duramycin reflect authentic alterations in mitochondrial volume (Fig. 2). Duramycin addition induced abrupt contraction of the mitochondria, associated with generation of parallel arrays of cristae (Fig. 2B). The contraction was quantitated morphometrically. In a representative experiment, addition of 5 μM duramycin decreased the average mitochondrial cross-sectional area from 0.825 ± 0.193 μm^2 to 0.493 ± 0.149 μm^2. When data from three separate experiments were pooled, control dimensions of 1.040 ± 0.259 μm^2 were reduced to 0.652 ± 0.109 μm^2 by 5 μM antibiotic. In both cases the effect of duramycin on mitochondrial size was significant at the $P < 0.05$ level. Contraction was followed by gradual swelling, vesicularization of the inner membrane (i.m.; Fig. 2C), and ultimately a decrease in matrix density (Fig. 2D).

Duramycin addition also induced marked, distinctive alterations in mitochondrial morphology. Foremost among these was ruffling or crenelation of the outer membrane (o.m.) and its dissociation from the i.m. (Fig. 2D, arrows). This effect was not mimicked by detergents, either Triton X-100 or the relatively o.m.-specific agent digitonin; by glycerol, which has been reported to disrupt contacts between the i.m. and o.m. [26]; or by agents/treatments which induce mitochondrial swelling such as glutathione or Ca^{2+} loading (data not shown).

The two components of the duramycin-induced decrease in A_{540} could be distinguished on the basis of their dependence on duramycin concentration (Fig. 3). The energization-dependent component was half-maximal at 2 μM duramycin (10 nmol/mg protein) and peaked at 5 μM (25 nmol/mg protein). In contrast, the energization-independent process had a C_{50} for duramycin > 5 μM. The mitochondrial contraction induced by duramycin was independent of energization (Fig. 1). A plot of the magnitude of this component as a function of duramycin concentration yielded a C_{50} identical to that for energization-dependent swelling (Fig. 3). For all three components, duramycin effects were dependent on the ratio of antibiotic to mitochondrial protein rather than on absolute antibiotic concentration (data not shown).

The concentration of duramycin required to elicit each of the components of the change in apparent absorbance at 540 nm was decreased by an increase in assay temperature. The effect of temperature on the energization-independent decrease in A_{540} was particularly large (Fig. 4). At higher temperatures, energy-dependent and energy-independent swelling were therefore difficult to resolve (not shown). The temperature data suggest in addition (Fig. 4) that pronounced energization-independent swelling occurred only when duramycin-induced contraction was maximal.

Mitochondrial swelling reflects altered i.m. permeability. Passive swelling in the presence of diverse solutes was used to determine the selectivity of the duramycin-

