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INHIBITION OF THE BIOENERGETIC FUNCTIONS OF ISOLATED RAT LIVER MITOCHONDRIA BY POLYAMINES*

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Abstract—The abilities of the naturally occurring polyamines, putrescine, spermidine and spermine, to affect variables related to the bioenergetic functions of isolated rat liver mitochondria were studied. At concentrations comparable to those present intracellularly, the polyamines inhibited state 4 respiration, but they had much less effect on state 3 or uncoupled respiration. The concentrations required to produce 25% inhibition (I_{25}) of state 4 respiration varied according to the polyamine, with putrescine being least effective (I_{25}, 20 mM) and spermidine and spermine being more effective and comparable (I_{25}, 7.5 and 7.0 mM respectively). This inhibition was antagonized by 15 mM potassium and enhanced by valinomycin and 4 mM magnesium. Inhibition of monoamine oxidase, an enzyme of outer mitochondrial membrane, was also observed to occur. Addition of polyamines to mitochondrial suspensions caused an increase in the optical density and protected against the swelling effects of sublytic concentrations of Triton X-100. By electron microscopy, polyamines were found to cause the outer mitochondrial compartment to collapse bringing the inner and outer membranes into apparent contact with one another. The electrophoretic mobility of mitochondria toward the anode was markedly slowed by polyamines (i.e. 50% by 1.25 mM spermine), indicating surface binding and neutralization of the negative surface charge. In almost all of the above mitochondrial effects, spermine and spermidine were similar in effectiveness and putrescine was less effective. It is suggested that polyamines may be capable of modulating respiration of isolated mitochondria by binding to non-specific anionic sites at the surface of the inner mitochondrial membrane. Neutralization of the net negative surface potential may interfere with cation fluxes across the membrane, particularly those of potassium.

The naturally occurring polyamines, putrescine, spermidine and spermine, are ubiquitous among eukaryotic cells and are known to reach intracellular concentrations approaching the millimolar range [1]. While their precise role in cellular physiology has not yet been defined, the polyamines, because of their polycationic nature, are known to substitute for inorganic cations, such as magnesium, calcium and potassium, in a variety of biosystems (see Refs. 2–4 for review). Conceivably, they might also exhibit a non-specific affinity for the negatively charged phospholipid groups and sialic acid residues associated with the membrane of cells and organelles. Given these considerations, it might be expected that polyamines could modulate mitochondrial function since the latter is intimately dependent on transmembranous proton and cation fluxes and on the presence of a charge differential across the inner mitochondrial membrane [5].

To date, the possibility that polyamines might have an intracellular modulating role in mitochondrial respiration has received only limited attention in the literature. Chaffee *et al.* [6, 7] have reported that spermidine inhibits state 4 respiration of isolated mitochondria of rat liver and kidney and state 3 respiration to a lesser extent. Using α-ketoglutarate as substrate, they found that 1.0 mM spermine and 1.25 mM spermidine enhanced the respiratory control ratios by suppression of respiration after conversion of ADP to ATP. In a subsequent study, using β-hydroxybutyrate as substrate, Chaffee *et al.* [8] reported that shifts in the respiratory control ratio and role of respiration during ADP–ATP conversion could be affected by much lower concentrations of spermine (15.7 μM). They suggested that alterations in intracellular polyamine levels may play a role in metabolic regulation of mitochondria *in vivo*. Finally, methylglyoxal-bis(guanylhydrazone) (MGBG)‖, an anticancer agent having a structural resemblance to spermidine [9, 10], was shown recently to inhibit respiration of isolated rat liver mitochondria at millimolar concentrations [11] and to affect mitochondrial structure and function in intact cells [12, 13]. Although none of the above studies indicates a role for polyamines in modulating mitochondrial function in the intact cells, the present investigation was undertaken to further define the effects of polyamines on isolated rat liver mitochon-

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‖ Abbreviations: MGBG, methylglyoxal-bis(guanylhydrazone); EGTA, ethyleneglycol-bis(beta-aminoethylether),*N*,*N*-tetraacetic acid; DNP, 2,4-dinitrophenol; and TMPD, *N*,*N*,*N*¹*N*¹-tetramethyl-*p*-phenylenediamine.

dria and to examine the molecular basis for these effects.

MATERIALS AND METHODS

Rat liver mitochondria were prepared according to the method of Weinbach (1961) as described elsewhere [14], and the homogeneity of the mitochondrial fraction was confirmed by electron microscopy. Isolated mitochondria were suspended in 0.25 mM sucrose (British Drug House Chemical Ltd., Poole, England) with 10 mM Tris–HCl buffer (pH 7.3) and 1 mM potassium ethyleneglycol-bis(beta-aminoethylether),*N*,*N*-tetraacetic acid (EGTA, Sigma Chemical Co., St. Louis, MO). Protein concentrations were determined by the method of Lowry *et al.* [15], and samples were diluted appropriately to be equal. The polyamines associated with the isolated mitochondria were separated and quantitated in 0.6 M perchloric acid extract by high pressure liquid chromatographic analysis as described elsewhere [16].

