Dual effect of spermine on mitochondrial Ca^{2+} transport

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1. A dual effect of the polyamine spermine on Ca^{2+} uptake by isolated rat liver, brain and heart mitochondria could be demonstrated by using a high-resolution system for studying mitochondrial Ca^{2+} transport. Depending on the experimental situation, spermine had an inhibiting or accelerating effect on mitochondrial Ca^{2+} -uptake rate, but invariably increased the mitochondrial Ca^{2+} accumulation. 2. Both effects were concentration-dependent and clearly discernible on the basis of their different kinetic characteristics. For mitochondria from all three tissues the half-maximally effective concentration for inhibition of the initial rate of Ca^{2+} uptake was approx. 180 μM , whereas that for the subsequent stimulation of Ca^{2+} accumulation was approx. 50 μM . 3. Acceleration of the initial uptake rate could be seen when the mitochondria were preloaded with spermine during a 2 min preincubation period and thereafter incubated in a medium without spermine. 4. When such spermine-preloaded mitochondria were incubated in a spermine-containing medium, the increase in Ca^{2+} -accumulation capacity was maintained in spite of an unchanged rate of Ca^{2+} uptake. 5. Mg^{2+} interacted with the effects of spermine in a differential manner, enhancing the initial inhibition of the rate of mitochondrial Ca^{2+} uptake and diminishing the subsequent stimulation of mitochondrial Ca^{2+} accumulation. 6. This dual effect of spermine on mitochondrial Ca^{2+} transport resolves the apparent paradox that a polycationic compound can act as a stimulator of Ca^{2+} uptake.

INTRODUCTION

Polyamines are natural constituents of eukaryotic cells (Schreiner, 1878; Tabor *et al.*, 1961; Tabor & Tabor, 1964) derived from ornithine through decarboxylation (Jänne *et al.*, 1978; Pegg, 1986; Heby & Persson, 1990). Spermine exerts a great variety of effects of cellular functions (Tabor & Tabor, 1964; Pegg & McCann, 1988; Schubert, 1989). Intracellular concentrations of spermine approach the millimolar range (Tabor & Tabor, 1964; Cannellakis *et al.*, 1979; Byczkowski *et al.*, 1982). Spermine is taken up into mitochondria (Toninello *et al.*, 1985) and in liver cells the intramitochondrial concentrations were found to be as high as those in the cytosol (Byczkowski *et al.*, 1982).

Spermine has a remarkable stabilizing action on mitochondria (Tabor, 1960; Chaffee *et al.*, 1977; Phillips & Chaffee, 1982; Byczkowski *et al.*, 1982; Toninello *et al.*, 1984). In an early review article on the polyamines, Tabor & Tabor (1964) considered similarities between the effects of polyamines and those of Ca^{2+} and Mg^{2+} , an area of continuous research efforts ever since (Schubert, 1989). Several reports have described interactions between effects of spermine and Mg^{2+} on mitochondrial function (Chaffee *et al.*, 1977; Byczkowski *et al.*, 1982; Toninello *et al.*, 1985). More recently, the effects of spermine on mitochondrial Ca^{2+} transport have attracted considerable attention. However, although an early study by Åkerman (1977) described an inhibition of mitochondrial Ca^{2+} uptake by spermine, many subsequent studies reported a stimulatory effect of spermine on Ca^{2+} uptake by mitochondria from liver (Nicchitta & Williamson, 1984; Lenzen *et al.*, 1986; Kröner, 1988; McCormack, 1989), hepatoma cells (Fiskum, 1985), brain (Lenzen *et al.*, 1986; Jensen *et al.*, 1987, 1989; Rottenberg & Marbach, 1990), heart (Lenzen *et al.*, 1986; McCormack, 1989), kidney (McCormack, 1989) and pancreatic islets (Lenzen & Rustenbeck, 1991). Surprisingly, however, for unknown reasons rather different half-maximally effective concentrations were reported (Nicchitta & Williamson, 1984; Lenzen *et al.*, 1986; Jensen *et al.*, 1987; Kröner, 1988; McCormack, 1989). In the range of the free Mg^{2+} concentration

in the cell (Åkerman & Nicholls, 1983; Corkey *et al.*, 1986; Gylfe, 1990; Jung *et al.*, 1990) an inhibitory effect of Mg^{2+} on mitochondrial Ca^{2+} uptake was observed, which counteracted the stimulatory effect of spermine on mitochondrial Ca^{2+} transport (Lenzen *et al.*, 1986; Kröner, 1988).

