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The Effects of Methylglyoxal-bis(guanylhydrazone) on Spermine Binding and Transport in Liver Mitochondria

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ABSTRACT. This study evaluated the effect of the anticancer drug methylglyoxal-bis(guanylhydrazone) (MGBG) on the binding of the polyamine spermine to the mitochondrial membrane and its transport into the inner compartment of this organelle. Spermine binding was studied by applying a new thermodynamic treatment of ligand–receptor interactions (Di Noto *et al.*, *Macromol Theory Simul* **5:** 165–181, 1996). Results showed that MGBG inhibited the binding of spermine to the site competent for the first step in polyamine transport; the interaction of spermine with this site, termed S_1, also mediates the inhibitory effect of the polyamine on the mitochondrial permeability transition (Dalla Via *et al.*, *Biochim Biophys Acta* **1284:** 247–252, 1996). In the presence of 1 mM MGBG, the binding capacity and affinity of this site were reduced by about 2.6-fold; on the contrary, the binding capacity of the S_2 site, which is most likely responsible for the internalization of cytoplasmic proteins (see Dalla Via *et al.*, reference cited above), increased by about 1.3-fold, and its binding affinity remained unaffected. MGBG also inhibited the initial rate of spermine transport in a dose-dependent manner by establishing apparently sigmoidal kinetics. Consequently, the total extent of spermine accumulation inside mitochondria was inhibited. This inhibition in transport seems to reflect a conformational change at the level of the channel protein constituting the polyamine transport system, rather than competitive inhibition at the inner active site of the channel, thereby excluding the possibility that the polyamine and drug use the same transport pathway. Furthermore, it is suggested that, in the presence of MGBG, the S_2 site is able to participate in residual spermine transport. MGBG also strongly inhibits ΔpH-dependent spermine efflux, resulting in a complete block in the bidirectional flux of the polyamine and its sequestration inside the matrix space. The effects of MGBG on spermine accumulation are consistent with *in vivo* disruption of the regulator of energy metabolism and replication of the mitochondrial genome. BIOCHEM PHARMACOL **58**;12:1899–1906, 1999.

KEY WORDS. spermine; MGBG; binding site; transport; mitochondria

MGBG‖, an organic polycation, garnered considerable attention during the 1960s as a possible antitumour and cytotoxic agent [1]. Although it was discarded from clinical use because of its severe toxicity, recent clinical trials have shown that a special administration schedule reduces its toxicity while preserving its antitumour activity in different organs [2]. Although the mechanism underlying its antiproliferative effect is not known, it has been observed that MGBG affects the metabolism of the natural polyamines in several ways [3 and bibliography therein]. In particular, MGBG reduces the intracellular content of polyamines [4 and bibliography therein], most likely by inducing the cytosolic enzyme spermidine/spermine N^1-acetyltransferase [5]. The drug is also known to be a powerful inhibitor of the key polyamine biosynthetic enzyme, *S*-adenosylmethionine decarboxylase [6]. Interestingly, the spectrum of antineoplastic effects exerted by MGBG suggests that the drug might exhibit increased antitumour activity in patients who are malnourished, possibly due to enhanced polyamine depletion [2]. It was very recently found that the antiproliferative effect of MGBG sensitizes a variety of human cell lines towards the apoptotic effect of tumour necrosis factor [7]. Besides its inhibition of DNA synthesis (e.g. see Ref. 8) and the production of free radical metabolites following its oxidation [9], the strong cytotoxic effects exhibited by MGBG have also been attributed to inhibitory effects on mitochondrial phosphorylation [10–13], impairment of certain mitochondrial membrane-linked enzymes such as carnitine palmitoyl transferase and carnitine acetyl transferase [14, 15], and to an early step in mitochondrial damage [16]

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‖ *Abbreviations:* MGBG, methylglyoxal-bis(guanylhydrazone); $\Delta\Psi$, mitochondrial membrane potential; and S_1 and S_2, spermine binding sites 1 and 2.

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associated with selective inhibition of mitochondrial DNA synthesis [17].