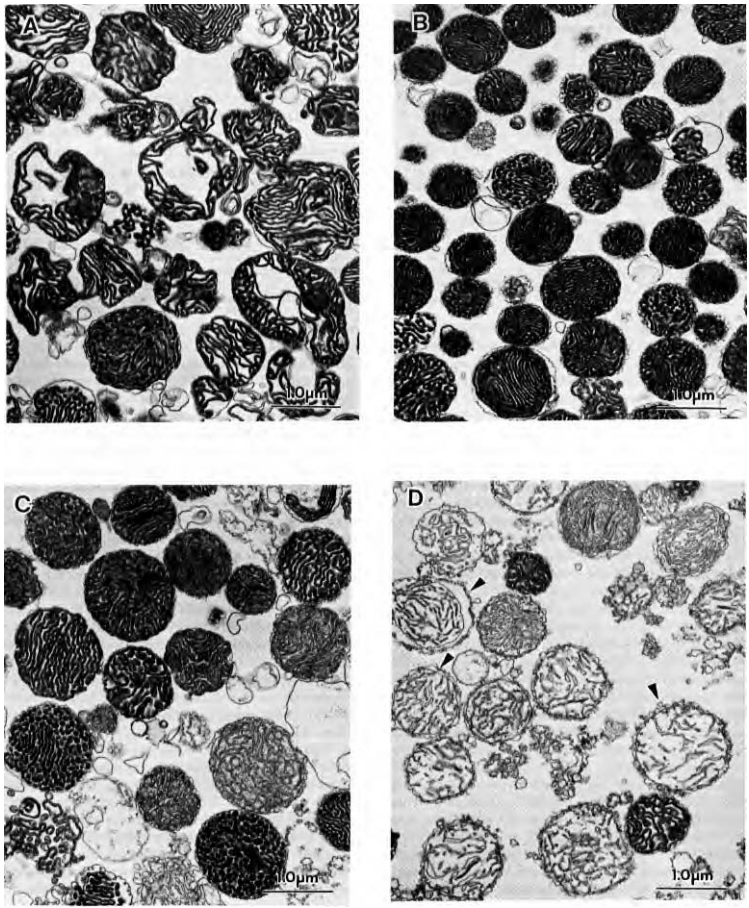

Fig. 2. Effect of duramycin on mitochondrial structure. (A) Control rat heart mitochondria fixed after 3 min incubation in standard reagent supplemented with rotenone. (B) Mitochondria fixed immediately upon addition of 5 μM duramycin and 5 mM succinate. (C) Mitochondria fixed 5 min after the addition of succinate plus 5 μM duramycin. (D) Mitochondria fixed after 12 min in the presence of succinate and 10 μM duramycin.

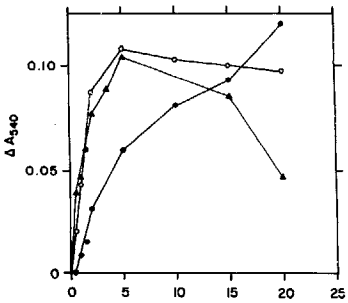

Fig. 3. Dependence on duramycin concentration of the three components of A_{540}. ○, Rapid increase in A_{540}; ●, energy-independent decrease in A_{540} (maximum A_{540} after duramycin addition minus A_{540} 6 min later, measured in the absence of succinate); ▲, energy-dependent decrease in A_{540} (difference at 6 min in the A_{540} values reached in the presence and absence of succinate).

induced permeability increases. For a series of salts, relative swelling rates obtained with 3–5 μM duramycin were $NH_4NO_3 > KNO_3$, $NaNO_3$, $LiNO_3 > NH_4Cl$, KCl, NaCl, LiCl > 0 (Fig. 5A). By comparison, valinomycin (2 μg/ml) induced much more rapid swelling in KNO_3 and no swelling in KCl. When mitochondria were suspended in solutions of neutral solutes,

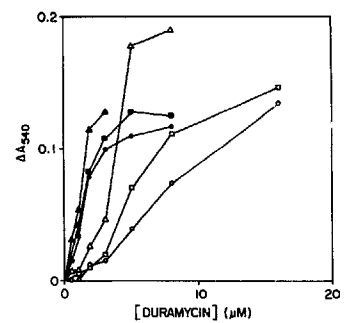

Fig. 4. Effect of temperature on the rapid increase in A_{540} (filled symbols) and the energization-independent decrease in A_{540} (open symbols) induced by duramycin. Data were obtained as outlined in the legend to Fig. 3. △, ▲, 34.3 °C; □, ■, 25.0 °C; ○, ●, 18.6 °C.

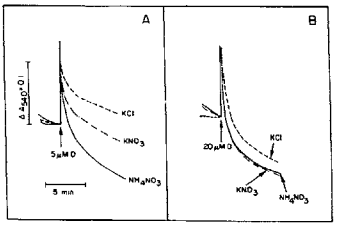

Fig. 5. Passive swelling of isolated rat heart mitochondria induced by duramycin. Mitochondria were pre-incubated in the indicated salts (150 mM) in the presence of rotenone for 3 min prior to the addition of 5 μM (panel A) or 20 μM (panel B) duramycin.