Using a high-resolution system for studying mitochondrial Ca^{2+} transport, we show that spermine has a dual effect on mitochondrial Ca^{2+} transport, both a modulation of the initial velocity of mitochondrial Ca^{2+} uptake and a subsequent increase in mitochondrial Ca^{2+} -storage capacity. Thus the effects of spermine on the regulation of mitochondrial Ca^{2+} are more complex than originally expected.

MATERIALS AND METHODS

Chemicals

Polyamines, ATP and Hepes were from Sigma, St. Louis, MO, U.S.A. All other reagents, of analytical grade, were from Merck, Darmstadt, Germany.

Isolation of mitochondria

Liver, heart and brain were from Wistar rats, homogenized in homogenization medium (210 mM-mannitol, 70 mM-sucrose, 20 mM-Hepes and 0.5 mM-EGTA, adjusted to pH 7.0 with KOH) and maintained on ice (Lenzen & Panten, 1985; Lenzen *et al.*, 1985). After centrifugation for 15 min at 660 *g*, the mitochondrial fraction was obtained by centrifugation of the supernatant for 15 min at 4000 *g* and 4 °C (Lenzen & Panten, 1985; Lenzen *et al.*, 1985). Such a preparation yields mitochondria with a Ca^{2+} content of 9.5 nmol/mg of protein (Lenzen *et al.*, 1986). After resuspension and re-centrifugation, the pellet was suspended in test medium (125 mM-KCl, 2 mM- KH_2PO_4 , 5 mM-succinate, 25 mM-Hepes, adjusted to pH 7.0 with KOH) (Becker *et al.*, 1980), which simulated the ionic composition of the cytosol. The protein content of 1 μl of the mitochondrial fractions, determined as described by McKnight (1977), was $29.1 \pm 1.3 \mu\text{g}$ ($n = 21$) for liver mitochondria, $21.8 \pm 3.0 \mu\text{g}$ ($n = 4$) for brain mitochondria and $19.4 \pm 2.0 \mu\text{g}$ ($n = 8$) for heart mitochondria.

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Fig. 1. Concentration-dependent effects of spermine in comparison with control (Con) on the kinetics of Ca^{2+} uptake by isolated rat liver mitochondria

The test medium with an initial Ca^{2+} concentration of $2 \mu\text{M}$ in the micro-incubation chamber contained spermine (50 , 100 , 200 or $400 \mu\text{M}$). At zero time the experiments were started by injection of mitochondria into the test medium. A delay of 2 s at the beginning of the experiment was due to injection and mixing. Results shown are recordings typical of 12 experiments.

Measurement of free Ca^{2+} concentration

Ca^{2+} uptake and efflux by isolated mitochondria were measured in test medium at 25°C with a specially designed Ca^{2+} -sensitive mini-electrode containing the Ca^{2+} ionophore ETH 1001 (Ammann *et al.*, 1983) by addition to the micro-incubation chamber ($40 \mu\text{l}$ volume) of mitochondria suspended in 1 – $2 \mu\text{l}$ of test medium as described in detail by Lenzen & Panten (1985). The potential difference between the electrode pair was displayed on a digital millivoltmeter (type PHM 84; Radiometer, Copenhagen, Denmark), which was connected to a data-acquisition board with a 12-bit analogue-digital converter (DAS 8 PGA with screw terminal box, STA 08 PGA; Megabyte Corp., Taunton, MA, U.S.A.) plugged into an IBM AT 03 personal computer. The data were recorded at a frequency of 20 Hz , and the free Ca^{2+} concentrations were calculated in real time by the data-acquisition program Labtech Notebook (Laboratory Technologies Corp., Wilmington, MA, U.S.A.). The Ca^{2+} -sensitive minielectrode was calibrated with Ca^{2+} buffer solutions ($\text{pCa } 5$ – 8). Accuracy of calibration for experiments with mitochondria was confirmed by simultaneous measurement of Ca^{2+} fluorescence with the indicator fluo 3. The electrode response time was not limiting, since complexation of Ca^{2+} by EGTA yielded a velocity of Ca^{2+} -concentration decrease at least twice the fastest velocity registered with mitochondria. Mg^{2+} and spermine did not interfere with electrode recordings. The initial velocity of Ca^{2+} uptake was defined as the peak value of the first derivative of the Ca^{2+} concentration ($d[\text{Ca}^{2+}]/dt$) and was usually achieved 2.5 – 3.0 s after starting the experiment.