Studies of Ehrlich ascites carcinoma cells indicated that the cytotoxic effects of an MGBG precursor, methylglyoxal, also arise from inhibition of electron flow through complex I of the mitochondrial respiratory chain [18]. Studies of liver mitochondria revealed that MGBG exhibits a clear-cut protective activity against permeability transition induced by Ca^{2+} in the presence of phosphate, oxalacetate, or *ter*-butylhydroperoxide [19], similar to the protection obtained with spermine [20, 21] or spermidine [21]. Spermine, spermidine, and putrescine are transported into liver mitochondria by a proteinaceous channel that operates by an electrophoretic mechanism with a non-ohmic flux–voltage relationship [22–24]. The observation that accumulated spermine can be released from mitochondria under conditions of a high electrochemical gradient suggests that the polyamine cycles back and forth across the inner membrane [25]. MGBG is also transported into rat liver mitochondria, by an as yet unknown mechanism. The fact that MGBG and spermine inhibit each other's transport into mitochondria, taken together with the enhanced uptake of both polycations following outer membrane lysis [26], would support the hypothesis as to the presence of a common transport system corresponding to the polyamine uniporter [22]. Recent analyses of polyamine binding by means of a new thermodynamic model of ligand–receptor interactions [27] demonstrated that energized mitochondria possess two spermine binding sites with distinct functions [28, 29].

The present study examined the effects of MGBG on the binding of spermine to both sites and on the bidirectional transport of the polyamine across the mitochondrial membrane.

MATERIALS AND METHODS

Rat liver mitochondria were isolated in 250 mM sucrose buffered with 5 mM HEPES (pH 7.4) by conventional differential centrifugation. Protein concentration was assayed by the biuret reaction with bovine serum albumin as a standard. All incubations were conducted at 20° with 1 mg of mitochondrial protein/mL in a low ionic strength medium, conditions used in previous permeability transition [19, 21, 30, 31], spermine binding [28], and spermine transport [22–24] studies. The incubation medium contained 200 mM sucrose, 10 mM HEPES (pH 7.4), 5 mM sodium succinate, 1.25 μM rotenone, and 1 mM sodium phosphate. Additions of [^{14}C] spermine are indicated in the description of specific experiments. Uptake of [^{14}C] spermine was determined by a centrifugal-filtration method as previously described [23, 24]. Membrane potential ($\Delta\Psi$) was measured as reported in [22]. All mitochondrial preparations exhibited $\Delta\Psi$ values in the range of 175 to 185 mV when incubated in the standard medium.

Binding parameters were calculated as previously reported [28] by applying a new thermodynamic treatment of ligand–receptor interactions [27]. Scatchard analyses were performed using the equation:

$$\frac{[B]}{[\mathrm{F}]} = \sum_{i=1}^{n_i} \{[B_{\max,i}] - [B_i]\} \cdot \left[\frac{1}{K_{i,1}(t)} + \epsilon_i(\mathrm{F}) \right] \qquad (1)$$

In this equation,

$$\epsilon_i(\mathrm{F}) = \sum_{k=2}^{n_i} \frac{[\mathrm{F}]^{k-1}}{\prod_{j=1}^{k} K_{i,j}(t)}$$

represents the appropriate measure of the extent of multiple coordination on the i-th site, $[B_{\max,i}]$ is the maximum concentration of i-th sites that may be bound by the ligand, $[B_i]$ is the concentration of i-th sites bound by the ligand, $[B_{\max}]$ is the maximum receptor-bound ligand concentration, $[B]$ is the receptor-bound ligand concentration, [F] is the free ligand concentration, $K_{i,j}(t)$ is the affinity constant of the ligand for the i-th site, j is the occupancy number, and t is time. Fitting was performed using a FORTRAN program developed in our laboratory as described in [27].

The distribution of total bound spermine on its respective binding sites was calculated by parameter $X_i(\mathrm{F})$, obtained by means of Eqn 2:

$$X_i(\mathrm{F}) = \frac{[B_{\max,i}] - [B_i]}{[B_{\max}] - [B]} = \frac{1}{1 + \beta_i[\mathrm{F}]} \qquad (2)$$

where β_i is a parameter that describes the influence of the parallel filling of the other k-th sites on filling of the i-th site; theoretically, β_i can range from 0 to infinity [27].