Respiration of mitochondria was measured polarographically by the method of Estabrook [17] using a Clark oxygen electrode at 25°. The reaction mixture (final volume, 1.5 ml) contained 15 mM KCl and 50 mM Tris–HCl (pH 7.2) as described by Slater and Holton [18]. In this half-isotonic medium, the respiratory measurements were the same as those made in isotonic medium except that the rate transitions were more sharply demarcated and, therefore, easier to quantitate. The specific composition of the media varied according to the experimental design and is given in the figure legends. The sensitivity of state 3 and state 4 respiration (established by the presence or absence of ADP, respectively, as defined by Chance and Williams [19]) to polyamines was measured. Oxygen consumption was recorded after addition of 50 μl of mitochondrial suspension (3.5 mg of protein) to the electrode chamber containing medium and 5 mM glutamate or 5 mM succinate (plus glutamate) as substrate. Two minutes after the addition of mitochondria, putrescine, spermidine or spermine (Sigma Chemical Co.) was added in a volume of 10–20 μl using a Hamilton microsyringe. Relative oxygen consumption was calculated by assuming that the respiration rate before the drug addition represented 100% of activity.

Changes in optical density of mitochondrial suspensions were followed using a recording spectrophotometer set at 540 or 800 nm. Decreases in optical density of mitochondrial suspensions at 540 nm were used as a measure of organelle swelling [20] while increases at 800 nm were taken as an indication of mitochondrial aggregation [21] which was confirmed microscopically. Mitochondria were suspended in media containing various concentrations of salts and/or sucrose and buffered to pH 7.2 with Tris–HCl (the specific compositions of the media are given in the figure legends). Subsequent additions were made in 10 μl volumes. Spectrophotometric measurements were made at room temperature immediately after the addition of 10 μl of mitochondrial suspension (0.7 mg of protein) into the medium (final volume, 3.0 ml).

Samples of the mitochondrial suspensions were observed by electron microscopy following drug treatment. Aliquots (1.5 ml) of control and drug-treated mitochondria were transferred from the electrode chamber to 3 ml of 0.1 M phosphate-buffered 3% glutaraldehyde at 4°. After 45 min of fixation, the mitochondrial suspension was centrifuged at 10,000 *g* for 5 min. Fixed mitochondrial pellets were washed overnight in phosphate buffer, postfixed in 1% phosphate-buffered osmium textroxide at 4° for 3 hr, dehydrated in graded ethanol series, and embedded in Epon-Araldite plastic resin. Thin sections (90 nm) were prepared using a Porter-Blum MT-1 ultramicrotome (Sorvall Corp., Norwalk, CT), stained with uranyl acetate-lead citrate, and examined with a Siemens Elmiskop 101 electron microscope.

For electrophoretic mobility studies, mitochondria (100 μl) were suspended in a medium containing 250 mM sucrose, 15 mM KCl, 2 mM succinate and 10 mM Tris–HCl (pH 7.2) at a final volume of 10 ml. Treated and control mitochondria were preincubated for 5 min at 37° prior to mobility measurements. Electrophoresis was carried out at 37° using a cylindrical tube apparatus as described elsewhere [22]. A constant voltage of 50 V was applied across gray sintered-platinum electrodes. A running distance of 10 μm was timed for each particle. Electrode polarity was reversed after each transit, and each particle was measured for two transits. Transit times were obtained using a recording timer, and minimum of 50 particles was measured (100 transits). Apparatus parameters were checked using human red blood cells in phosphate-buffered saline ($\mu = -1.38$ μm per sec per V per cm). All mobilities were measured at the proximal stationary level.

Monoamine oxidase activity in rat liver mitochondria was measured by the method of Weetman and Sweetman [23] using 5-hydroxytryptamine as substrate. The initial velocity of the enzyme activity was measured at 25° in the absence and presence of inhibitor with an oxygen electrode in a medium containing 15 mM KCl, 50 mM Tris–HCl (pH 7.2), and rotenone plus antimycin or KCN. The reaction was initiated by the addition of 5-hydroxytryptamine at final concentrations ranging up to 1 mM.

RESULTS

High pressure liquid chromatographic analysis, performed as described elsewhere [13], revealed that significant quantities of all three polyamines were present in rat liver and that they were found in similar proportion and amount in mitochondria following the isolation procedure (Table 1). Spermine and spermidine were present in approximately equal quantities which were about twenty times greater than that of putrescine. Given the rigors of the isolation and washing procedures, it is assumed that the polyamines measured here represent tightly bound molecules, perhaps associated with mitochondrial membranes. Their quantity, however, is probably a reflection of their binding affinity rather than the amount present prior to tissue homogenization. Thus, in addition to those polyamines exogenously added to mitochondrial suspensions,

Table 1. Endogenous polyamine content of rat liver and isolated rat liver mitochondria

Polyamines	Liver		Mitochondria	
	nmoles/mg wet wt	μM*	nmoles/mg protein	μM*
Putrescine	0.03 ± 0.003†	38	0.18 ± 0.04†	27
Spermidine	0.52 ± 0.07	660	3.56 ± 0.34	534
Spermine	0.48 ± 0.07	600	3.80 ± 0.29	570

* Based on a calculated liver density of 1.26 mg/ml and a mitochondrial protein density of 1 mg/14 μl. Concentration estimates assume uniform polyamine distribution in liver and mitochondria.