Calculations

Values are presented as mean values $\pm$ S.E.M., were tested for statistical significance by Student's *t* test or analysis of variance (ANOVA), and were calculated with the ASEASYAS spreadsheet program (Trius Inc., North Andover, MA, U.S.A.).

RESULTS

Effects of spermine on mitochondrial Ca^{2+} transport

Spermine affected the kinetics of Ca^{2+} uptake by isolated rat liver mitochondria in a time- and concentration-dependent manner (Fig. 1). An initial inhibition of the rate of mitochondrial Ca^{2+} uptake was followed by a subsequent increase in mitochondrial Ca^{2+} accumulation (Fig. 1). Ca^{2+} uptake by control mitochondria virtually ceased at 10 s , whereas Ca^{2+} uptake induced by above $200 \mu\text{M}$ -spermine Ca^{2+} continued to the end of the incubation period. The effects of spermine were not affected by removal of phosphate from the test medium (results not shown).

The same effects were seen with isolated mitochondria from rat brain and heart (Figs. 2 and 3). The inhibition of the initial velocity of Ca^{2+} uptake by mitochondria from all three tissues was comparable and concentration-dependent in the range up to $1000 \mu\text{M}$ -spermine (Fig. 2). The half-maximally effective concentrations were $182 \pm 17 \mu\text{M}$ ($n = 12$) for liver mitochondria, $185 \pm 30 \mu\text{M}$ ($n = 5$) for brain mitochondria and $179 \pm 21 \mu\text{M}$ ($n = 5$) for heart mitochondria. The subsequent spermine-induced increase in Ca^{2+} accumulation by isolated rat liver, brain and heart mitochondria from all three tissues yielded lower minimal free Ca^{2+} concentrations in the incubation medium (Fig. 1). This effect was also concentration-dependent and was maximal at around $200 \mu\text{M}$ -spermine (Fig. 3). At high spermine concentrations the uptake velocity was decreased to such an extent that the minimal Ca^{2+} concentration achieved during the incubation period rose again. However, the efficacy of spermine decreased in the order liver > brain > heart mitochondria (Fig. 3). The half-maximally effective concentrations were $45 \pm 3 \mu\text{M}$ ($n = 12$) for liver mitochondria, $52 \pm 9 \mu\text{M}$ ($n = 5$) for brain mitochondria and $54 \pm 9 \mu\text{M}$ ($n = 5$) for heart mitochondria.

At all starting free Ca^{2+} concentrations between 0.5 and $10 \mu\text{M}$ the initial velocity of Ca^{2+} uptake by isolated rat liver mitochondria was lower in the presence of spermine ($100 \mu\text{M}$) (results not shown). In the same way, the minimal free Ca^{2+} concentration

Fig. 2. Concentration-dependent effects of spermine on the initial velocity of Ca^{2+} uptake by isolated rat liver (●), brain (■) and heart (◆) mitochondria

The test medium with an initial Ca^{2+} concentration of $2 \mu\text{M}$ contained increasing concentrations of spermine. Results shown are mean values $\pm$ S.E.M. of 4 experiments. The initial velocity of mitochondrial Ca^{2+} uptake in the absence of spermine was set at 100% (▲) and represented 47.6 ± 4.9 nmol/min per mg of protein in liver mitochondria, 44.2 ± 5.9 nmol/min per mg in brain mitochondria and 15.1 ± 5.9 nmol/min per mg in heart mitochondria. Spermine significantly inhibited the initial velocity of Ca^{2+} uptake by mitochondria from all three tissues ($P < 0.01$; ANOVA).