RESULTS

As shown in Fig. 1, addition of spermine to rat liver mitochondria suspended in standard medium and energized by succinate respiration ($\Delta\Psi \cong 180$ mV) resulted in almost instantaneous uptake of the polyamine (i.e. about 12 nmol/mg prot). This amount, representing the extent of binding of 1 mM spermine to the mitochondrial membrane [28], is substantially reduced in the presence of uncouplers or respiratory chain inhibitors [29]. Following this initial rapid uptake phase, the polyamine is transported with slower kinetics, linear with time in the first 10 min, and reaching the maximum extent of accumulation (≅50 nmol/mg prot) after 40 min of incubation. The total amount of spermine accumulated in mitochondria is strongly inhibited by deenergizing agents [29] and represents the amount of spermine transported into the matrix space [24]. The presence of 1 mM MGBG in the incubation medium inhibited spermine transport and reduced its initial binding by 3 nmol/mg prot. The inset of Fig. 1 shows the dose–response effect of MGBG on spermine accumulation after 40 min of incubation, reported as the percentage of

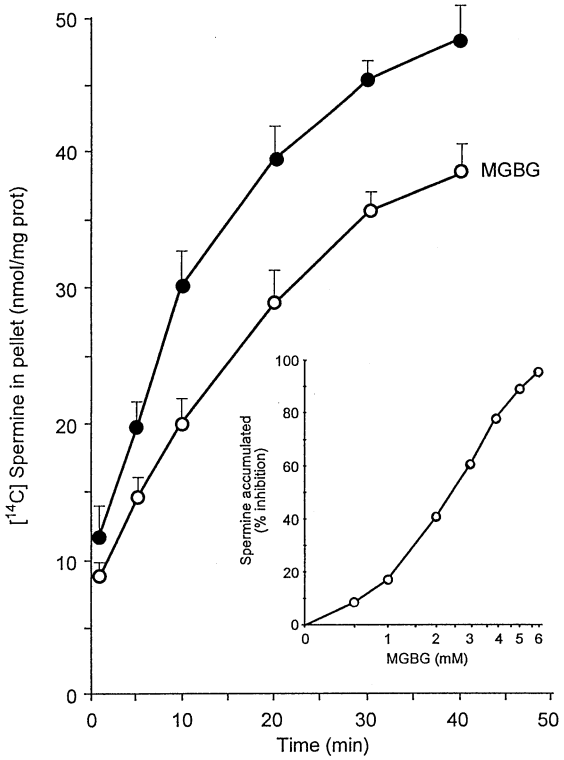

FIG. 1. Effect of MGBG on spermine uptake by liver mitochondria. Rat liver mitochondria were incubated in standard medium as described in Materials and Methods in the presence of 1 mM [^{14}C] spermine (50 μCi/mmol); when present, the concentration of MGBG was 1 mM. The inset reports the dose–response curve of MGBG-mediated inhibition of spermine transport into the matrix, expressed as percentage of inhibition as a function of the log of the MGBG concentration. The points refer to the mean values of the calculated percentages. MGBG was tested at the concentrations indicated in the figure. The concentration of spermine present in the matrix was calculated by subtracting the aliquots bound at zero time from the total amounts taken up after 40 min (see description of Fig. 3 in the Results section). The values shown represent averages of five separate experiments. The open and closed circles represent the presence and absence of MGBG, respectively.

inhibition. As shown in the figure, spermine accumulation in the matrix was completely abolished in the presence of 5–6 mM MGBG.

Figure 2 shows the kinetics of the initial spermine transport phase both in the absence and presence of 0.5 mM MGBG. As previously reported [22], the initial phase of polyamine transport exhibited saturable kinetics, with an apparent K_m of about 0.13 mM (see inset in the figure). In the presence of the polycationic drug, the kinetics assumed an apparent sigmoidal trend. This shift in the kinetics was confirmed by the curvilinear or biphasic trend of the double-reciprocal plot obtained with MGBG (see inset in Fig. 2). Analysis of the transport rates in the absence and presence of MGBG yielded Hill factors of about 1 and 2, respectively.