† Standard deviation where N = five rats.

pre-existing polyamines were presumed bound to the isolated mitochondria.

At millimolar concentrations, the polyamines inhibited selectively state 4 respiration of rat liver mitochondria. Typical oxygraph tracings, showing the effects of 20 mM spermidine on mitochondrial respiration, are given in Fig. 1. The effects of the other polyamines, spermine and putrescine, were qualitatively similar and are not shown. Under conditions suitable for oxidative phosphorylation (in the presence of phosphate ions), there was only slight inhibition by spermidine on state 4 respiration and no effect on state 3 or uncoupled respiration (Fig. 1, A–C). Inhibition of state 4 respiration occurred whether the polyamines were added after (Fig. 1A) or before (Fig. 1B) ADP. In the presence of valinomycin and potassium, marked inhibition occurred (Fig. 1D). In the absence of phosphate ions, spermidine inhibited state 4 respiration to a greater extent (Fig. 1E) but still had no effect on respiration uncoupled with DNP (Fig. 1, E and F). The inhibition was dose-dependent and the concentration required to produce a 25% inhibition (I_{25}) of state 4 respiration varied according to the polyamine, with putrescine being least effective (I_{25}, 20 mM) and spermidine being more effective and comparable (I_{25}, 7.5 and 7.0 mM respectively).

The inhibitory action of the polyamines on state 4 respiration was found to be strongly dependent on the potassium concentration in the medium. The dose-dependent nature of spermine effects on the reciprocal relative respiratory activity of mitochondria aged 12 hr at 0° in 250 mM sucrose, in the presence and absence of 100 mM KCl, is shown in Fig. 2. Aged mitochondria lose their endogenous potassium and membrane-bound magnesium. It appears that potassium protects mitochondria from the inhibitory action of spermine, even at high concentrations of the polyamine. By contrast, low concentrations of magnesium ions enhanced the inhibitory effects of polyamines on state 4 respiration. Figure 3 shows the dose-dependent effects of spermidine on the reciprocal of relative oxygen uptake by mitochondria, aged 24 hr at 0° in 250 mM sucrose + 1 or 4 mM magnesium sulfate in the presence of 15 mM potassium. At 4 mM, magnesium increased inhibition by spermidine, and at 1 mM the kinetics of respiratory inhibition became parabolic. Such a

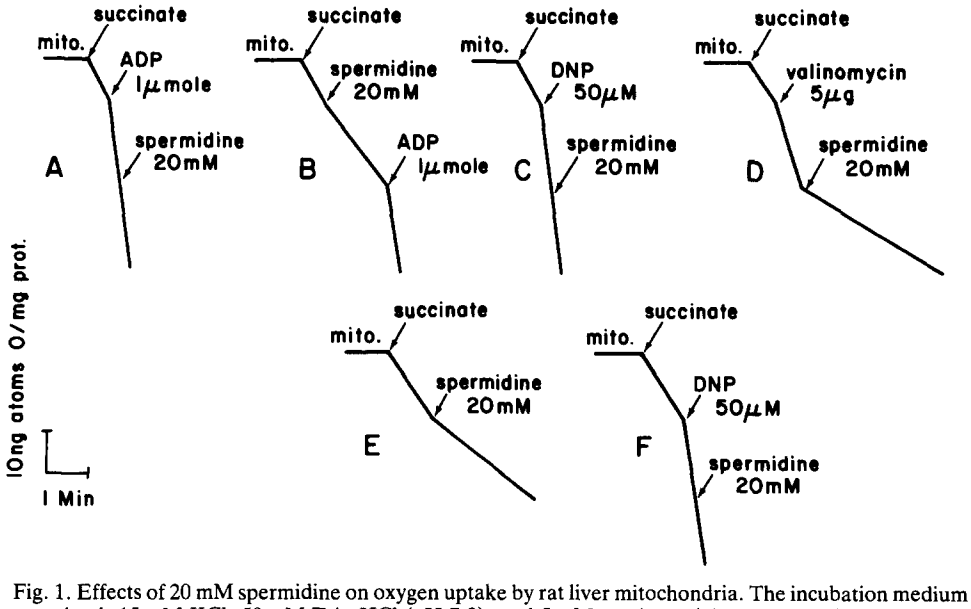

Fig. 1. Effects of 20 mM spermidine on oxygen uptake by rat liver mitochondria. The incubation medium contained: 15 mM KCl, 50 mM Tris–HCl (pH 7.2), and 5 mM succinate (plus glutamate) as substrate. In experiments A, B, C and D, 5 mM $MgSO_4$ and 5 mM K^+ phosphate buffer (pH 7.2) were added (final volume, 1.5 ml).