Fig. 3. Concentration-dependent effects of spermine on the minimal Ca^{2+} concentration achieved through Ca^{2+} accumulation by isolated rat liver (●), brain (■) and heart (◆) mitochondria

The test medium with an initial Ca^{2+} concentration of $2 \mu\text{M}$ contained increasing concentrations of spermine. Results shown are mean values $\pm$ S.E.M. of 4 experiments. The minimal Ca^{2+} concentration achieved was set at 100% (▲) and represented $0.44 \pm 0.02 \mu\text{M}$ in liver mitochondria, $0.38 \pm 0.01 \mu\text{M}$ in brain mitochondria and $0.41 \pm 0.02 \mu\text{M}$ in heart mitochondria. Spermine significantly lowered the minimal Ca^{2+} concentration achieved through Ca^{2+} accumulation by mitochondria from all three tissues ($p < 0.01$; ANOVA).

in the incubation medium achieved within a 2 min test period in the presence of spermine ($100 \mu\text{M}$) was lower by at least $0.1 \mu\text{M}$ than that in its absence at all starting free Ca^{2+} concentrations between 0.5 and $10 \mu\text{M}$ (results not shown).

Exposure of the mitochondria to spermine was separated from that to Ca^{2+} by the following experimental design. Isolated liver

Fig. 4. Effect of preloading with spermine on the kinetics of Ca^{2+} uptake by isolated rat liver mitochondria

The test medium was adjusted to $10 \mu\text{M}$ before the start of the experiment. At time zero the experiments were started by addition of mitochondria to the test medium, which had previously been preincubated for 2 min in the absence (Con) or presence (Sper) of $1000 \mu\text{M}$ -spermine. Results shown are recordings typical of 8 experiments.

Table 1. Effects of preloading of mitochondria with spermine on the initial velocity of Ca^{2+} uptake and on Ca^{2+} accumulation capacity in the absence or presence of spermine in the incubation medium

Spermine was absent (control) (A), present only in the incubation medium (B), present only in the preincubation medium (C), or present in both the incubation and the preincubation media (D). Mitochondria were preincubated for 2 min at 25°C in the presence of 1 mM -spermine; the spermine concentration in the incubation medium was $400 \mu\text{M}$. The design of experiment (B) was identical with the experimental design used in Fig. 1. Results shown are mean values $\pm$ S.E.M. of 7–12 experiments. The numbers in parentheses express the values as percentages of control.

	A	B	C	D
Initial velocity (nmol/min per mg)	158.6 ± 5.3 (100%)	121.0 ± 3.9 (76%)	200.6 ± 3.5 (127%)	162.0 ± 7.2 (102%)
Minimal $[\text{Ca}^{2+}]$ (nM)	479 ± 21 (100%)	265 ± 14 (55%)	305 ± 14 (64%)	259 ± 13 (54%)

mitochondria in a volume of $200 \mu\text{l}$ were preincubated in the absence or presence of $1000 \mu\text{M}$ -spermine for 2 min, and thereafter a $2 \mu\text{l}$ portion of this mitochondrial suspension was transferred into the micro-incubation chamber of the Ca^{2+} mini-electrode containing $40 \mu\text{l}$ of test medium. This decreased the final spermine concentration of the incubation medium to less than $50 \mu\text{M}$. Such a concentration of spermine caused no significant decrease in the initial velocity of Ca^{2+} uptake (less than 10%; Fig. 7). Mitochondria preloaded with spermine during this preincubation period took up Ca^{2+} faster from the beginning of the experiment (Fig. 4). The maximal velocity of Ca^{2+} uptake was significantly increased ($P < 0.01$; Student's t test) and the minimal free Ca^{2+} concentration in the incubation medium was significantly decreased ($P < 0.01$; Student's t test) by the action of spermine (Table 1, column A versus column C). However, when such preloaded mitochondria were used to measure Ca^{2+} uptake in a spermine-containing medium, the initial velocity was unchanged as compared with control mitochondria, whereas the

Fig. 5. Concentration-dependent effects of Mg^{2+} on the kinetics of Ca^{2+} uptake by isolated rat liver mitochondria

The test medium with an initial Ca^{2+} concentration of $2 \mu\text{M}$ contained Mg^{2+} (0.5, 1.0 or 2.0 mM) or no Mg^{2+} (Con). Under these experimental conditions the Mg^{2+} is free (Lenzen *et al.*, 1986). Results shown are recordings typical of 4 experiments.