Figure 3 reports the effects of MGBG on the amount of spermine that bound to the mitochondrial membranes at zero time as a function of the total, external polyamine concentration. These results were obtained by extrapolating at zero time the concentration-dependent spermine uptake by mitochondria, which was linear with time in the first 5 min of incubation, on the y-axis of an uptake versus time diagram (see Fig. 1 in Ref. 28). As illustrated in the figure, spermine binding appeared to be saturable in both the absence and presence of the drug. The inset in Fig. 3 reports a comparison of spermine binding in the absence and presence of MGBG, performed using the Scatchard analysis-based thermodynamic model [27]. Binding data, plotted as the dependence of [*B*]/[F] on [*B*], were simulated with a series of curve profiles belonging to Eqn 1, obtained via computer simulation for several ranges of parameters *s* and n_i. The theoretical curves that satisfactorily fit the experimental data (solid lines in the insets) are typical of two binding sites, S_1 and S_2, both with monocoordination; their equation is reported in Ref. 28, Fig. 2.

Table 1 reports spermine binding parameters in the absence and presence of MGBG derived by Scatchard analysis. These analyses demonstrated the presence of two binding sites on the mitochondrial membranes, each binding one equivalent of spermine. As evidenced in this table, the presence of 1 mM MGBG diminished both the spermine binding capacity and affinity for the S_1 site by about 2.6-fold; in contrast, the S_2 site increased its spermine binding capacity by about 1.3-fold, while its binding affinity

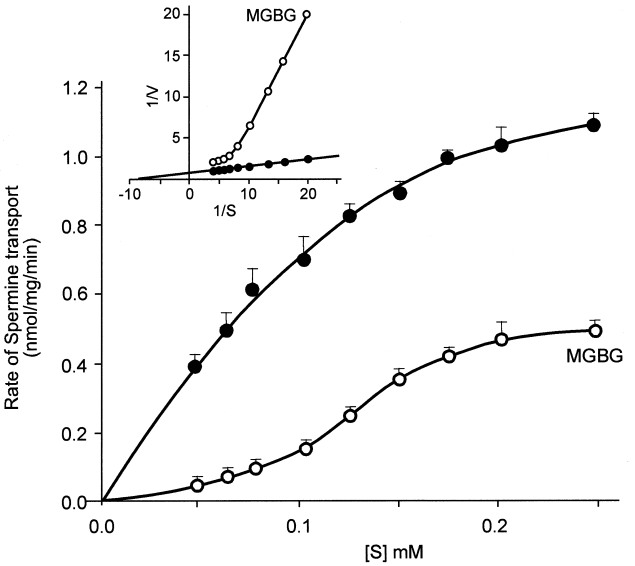

FIG. 2. Effect of MGBG on the kinetics of spermine transport. Rat liver mitochondria were incubated in standard medium as described in Materials and Methods in the presence of different concentrations of [^{14}C] spermine (0.05 μCi/mL), as indicated in the figure. MGBG was present at a concentration of 0.5 mM. The inset reports the double-reciprocal plot of the mean value of the initial transport rate. The values shown represent the averages of four separate experiments. The calculated K_m for spermine transport in the absence of MGBG is 0.13 mM. The open and closed circles represent the presence and absence of MGBG, respectively. S is the total exogenous spermine concentration and V the rate of spermine transport.