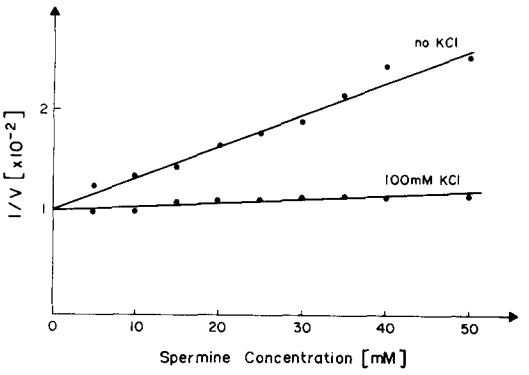

Fig. 2. Changes in the dose-dependent effect of spermine on reciprocal relative oxygen uptake by potassium, using aged rat liver mitochodria with succinate (plus glutamate) as substrate. The incubation medium contained 250 mM sucrose with 10 mM Tris–HCl (pH 7.2) and 1 mM EGTA or 100 mM KCl with 20 mM Tris–HCl (pH 7.2) in a final volume of 1.5 ml.

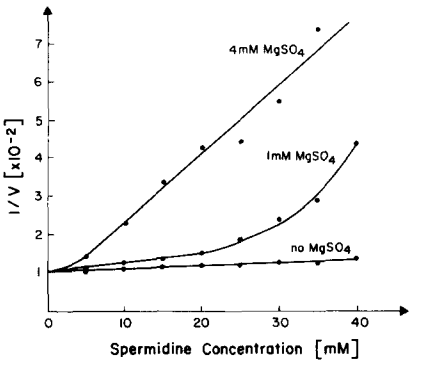

Fig. 3. Changes in the dose-dependent effect of spermidine on reciprocal relative oxygen uptake by magnesium, using aged rat liver mitochondria with succinate (plus glutamate) as substrate. The incubation medium contained 15 mM KCl, 50 mM Tris–HCl (pH 7.2), and $MgSO_4$ at concentrations shown, in a final volume of 1.5 ml.

non-linear inhibition curve usually suggests a complex mechanism of action involving more than one step of interaction between the inhibition and the organelle.

Since the effects of polyamines on state 4 respiration could be modified by varying the concentration of potassium in the medium, it was of interest to examine the interaction of polyamines with valinomycin, a cationophore specific for potassium [24]. Inhibition of respiration by 20 mM spermine was markedly increased in mitochondria which were de-energized with valinomycin in the presence of 15 mM potassium (Fig. 4D). A double-reciprocal plot showing the dependence of respiratory inhibition on polyamine concentration in the presence and absence of valinomycin is shown in Fig. 5. In the absence of valinomycin the relationship is non-linear, while in its presence the relationship became linear and the inhibition markedly increased. The same order of effectiveness among the polyamines was

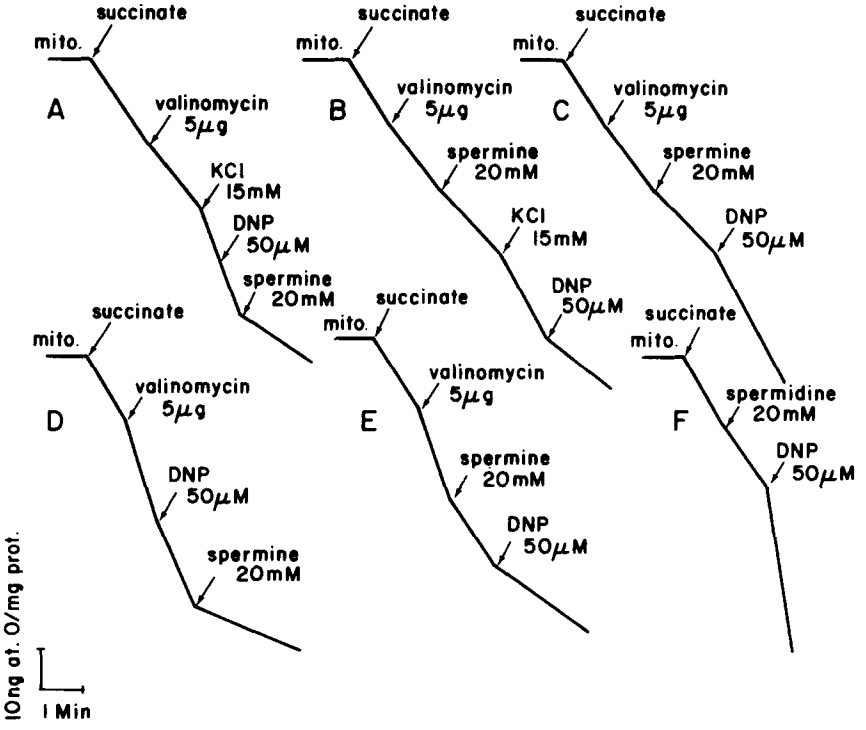

Fig. 4. Effects of spermine on oxygen uptake by rat liver mitochondria de-energized by valinomycin or dinitrophenol in different potassium concentrations. Incubation medium contained: A, B and C, 250 mM sucrose, the same as in Fig. 2; D, E and F, 110 mM KCl and 20 mM Tris–HCl (pH 7.2), in a final volume of 1.5 ml.