Fig. 6. Effects of spermine in the absence and presence of Mg^{2+} on the kinetics of Ca^{2+} uptake by isolated rat liver mitochondria

The test medium with an initial Ca^{2+} concentration of $2 \mu\text{M}$ contained spermine ($400 \mu\text{M}$) in the absence (A) and presence (B) of Mg^{2+} ($0.5 \mu\text{M}$) compared with no addition (C). Results shown are recordings typical of 4 experiments.

minimal free Ca^{2+} concentration in the incubation medium was virtually identical with that achieved when spermine was present in the incubation medium only. (Table 1, column A versus column D). A comparison of column A with column B in Table 1 confirms the existence of the inhibitory action of spermine on initial Ca^{2+} -uptake velocity when mitochondria were exposed simultaneously to Ca^{2+} and spermine in the incubation chamber. This is identical with the experimental design depicted in Figs. 1–3.

Interactions between spermine and Mg^{2+} in the regulation of mitochondrial Ca^{2+} transport

Mg^{2+} both inhibited the initial velocity of mitochondrial Ca^{2+} uptake and raised the minimal free Ca^{2+} concentration achieved through mitochondrial Ca^{2+} accumulation in a concentration-dependent manner (Fig. 5). Compared with the control, a free Mg^{2+} concentration of 0.5 mM, which is in the lower physiological range (Corkey *et al.*, 1986; Gylfe, 1990; Jung *et al.*, 1990), did not raise the minimal free Ca^{2+} concentration, although it already inhibited the initial velocity of mitochondrial Ca^{2+} uptake. However, such a low Mg^{2+} concentration stabilized the steady-state free Ca^{2+} concentration in the range of $0.5 \mu\text{M}$ and prevented

Fig. 7. Concentration-dependent effects of spermine in dependence on the Mg^{2+} concentration on the initial velocity of Ca^{2+} uptake by isolated rat liver mitochondria

The test medium with an initial Ca^{2+} concentration of $10 \mu\text{M}$ contained increasing concentrations of spermine at different Mg^{2+} concentrations ($\circ$, 0 mM; $\bullet$, 0.5 mM; $\blacksquare$, 1.0 mM; $\blacklozenge$, 2.0 mM). Results are mean values for 4 experiments. The initial velocity of mitochondrial Ca^{2+} uptake by controls in the absence of spermine and Mg^{2+} was set at 100% and represented $283.7 \pm 2.4 \text{ nmol/min}$ per mg of protein. Spermine significantly inhibited the velocity of Ca^{2+} uptake at all Mg^{2+} concentrations ($P < 0.01$; ANOVA).

Fig. 8. Concentration-dependent effects of spermine in dependence on the Mg^{2+} concentration on the minimal Ca^{2+} concentration achieved through Ca^{2+} accumulation by isolated rat liver mitochondria

The test medium with an initial Ca^{2+} concentration of $10 \mu\text{M}$ contained increasing concentrations of spermine at different Mg^{2+} concentrations ($\circ$, 0 mM; $\bullet$, 0.5 mM; $\blacksquare$, 1.0 mM; $\blacklozenge$, 2.0 mM). Results shown are mean values for 4 experiments. The minimal Ca^{2+} concentration achieved by controls in the absence of spermine and Mg^{2+} was set at 100% and represented $0.50 \pm 0.01 \mu\text{M}$. Spermine significantly lowered the minimal Ca^{2+} concentration achieved through Ca^{2+} accumulation in the presence of 0 and 0.5 mM- Mg^{2+} ($P < 0.01$; ANOVA), had no significant effect in the presence of 1.0 mM- Mg^{2+} and significantly increased the minimal Ca^{2+} concentration achieved through Ca^{2+} accumulation in the presence of 2.0 mM- Mg^{2+} ($P < 0.01$; ANOVA).

the slight increase in free Ca^{2+} concentration through Ca^{2+} release from the mitochondria, as evident in experiments with control mitochondria devoid of free Mg^{2+} in the incubation medium (Fig. 5).

Fig. 6 shows that Mg^{2+} (0.5 mM) enhanced the ability of spermine (400 μM) to inhibit the initial velocity of mitochondrial Ca^{2+} uptake. On the other hand, Mg^{2+} (0.5 mM) counteracted the ability of spermine (400 μM) to lower the minimal free Ca^{2+} concentration achieved through mitochondrial Ca^{2+} accumulation. Assuming free intracellular spermine concentrations in the range of 100–400 μM and free intracellular Mg^{2+} concentrations in the range of 0.5–1.0 mM, the combined effect of the two agents at these concentrations favours a stable steady-state free Ca^{2+} concentration around 0.5 μM , as depicted in Fig. 6.