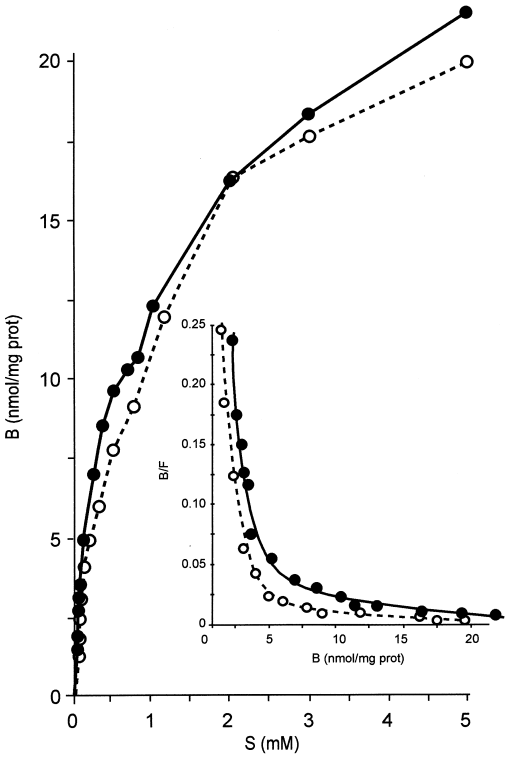

FIG. 3. Effect of MGBG on concentration-dependent spermine binding to mitochondrial membranes. Experimental conditions are described in Fig. 2, with MGBG present at a concentration of 1 mM. The data points were obtained by extrapolating at zero time the concentration-dependent spermine uptake, determined from the line best-fitting the mean value of five experiments. (—●— without MGBG; —○— with MGBG). Further details of the method used to construct the plots are provided in Fig. 1 of Ref. 32. The inset reports spermine binding analyses with the thermodynamic treatment of the Scatchard method. Construction of the diagrams is described in Fig. 2 of Ref. 28. S is the exogenous [^{14}C] spermine concentration present in the range of 10 μM–5 mM (0.05 μCi/mL). B is the amount of spermine (nmol/mg prot) that binds instantaneously at zero time. F is free spermine concentration.

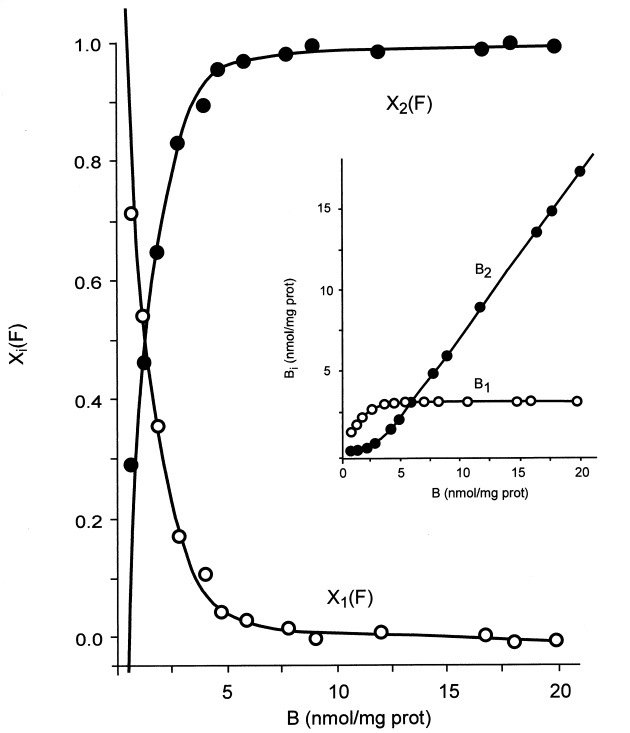

FIG. 4. Molar fraction ratio of spermine binding to mitochondria in the presence of MGBG. The calculations refer to the amount of spermine required to fill the binding sites. $X_1(F)$ and $X_2(F)$ are the molar fraction ratios required to fill the first (S_1) and second (S_2) spermine binding sites, respectively. The inset reports the subdivision of total bound spermine between the S_1 and S_2 sites. The two aliquots, B_1 and B_2, bound to sites S_1 and S_2, respectively, were determined by Eqn 2; B was calculated as described in the legend to Fig. 3, and the molar fraction ratios $X_1(F)$ and $X_2(F)$ of zero time bound spermine and B_{max1} and B_{max2} are reported in Table 1. An example of this calculation is reported in Fig. 3 of [28].

remained unchanged. Calculation of parameter β_1, which describes the possible influence of the parallel filling of S_2 on filling of S_1 (see Eqn 2) was not affected by MGBG. Thermodynamic analyses of data obtained at high MGBG concentrations (5–6 mM) show that the drug almost completely inhibited binding of spermine to the S_1 site and, in contrast to observations made using a concentration of 1 mM, also abolished binding to the S_2 site (results not reported).