3–5 μM duramycin enhanced swelling in the presence of erythritol and arabinose but had limited effects in the presence of mannitol or sucrose (Table I). These observations on the effects of low concentrations of duramycin can be interpreted as follows. (1) Duramycin selectively enhanced i.m. cation permeability; for any cation, swelling measured in the presence of the permeant NO_3^- anion exceeded that observed with Cl^-. (2) The duramycin-induced permeability increase favored H^+ over the alkali cations; among the NO_3^- salts tested, swelling was most rapid in NH_4NO_3. (3) Anion permeability of the i.m. was increased to a limited extent, as indicated by the modest swelling recorded in the presence of Cl^- salts. (4) Duramycin increased i.m. permeability to small (< 200 dalton) neutral solutes. At high duramycin concentrations (> 10 μM) rapid and equivalent swelling was observed in the presence of all monovalent salts tested (Fig. 5B), indicating a more generalized increase in i.m. permeability. The dependence on duramycin concentration of the increases in cation-selective and generalized i.m. permeability (data not shown) were identical to those determined for en-

TABLE I

Effect of 5 μM duramycin on swelling of mitochondria in neutral solutes

Substantial swelling was observed in erythritol in the absence of duramycin. Swelling rates were therefore determined immediately upon adding mitochondria to the specific buffered solution containing rotenone (0.8 μM) and duramycin (5 μM).

Solute	ΔA_{540}/min	
	control	+5 μM duramycin
Erythritol	0.170	0.440
Arabinose	0.023	0.290
Mannitol	0.000	0.036
Sucrose	< 0.000 [a]	0.067

[a] In sucrose, A_{540} increased over the first one min.

TABLE II

Inhibition of mitochondrial swelling induced by duramycin

Swelling was measured in standard buffer in the presence of rotenone and succinate using the protocol in Fig. 1. Inhibitors were added prior to mitochondria. ΔA_{540} is the difference between the maximum A_{540} obtained after duramycin addition and the value attained 6 min later. In the absence of duramycin, $\Delta A_{540} = 0.001$. n.d., not determined.

Inhibitor	ΔA_{540}	
	3 μM duramycin	20 μM duramycin
None	0.164	0.153
10 μM palmitoyl-CoA	0.043	0.148
500 μM atractyloside	0.093	0.148
400 μM *N*-ethylmaleimide	0.085	n.d.
100 μM ATP	0.127	n.d.
0.5 mM $MgCl_2$	0.159	n.d.
100 μM ATP + 0.5 mM $MgCl_2$	0.075	0.158

ergization-dependent and energization-independent swelling, respectively (Fig. 3).

The energy-dependent swelling induced by 3 μM duramycin was inhibited by atractyloside, a specific inhibitor of the adenine nucleotide translocator (AdNT) [27], by palmitoyl-CoA, by *N*-ethylmaleimide, and by ATP (Table II). The effect of ATP was enhanced by Mg^{2+}, which exerted minimal inhibition on its own, and was 50% maximal at 30–50 μM nucleotide. ADP and AMP produced inhibition similar to that obtained with ATP; GDP and GTP were not inhibitors. Energization-dependent swelling was also inhibited if either K^+ or inorganic phosphate was omitted from the standard reagent (not shown). In no case was inhibition complete. This is to be expected since, at 3 μM duramycin, approx. 30% of the total swelling observed can be attributed to the energy-independent process (Fig. 3). Swelling induced by 20 μM duramycin and swelling obtained at lower duramycin concentrations in the absence of succinate, i.e., energy-independent swelling, were not altered by any of these manipulations, nor was the initial rapid increase in A_{540}.

The results obtained with duramycin parallel results of earlier investigations using the sulfhydryl-modifying reagent *p*-chloromercuribenzoate (PCMB). At 10–20 nmol/mg protein, an amount sufficient to titrate both the phosphate and adenine nucleotide translocators, PCMB stimulates energy-linked swelling in potassium acetate, i.e., enhances K^+ permeability. At 15–30 nmol/mg protein, PCMB induces passive swelling of heart mitochondria in KCl, i.e., increases i.m. permeability to both cations and anions (Ref. 28, especially Fig. 13).