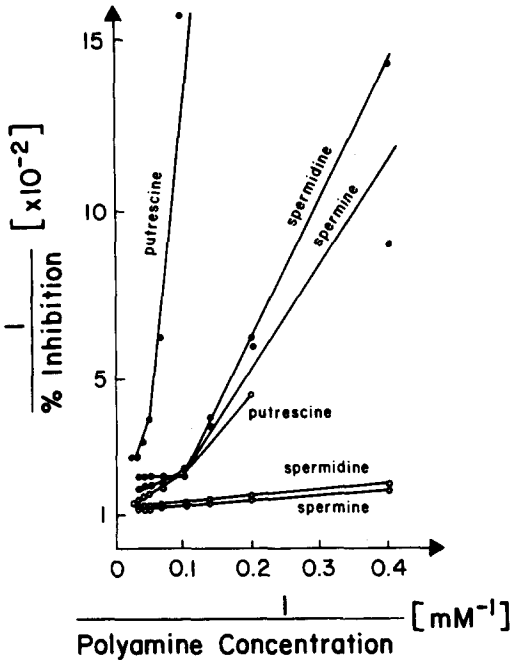

Fig. 5. Double-reciprocal plot of the inhibition of respiratory activity of rat liver mitochondria by polyamines in the presence (○) or absence (●) of valinomycin. The incubation medium was the same as in Fig. 1. Valinomycin was present at 5 μg/1.5 ml of final volume.

maintained in the presence of valinomycin, with spermine and spermidine being much more potent inhibitors than putrescine (Fig. 5).

The polyamines had practically no effect on the respiration of valinomycin-treated mitochondria in isotonic sucrose without potassium (Fig. 4, B and C). However, when mitochondria were made permeable to potassium and protons (in the presence of valinomycin, KCl and DNP potassium ions may enter passively) and then exposed in isotonic sucrose to polyamines, their respiration was inhibited markedly (Fig. 4A). Unexpectedly, in mitochondria pretreated with valinomycin and spermine, DNP opposed the stimulatory effect of potassium on respiration whether the medium was isotonic sucrose (Fig. 4B) or potassium chloride (Fig. 4E). DNP, alone, stimulated the respiration of mitochondria pretreated with spermine in isotonic potassium chloride (Fig. 4F). In the same medium, spermine caused a more pronounced inhibition of respiration by mitochondria pretreated with valinomycin in the presence of DNP (Fig. 4D).

Addition of polyamines to rat liver mitochondria increased the optical density of the suspension (Fig. 6). By light microscopy, it was observed that this increase in optical density correlated with the aggregation of mitochondria into clusters of 20–30 organelles which may contribute to the spectrophotometric changes. An increase in optical density at 800 nm with polyamine addition occurred with either

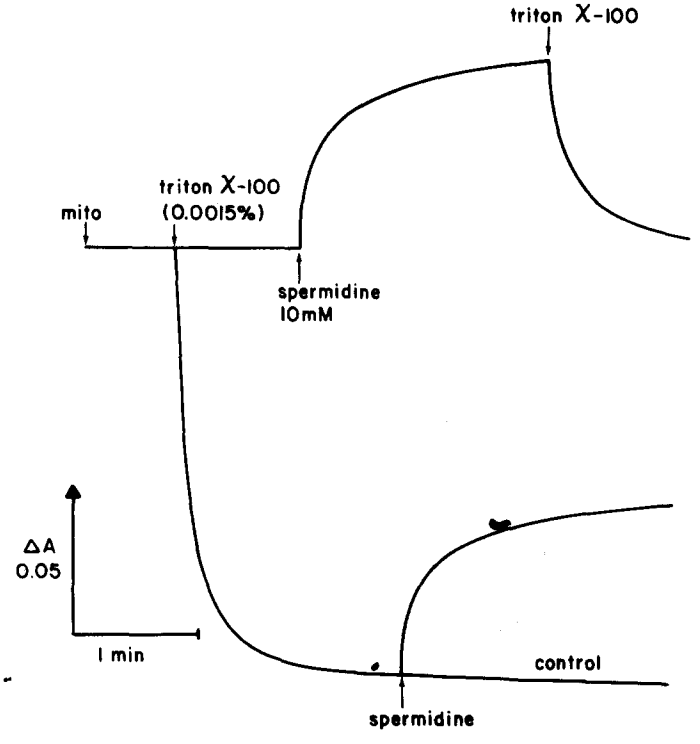

Fig. 6. Changes in optical density of a suspension of rat liver mitochondria at 800 nm treated with 10 mM spermidine and/or 0.0015% Triton X-100. Spermidine stabilizes the mitochondria so that the effects of Triton X-100 are decreased. The incubation medium contained: 250 mM sucrose, 10 mM Tris–HCl (pH 7.2), and 1 mM EGTA in a final volume of 3.0 ml. Spermine gave comparable results, while putrescine was less effective in producing the same effect. For further experimental details, see Materials and Methods.