Mg^{2+} further decreased the spermine-inhibited initial velocity of Ca^{2+} uptake in a concentration-dependent manner (Fig. 7). The ability of spermine to decrease the minimal free Ca^{2+} concentration achieved through mitochondrial Ca^{2+} accumulation gradually disappeared with increasing Mg^{2+} concentrations and turned into an impaired Ca^{2+} accumulation in the presence of high Mg^{2+} and spermine concentrations (Fig. 8).

DISCUSSION

The initial velocity of Ca^{2+} uptake, i.e. the maximal rate of Ca^{2+} -concentration decrease, was reached 2.5–3.0 s after the beginning of the experiment (Fig. 1). This parameter is an indicator of Ca^{2+} transport, since the Ca^{2+} concentration of the incubation medium was decreased by more than 50% at this time and any saturation of membrane binding sites would be exceeded. Only after the first 10–15 s of incubation was the stimulatory effect of spermine on mitochondrial Ca^{2+} accumulation visible (Fig. 1). This increased mitochondrial Ca^{2+} accumulation has been interpreted as an enhanced uptake velocity when the first measurement was made only after such a large time interval (Kröner, 1988). Nevertheless, when the uptake velocity is expressed as a function of the Ca^{2+} concentration actually present at each particular time point during the course of uptake, the inhibitory action of spermine is evident.

The half-maximally effective concentrations of spermine for inhibition of the initial velocity of Ca^{2+} uptake were more than 3 times higher than for the subsequent stimulation of Ca^{2+} accumulation for mitochondria from all three tissues. This may explain why earlier investigations with a lower time resolution yielded somewhat controversial half-maximally effective concentrations (for discussion, see McCormack, 1989).

The present study shows that the well-established enhanced Ca^{2+} uptake by spermine rather represents an enhanced accumulation of Ca^{2+} by the mitochondria, but not necessarily an increased velocity of Ca^{2+} uptake. In enzymological terms this would indicate a change in the equilibrium rather than in the reaction velocity. The enhancement of Ca^{2+} accumulation is the consistent feature of the spermine action, whereas the uptake velocity changes in dependence on the prior exposure to spermine (Fig. 4; Table 1). Since the enhanced accumulation capacity is not dependent on the uptake velocity, we consider a direct allosteric interaction between spermine and the uniporter protein to be unlikely as the mechanism of action which has been assumed up to now by other authors and ourselves (Lenzen *et al.*, 1986; Kröner, 1988; McCormack, 1989; Jensen *et al.*, 1989).

The experiments with spermine-preloaded mitochondria show that the inhibition or acceleration of the Ca^{2+} uptake is dependent not so much on the absolute spermine load of the mitochondria, but rather on the spermine load relative to the spermine concentration in the incubation medium. The underlying event may therefore be an association of spermine with unloaded mitochondria, or a dissociation of spermine from loaded mitochondria into the spermine-devoid medium, or a net equilibrium between loaded mitochondria and spermine-containing medium.

How this translates into changes in the Ca^{2+} -transport velocity remains to be elucidated. Changes of the mitochondrial membrane potential are unlikely to be responsible, because it is only marginally increased (by about 5%) through spermine action (Lenzen *et al.*, 1986). Concerning the increase in mitochondrial Ca^{2+} accumulation, one may speculate that uptake of spermine and an intramitochondrial site of action are of importance. However, during a 2 min incubation period, there was no further increase in spermine associated with the mitochondria after the first measurement at 20 s (I. Rustenbeck, unpublished work). This concurs with the slow mitochondrial uptake kinetics of spermine reported by Toninello *et al.* (1985). It appears therefore that spermine is only externally bound to the mitochondria during this short incubation period of 2 min and probably exerts its effects by interaction with other cations.

In the cell, the spermine concentration might determine how sensitive mitochondria react to oscillations in the cytosolic Ca^{2+} concentration. In conjunction with a modulatory role of Mg^{2+} (Gylfe, 1990; Jung *et al.*, 1990) this could provide the basis for a flexible coupling between the extramitochondrial and intramitochondrial free Ca^{2+} concentration. This would be particularly relevant in the case of fast changes in the spermine concentration which may occur upon rapid activation of ornithine decarboxylase (Mustelin *et al.*, 1987; Fan & Koenig, 1988). Polyamines may thus be of major physiological importance through coupling of oxidative metabolism to the energetic requirements of the cell.

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