Several lines of evidence (data not shown) suggest that the mechanisms of action of the thiol-reactive mercurial PCMB and duramycin are distinct even though the antibiotic does contain three thioether amino acids [29]. (1) Swelling induced by duramycin was immediate (Figs. 1 and 5); that seen with PCMB occurs after an initial lag [28]. (2) PCMB blocks the phosphate translocator, monitored indirectly via swelling in $(NH_4)_2HPO_4$, whereas duramycin did not. (3) All of the effects of PCMB, including swelling in KCl, inhibition of the FCCP-stimulated ATPase, and inhibition of succinate-dependent respiration, are prevented by dithiothreitol (DTT). Duramycin effects on the same parameters were insensitive to DTT. (4) The number of mitochondrial thiols, measured with 5,5′-dithiobis-(2-nitrobenzoic acid), was not altered by duramycin. (5) The permeability induced by PCMB favors K^+ over Na^+ and H^+ [30]. Duramycin-induced passive swelling favored H^+ over K^+ and Na^+ (Fig. 5A).

Discussion

The polypeptide antibiotic duramycin, which has been reported to interact specifically with PE [3], had marked effects on isolated rat heart mitochondria. At low concentrations (< 5 μM), duramycin selectively increased inner membrane permeability to monovalent cations and small neutral solutes. This resulted in passive swelling in the presence of salts of permeant anions as well as in energization-dependent swelling. A limited increase in i.m. permeability to Cl^- was also observed, but this may reflect the general increase in permeability which becomes more prominent at higher antibiotic concentrations. The apparent dependence of energization-dependent swelling on K^+, and inorganic phosphate (P_i) suggests that the ion movements involved include active H^+ extrusion, OH^-/P_i^- exchange, and K^+ influx, resulting in net electroneutral potassium phosphate accumulation.

The duramycin-induced permeability increase occurred rapidly; no lag was apparent in the onset of either passive or energized swelling. The permeability increase was correlated with abrupt contraction of the mitochondria. Both effects were half-maximal at 2 μM duramycin, or 10 nmol antibiotic per mg protein. Mitochondria are thus the most sensitive antibiotic target yet reported. Assuming phospholipid levels of 0.25 mg/mg protein of which 44% is PE [16], a PE concentration of 30 μM can be calculated for these experiments. Inner membrane permeability to cations was therefore half-maximally increased at a duramycin to PE ratio of 1 : 15.

Adenine nucleotide addition has been reported to elicit a rapid increase in the apparent absorbance of suspensions of heart mitochondria which is correlated with nucleotide binding to the AdNT [31,32]. The absorbance increases induced by adenine nucleotides and by duramycin differ in several important respects. First, adenine nucleotide effects are small and have, in fact, been classically termed low-amplitude changes [33,34].

In our experimental system, the maximal increase in A_{540} induced upon ADP addition was less than 10% of the maximal signal obtainable with duramycin (data not shown). Second, absorbance increases induced by duramycin reflect a marked decrease in mitochondrial volume; those induced by adenine nucleotides do not, representing instead "ultrastructural reorganization of the inner membrane" [34]. Finally, atractyloside blocks adenine nucleotide-induced absorbance increases [31] but not those induced by duramycin.

The mitochondrial AdNT is clearly implicated in the selective increase in i.m. permeability induced by low duramycin concentrations. The permeability increase was blocked by atractyloside, a highly specific inhibitor of the adenine nucleotide translocator [27] and by palmitoyl-CoA. Palmitoyl-CoA exerts multiple effects on mitochondria, but among these is inhibition of the AdNT [35]; atractyloside and palmitoyl-CoA stabilize the translocator in the same conformation [27]. The ability of AMP, ADP and ATP, but not GDP or GTP, to block this action of duramycin also indicates participation of an adenine nucleotide binding protein in the cation-selective permeability increase.