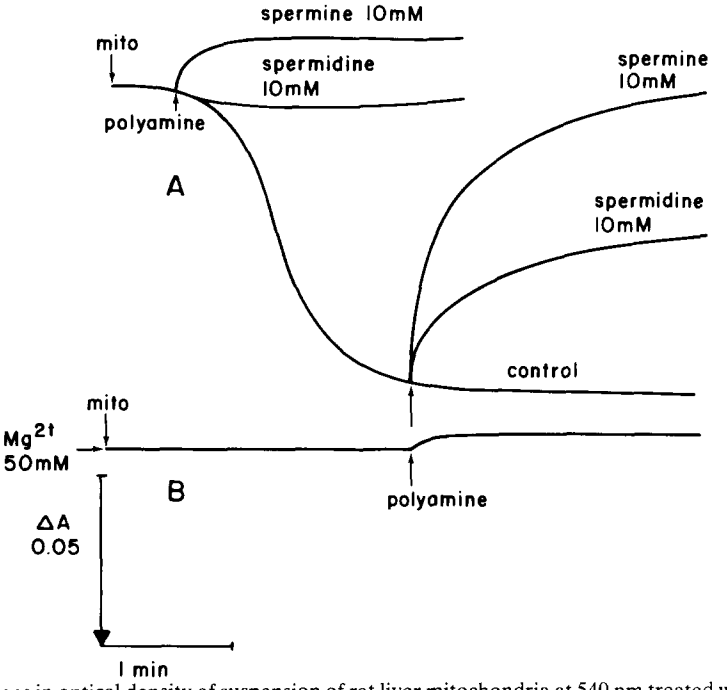

Fig. 7. Changes in optical density of suspension of rat liver mitochondria at 540 nm treated with spermine or spermidine. Spermidine and spermine prevented the swelling induced by hypotonic potassium nitrate (A) and swollen mitochondria were more susceptible to polyamine effects (A). The latter was prevented by 50 mM $MgCl_2$ (B). The incubation medium contained: (A) 50 mM KNO_3 and 20 mM Tris–HCl (pH 7.2); (B) 50 mM $MgCl_2$ and 20 mM Tris–HCl, both at a final volume of 3.0 ml. For further experimental details, see Materials and Methods.