Figure 4 shows the molar fraction ratios $X_1(F)$ and $X_2(F)$ for the amount of free spermine that can bind to sites S_1 and S_2, respectively, determined in the presence of MGBG. These values were obtained by using the values for β_1

TABLE 1. Effects of MGBG on spermine binding parameters

	B_{max} (nmol/mg protein)	B_{max1} (nmol/mg protein)	B_{max2} (nmol/mg protein)	$K_{1,1}$ (mol/L)	$K_{2,1}$ (mol/L)	χ†	β_1
Control	23.10 (5)*	8.30 (2)	14.80 (3)	$42.5\ (4) \cdot 10^{-6}$	$915\ (6) \cdot 10^{-6}$	0.0415	0.16 (3)
MGBG	23.55 (6)	3.15 (3)	20.40 (3)	$120\ (5) \cdot 10^{-6}$	$937\ (11) \cdot 10^{-6}$	0.0352	0.12 (2)

$$\chi = \frac{\sum |(N_0) - (N_c)|}{\sum |(N_c)|}$$

where (N_0) is the experimental value and (N_c) is the calculated value, N being given by B/F.

*Standard deviations in the least significant digits are given in parentheses.

†χ indicates the closeness of fit.

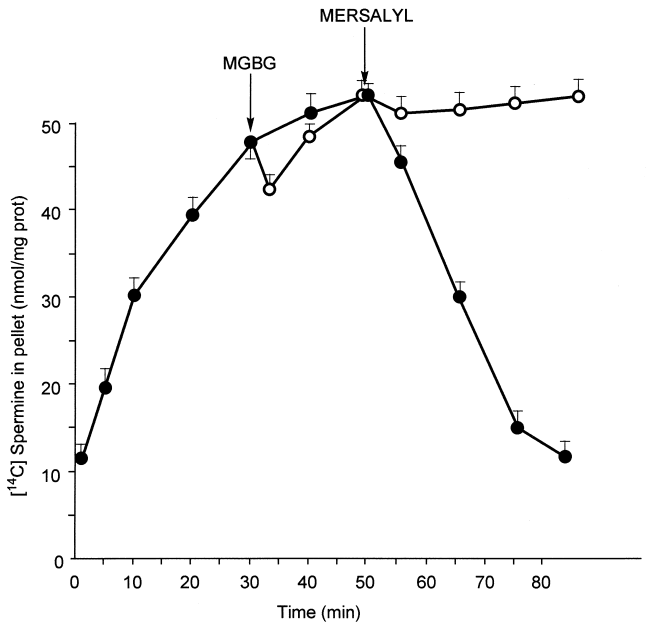

FIG. 5. Effect of MGBG on spermine efflux induced by mersalyl. Experimental conditions were as described in Fig. 1. Mersalyl and MGBG were present at concentrations of 10 μM and 1 mM, respectively (—●— without MGBG; —○— with MGBG). The values shown represent the average of five separate experiments.

reported in Table 1. As previously observed in other spermine binding experiments [28], these calculations show that, in the presence of MGBG, $X_1(F)$ decreased, but $X_2(F)$ increased with increasing amounts of bound spermine. This signifies that the S_1 site was filled before the S_2 site. However, as demonstrated in the inset of Fig. 4, the S_2 site began to bind spermine before the S_1 site was completely filled.

As shown in Fig. 5, addition of mersalyl, an inhibitor of phosphate transport, to mitochondria promptly blocked the spermine uptake process and induced a sustained efflux of the polyamine. The addition of 1 mM MGBG prior to mersalyl promoted a sudden but limited and transitory release of spermine; subsequent addition of mersalyl did not induce any further efflux of spermine, which remained segregated within the mitochondria until anaerobiosis took place.

The experiments described above were carried out in a hypotonic sucrose medium. When the assays were repeated using an iso-osmotic saline medium, the amounts of bound and transported spermine were substantially reduced. However, the binding constants and kinetics constant of spermine transport and the observed effects of MGBG were almost identical under these experimental conditions.