Two observations are difficult to reconcile with involvement of the AdNT in duramycin-induced mitochondrial swelling. First, Mg^{2+} enhanced the inhibitory effects of the adenine nucleotides, yet the true substrates of the AdNT are reported to be the free forms of ADP and ATP [36]. Second AMP, which is reported not to bind to the AdNT [36], blocked duramycin action as effectively as ADP or ATP. Nucleotide binding to the AdNT is inferred from a substrate-induced change in the fluorescence of the isolated AdNT [36]. AMP and the Mg^{2+} complexes of ADP and ATP may interact with the substrate binding site in a manner sufficient to block duramycin action but not to promote the conformational change resulting in altered fluorescence.

Two qualitatively very different models for the interaction of low concentrations of duramycin with the mitochondrial i.m. can be outlined. Both models are consistent with the data presented here and take into account prior reports on the effects of PCMB. One model postulates that duramycin alters the conformation of the AdNT such that the translocator itself permits the passage of monovalent cations and small solutes. Panov et al. [37] have reported that the AdNT can function as a gated pore for K^+ and H^+, and participation of the AdNT in calcium-induced perturbations of i.m. permeability has also been proposed [38]. Such a direct effect of duramycin on AdNT behavior could be mediated by interaction of duramycin either with the protein or with PE. PE is known to be required for optimal function of the reconstituted AdNT [39]. Discrimination between these alternative mechanisms will require determination of duramycin effects on the function of a reconstituted AdNT system.

At concentrations sufficient to inhibit both the P_i and adenine nucleotide-translocators, PCMB has also been reported to enhance i.m. permeability to cations [30]. Several observations, outlined above, most notably insensitivity of duramycin effects to dithiothreitol and failure of the antibiotic to alter mitochondrial sulfhydryl content, indicate that duramycin is not a sulfhydryl reagent. This model would propose that PCMB, by interacting with -SH groups, and duramycin, by interacting either with PE or with the AdNT, produce similar changes in i.m. permeability specifically by altering AdNT behavior.

The second model proposes that the enhanced permeability elicited by low concentrations of duramycin is mediated by the lipid constituents of the i.m. Oxidation of red blood cell membranes by diamide enhances membrane permeability to small molecules [40] while at the same time favoring PE movement between the inner and outer leaflets of the bilayer [41]. These effects are attributed to modification of sulfhydryl groups in a particular membrane-associated protein, namely, spectrin. It can be proposed that transbilayer movement of PE is the key element in increased membrane permeability in both the erythrocyte and the mitochondrial i.m. (the two processes have been linked in other reports as well [42]). Perhaps, in the mitochondrial i.m., lipid asymmetry [43] is maintained by the AdNT, the most abundant integral i.m. protein [44] just as spectrin controls lipid asymmetry in the red blood cell. Then, both duramycin and PCMB would be operating by increasing the transbilayer mobility of PE and thus inner membrane permeability, duramycin by acting directly on the lipid, PCMB by altering the AdNT. Evaluation of this model will require determination of the extent to which duramycin effects on membrane permeability require the presence of the AdNT as well as measurements of transbilayer lipid mobility.

In addition to its effects on cation permeability, duramycin induced dramatic changes in mitochondrial morphology, particularly o.m. appearance, and at higher concentrations the antibiotic induced a generalized increase in membrane permeability. The latter effect may simply reflect membrane disruption, although preliminary data showing inhibition of the uncoupled F_1/F_0-ATPase [19] suggest a more complex mode of action.

To the extent that duramycin interacts specifically with PE, the findings reported here demonstrate the antibiotic's potential as a probe of PE function in mitochondria. These studies are currently being enlarged to examine the effect of duramycin on mitochondrial energy conversion reactions, to measure directly the influence of duramycin on lipid phase behavior, and to document the extent to which, in this system, duramycin effects represent duramycin–lipid as opposed to duramycin–protein interactions.

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