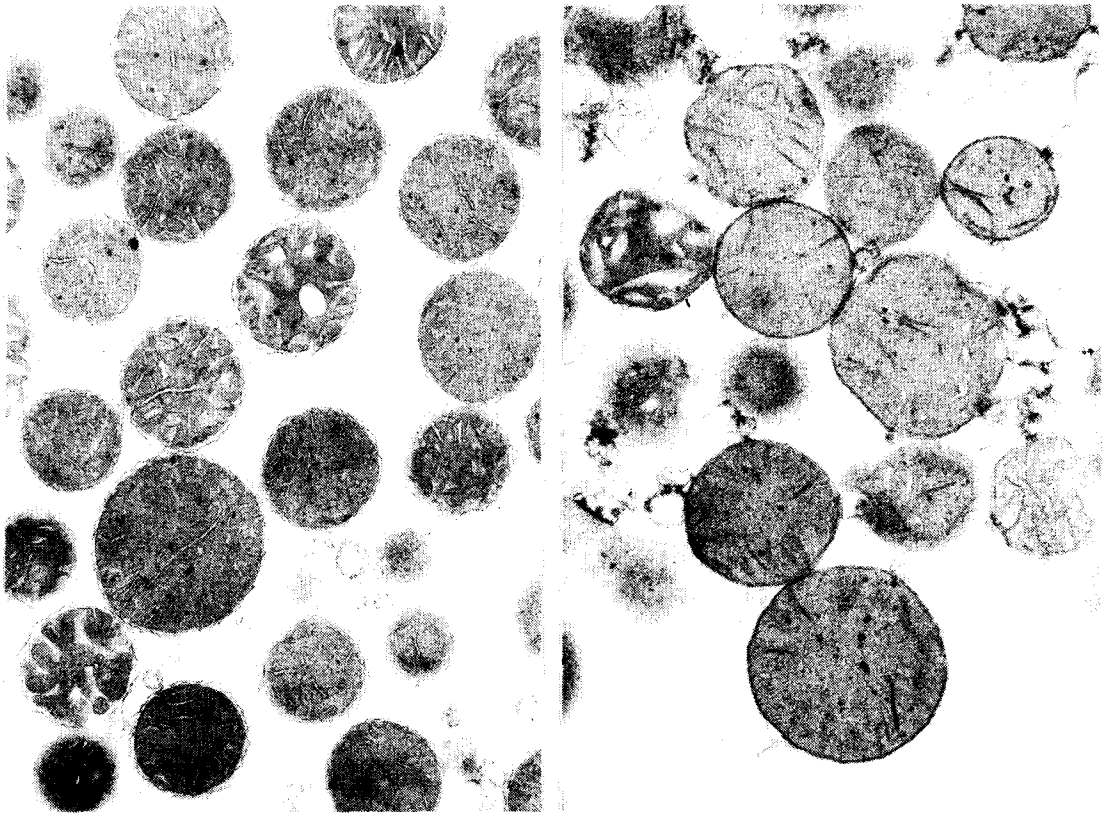

Fig. 8. Electron micrographs of untreated rat liver mitochondria (left) and mitochondria treated for 10 min with 60 mM spermine (right). The incubation medium was the same as in Fig. 6. In the untreated mitochondria, the intracristae spaces are obvious, while in the spermine-treated organelles these spaces are collapsed in most cases and the inner and outer membranes are not distinguishable.

Table 2. Drug effects on the electrophoretic mobility of rat liver mitochondria

Treatment	Mobility* (μm/sec/V/cm)	% Slowing
None	−1.37 ± 0.02	0
DNP (50 μM)	−1.31 ± 0.02	4
Spermine (1.25 mM)	−0.71 ± 0.01	48
Spermine (10.0 mM)	0.00	100

* Based on a minimum of 100 transit measurements per sample (± S.E.).

untreated mitochondria or those swollen with Triton X-100 (Fig. 6). Pretreatment of mitochondria with a polyamine prevented the swelling effects of sublytic concentrations of Triton X-100 by some 50% (Fig. 6). All three polyamines had qualitatively similar effects but differed quantitatively. Spermidine and spermine were essentially comparable in their potency, while putrescine was much less effective.

All three polyamines prevented the spontaneous swelling of mitochondria suspended in hypotonic potassium nitrate (Fig. 7A), and mitochondria already swollen in this medium became more susceptible to the optical density changes induced by the polyamines. This latter effect was completely prevented by high concentrations of magnesium cations (Fig. 7B).

Because of their negative surface potential, suspended mitochondria migrate in an electrophoretic field, at an average mobility of −1.37 μm per sec per V per cm in the direction of anode (Table 2). Pretreatment of mitochondria with a polyamine caused a slowing of their electrophoretic mobility. At 1.25 mM, spermine reduced by 50% the mitochondrial mobility, and at 10 mm this polyamine caused a total cessation of mobility (Table 2), presumably by completely neutralizing the surface potential of the organelles. Although polyamines were observed by light microscopy to cause an aggregation of mitochondrial particles, this effect should not intefere with electrophoretic measurements since the latter are dependent on the surface charge density of the particle and not on its size or overall charge. Moreover, care was taken during these experiments to restrict measurements to single mitochondria which were present among the aggregated organelles.

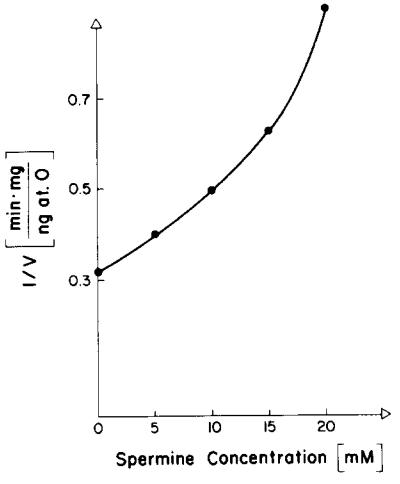

Fig. 9. Dose-dependent effect of spermine on reciprocal initial velocity of monoamine oxidase in rat liver mitochondria using 1 mM 5-hydroxytryptamine as substrate.

By electron microscopy the rat liver mitochondrial preparations were found to be relatively free of other cellular organelles. Vesicles of rough and smooth endoplasmic reticulum were the most frequent contaminants. In the mitochondria themselves, the matrix appeared condensed and the intracristal spaces were obvious (Fig. 8). The inner and outer membranes were clearly discernible from one another. Following treatment with polyamines, in particular spermine or spermidine, the mitochondria appeared aggregated to one another, thus confirming light microscopic observations. In many of the organelles, the intracristal spaces appeared collapsed (Fig. 8). The outer mitochondrial compartment was obliterated, and the inner and outer membranes could no longer be distinguished. The effects with putrescine were similar but less obvious.

The effects of polyamines on mitochondria were not confined to the inner mitochondrial membrane. All of the polyamines, but especially spermidine and spermine, were effective inhibitors of the outer membrane enzyme, monoamine oxidase. Enzyme measurements were made from initial velocity estimates of 5-hydroxytryptamine oxidation using the oxygen electrode. A Dixon plot of the inhibition of mitochondrial monoamine oxidase activity by spermine (Fig. 9) reveals a parabolic dependence on concentration.