DISCUSSION

The results of the present study clearly demonstrate that MGBG inhibits both the binding of spermine to mitochondrial membranes (Figs. 1 and 3) and its transport into the matrix space (Figs. 1 and 2). Furthermore, the drug is also able to block spermine efflux after its accumulation in the matrix (Fig. 5). This inhibition of spermine transport is not due to non-specific alterations of the inner membrane, given that previous experiments demonstrated a protective effect of MGBG against mitochondrial permeability transition induced by Ca^{2+} plus phosphate or other agents [19]. The observed sequestration of spermine inside the mitochondria due to MGBG-induced inhibition of its efflux provides further evidence against non-specific alterations of the inner membrane as a mechanism explaining the drug's effects. The inhibition of spermine accumulation in the inner compartment, which becomes completely blocked at high MGBG concentrations (Fig. 1B), probably reflects the main actions of the drug: the inhibition of the preliminary step of polyamine transport.

As pointed out previously [28], spermine uptake is determined by the sum of two processes, i.e. electrophoretic matrix transport and membrane binding. Correct evaluation of spermine binding is very difficult as a specific inhibitor of the transport step has not yet been identified; deenergizing agents are unsuitable for this purpose, as they block transport and change binding site conformation [29]. The thermodynamic model [27] used in this and previous studies of polyamine binding [28, 29] avoids problems associated with such analyses. The Scatchard plot analyses reported in Fig. 3 and summarized in Table 1 demonstrate that the experimental data fit the theoretical curves perfectly and confirm the previous description of two spermine binding sites, termed S_1 and S_2, on mitochondrial membranes [28]; both of these sites exhibit monocoordination and relatively high binding capacity and low binding affinity compared to the polyamine binding sites found on other membranes [32]. The very low β_1 values demonstrate that filling of both sites is not reciprocally influenced, as previously proposed [28].

It has been demonstrated that the binding of spermine to the S_1 site represents the preliminary event in spermine transport and is also involved in spermine-mediated inhibition of permeability transition [28]. On the other hand, the S_2 site appears to be responsible for the polyamine-mediated uptake of certain cytosolic enzymes such as the precursor of ornithine carbamoyl transferase [33] and casein kinase II [34], and for other effects such as the activation of phosphate efflux from the matrix space [25, 35]. The concentrations of spermine required to exhibit these latter effects are 1 mM or higher, and thus probably involve the S_2 site. The proposed distinct function of the S_2 site is supported by the fact that it begins to fill after the complete filling of S_1 [28], and that the S_1 site begins to operate at lower spermine concentrations ($\leq$50 μM) [21, 22]. Different functions for the two mitochondrial spermine binding sites have also been proposed by others [36].

The present analysis demonstrates that 1 mM MGBG exerts different effects on binding of spermine to the two sites. The observed decrease in the binding capacity and affinity of the S_1 site in the presence of the drug may

contribute to the reduction in the initial rate of spermine transport and consequently to the reduced accumulation of the polyamine (Fig. 2). This effect on the binding parameters of the S_1 site should counteract the protective effects of spermine against mitochondrial permeability transition [21]. However, this supposition is not confirmed by experimental data demonstrating that MGBG completely inhibits permeability transition [19]. This observation raises the possibility that MGBG might be able to substitute for spermine binding to the S_1 site. Recent studies demonstrate that the natural polyamines bind at different proportions to the S_1 site: while 40% of the total amount of bound spermine is found at the S_1 site [28], only 6% of bound spermidine is found at this site, and putrescine does not bind to it [37]. Differences in the flexibilities of the spermine, spermidine, and putrescine molecules might account for their distinct abilities to bind to the S_1 site. As previously reported [38], the presence of two *N* atoms in the middle of the spermine molecule renders it the most flexible of the three polyamines, with the relative distances between N_1 and N_4 able to vary up to 56% of its maximum length. In spermidine, the relative distance between N_1 and N_3 can vary up to 8%, thus making the molecule much more rigid, while putrescine, having a fixed distance between N_1 and N_2, is completely rigid. MGBG has the same number of atoms in the main chain as spermidine, with four *N* atoms in the middle. However, the presence of double bonds forming resonance hybrids would make MGBG almost completely inflexible. Furthermore, other very recent results demonstrate that the S_1 site is able to bind remarkable amounts of polyamine molecules that possess a symmetric structure and contain at least two aminopropyl groups.* Given that MGBG does not exhibit any flexibility and lacks aminopropyl groups, its binding to the S_1 site (responsible for spermine transport) appears to be very unlikely; hence, the drug is probably transported in mitochondria via a separate pathway. This leads us to predict that MGBG's inhibitory effect on spermine binding probably arises from a conformational change in the S_1 site induced upon binding of the drug to a distinct site(s). The fact that the binding of spermine to the S_2 site is increased upon addition of MGBG (see Table 1) clearly demonstrates that the drug does not bind to this site; instead, it apparently promotes a change in its conformation that has the opposite effects compared to those on the S_1 site. The observed effects of the drug indicate that its binding site is located in the proximity of the spermine binding sites, which, as very recently proposed, are probably contained within a single protein [37]. Evidence for such conformational changes is also provided by the results presented in Fig. 4, which demonstrate that, upon MGBG addition, spermine begins to fill the S_2 site before S_1 is completely filled. In this regard, MGBG resembles carbonyl cyanide *P*-trifluorometoxyphenylhydrazone and antimycin A, two inhibitors of spermine transport that act via different mechanisms [29]. Experiments are in progress in our laboratory to verify whether the increased binding capacity of the S_2 site in the presence of MGBG alters the functional activities of this site.