DISCUSSION

At millimolar concentrations, biological polyamines inhibited respiration of isolated rat liver mitochondria, with spermidine and spermine being comparable in effectiveness and putrescine considerably less potent. The greater suppression of state 4, as opposed to state 3 or uncoupled, respiration by polyamines has also been observed with MGBG, the anticancer agent [11] considered by some [9, 10] to be a structural analog of spermidine. In both cases, the differential inhibition may be rationalized by the chemiosmotic theory for oxidative phosphorylation [5]. In the absence of ADP (state 4), mitochondria generate a significant electrochemical gradient across the inner mitochondrial membrane. Polyamines being cations under physiological conditions would be electrophoretically drawn to the inner mitochondrial membrane by its negative potential at its interior and thus would bind to the outer membrane surface. Similar binding characteristics have also been noted for other cationic compounds. Rhodamine dyes, which are positively charged at physiological pH, specifically stain mitochondria whereas uncharged rhodamines do not [25]. Moreover, rhodamine 6G, which is positively charged, inhibits oxidative phosphorylation and blocks adenine nucleotide translocation, whereas the uncharged rhodamine B does not [26].

Once drawn to the periphery of the mitochondrial surface by the transmembrane potential, the polyam-

ines may bind to anionic sites available there. These could include sialic acid residues of the membrane [27] or, more likely, the negatively charged phosphate groups of membrane phospholipids. The latter have been postulated as natural binding sites for biguanides [28] and for certain of the bis-(guanylhydrazones) [11, 29], including MGBG. In the case of biguanides, Schafer [28] has suggested that alterations in the physical properties of the membrane as a consequence of drug binding may provide a basis for the mitochondrial effects which include inhibition of respiration.

In fact, considerable data indicate that polyamines bind to the membrane of mitochondria and alter certain of its physical properties. There is a marked slowing of the mitochondrial electrophoretic mobility following binding of polyamines to mitochondria resulting from neutralization of the negative membrane surface potential. This neutralization phenomenon may account for the ultrastructural appearance of mitochondria treated with polyamines, in particular spermidine or spermine. There is an apparent collapse of the outer mitochondrial compartment so that the spaces separating the inner and outer membranes and the membranes of the cristae are lost. Binding of polyamine cations may neutralize the repulsive forces which might otherwise keep the membranes separated. Similar findings have been noted with protamine [30], another polycationic agent, and with certain inorganic cations [21]. Polyamines are also capable of preventing the swelling effect of sublytic concentrations of Triton X-100 (Fig. 6) indicating that, like MGBG [11], their binding stabilizes the membrane in some manner. Finally, inhibition of monoamine oxidase activity by the polyamines confirms the involvement of the outer mitochondrial membrane.

The inhibitory effects of polyamines on the respiration of mitochondria were virtually abolished by the presence of higher concentrations of potassium (Fig. 2), suggesting competition for the same binding sites at the mitochondrial membrane. The exchange of potassium for protons across the mitchondrial membrane is known to be a critical event during mitochondrial respiration. As proposed by Azzi and Scarpa [31], potassium cations bind temporarily to superficial anionic sites, such as phospholipids, prior to being transported into the mitochondria during respiration. As a consequence of neutralization of these membrane anionic sites by polyamines, the cloud of potassium ions at the membrane may dissipate decreasing transport. Thus, the polyamines could ultimately slow mitochondrial respiration by binding to the inner membrane and displacing potassium ions.

Additional support for this interpretation is available from experiments using valinomycin, a cationophore specific for potassium [24]. In isotonic potassium chloride, inhibition of respiration by polyamines was greater when mitochondria were pretreated with valinomycin, especially when membranes were made more permeable to protons with DNP (Fig. 4). Since valinomycin pretreatment enhanced the inhibitory effect of polyamines on mitochondrial respiration, it appears that a site within the mitochondrial membrane which is sensitive to the polyamines is the potassium-proton exchange system. Valinomycin increases the permeability of the mitochondrial membrane to potassium cations and results in the depletion of potassium from the surface of the inner membrane. Thus, under these conditions, competition for cation binding sites at the membrane is decreased for the polyamines.

The present data suggest that polyamines can alter the function of isolated mitochondria by binding to anionic sites at the inner membrane and, thereby, intefere with cation exchange, particularly that of potassium. Whether or not polyamines are capable of similarly modulating the respiration of mitochondria within the context of an intact cell is not known. Although measurements of polyamines associated with the isolated mitochondria were found to be in the micromolar range (Table 1), it is unlkely that they reflect qualitatively or quantitatively the polyamine levels prior to cell homogenization, given the rigors of the isolation procedures. It is interesting that the concentrations of spermidine or spermine required for inhibition of state 4 respiration, although in the millimolar range, were comparable to those required by MGBG which has been shown to inhibit cell growth by inteference with mitochondrial function [13]. It is possible, therefore, that mitochondrial function could be affected by polyamines in the intact cell. We note also that many of the drug effects observed here were almost immediate, whereas those within the cell itself might occur more slowly and in the presence of lower polyamine concentrations (Table 1). At present, the potential of polyamines to modulate intracellular mitochondrial function in the intact cell is being addressed by experiments using the irreversible inhibitor of ornithine decarboxylase, α-difluoromethylornithine, which markedly lowers intracellular polyamine pools after two cell generations [32].

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