As shown in Fig. 2 and reported previously [24], the initial rate of spermine transport exhibits apparently hyperbolic kinetics. The MGBG-mediated inhibition of the initial rate produces a kinetic profile having an apparent sigmoidal trend with a non-linear double-reciprocal plot (see inset in Fig. 2) and with a Hill factor of about 2. Furthermore, the V_{max} is clearly lower in the presence of the drug. Most likely, MGBG inhibits spermine transport by decreasing its ability to bind to the S_1 site (Table 1). However, the perturbation provoked by MGBG binding would also render the S_2 site competent for spermine transport, which likely accounts for the observed sigmoidal kinetics of the residual transport. This hypothesis is supported by the observation that MGBG increases binding of spermine to S_2 (Table 1) and allows this site to begin binding before saturation of S_1 (Fig. 4); under normal conditions, S_2 begins to bind only upon complete filling of S_1 [28]. The possibility that the S_2 site can participate in spermine transport when other polycations are present has been confirmed in a very recent paper [38]. It is also to be pointed out that participation of S_2 in spermine transport does not completely substitute for S_1, as indicated by the reduction in V_{max} observed in the presence of MGBG (Fig. 2). This deficiency probably reflects the much lower binding affinity of S_2 compared to S_1 (Table 1). Similar inhibition kinetics have also been observed in measurements of the activity of the mitochondrial permeability transition channel upon addition of ADP [39].

The almost complete inhibition of spermine transport induced by very high concentrations of MGBG (see inset in Fig. 1) is most likely due to the strong reduction in spermine binding to the S_1 site and, in this case, to the S_2 site as well. These contrasting effects at the level of the S_2 site probably reflect a general property exhibited by high concentrations of MGBG and other bis(guanylhydrazones) [40]. Although mitochondria exposed to 5 mM MGBG maintain a stable $\Delta\Psi$ and are able to accumulate and retain Ca^{2+}, both $\Delta\Psi$ and Ca^{2+} retention are reduced compared to levels measured in the presence of 1 mM MGBG [19]. These circumstances would indicate a decrease in the overall active surface of suspended mitochondria as might occur upon aggregation, as previously described [40], and would account for the strong inhibition of spermine binding to the S_2 site.

A previous study proposed that an increase in ΔpH, following an electrophoretic efflux of phosphate from the matrix space, is able to drive an electroneutral efflux of spermine [25]. This phenomenon is evidenced by results of assays carried out in the presence of mersalyl, which, under the conditions utilized, prevents the uptake but not the release of phosphate. The results reported in Fig. 5 demonstrate that the movement of spermine towards the outer side of the mitochondrial membrane is completely blocked

* Tassani V, Dalla Via L and Toninello A, manuscript in preparation.

in the presence of MGBG. The transient release of small amounts of spermine (a few nmol) upon the addition of MGBG most likely results from detachment of the polyamine from the S_1 site and re-uptake by the S_2 site, due to the conformational alterations promoted by binding of the drug.

Although MGBG has been demonstrated to prevent permeability transition, thus exerting a beneficial effect on mitochondrial membranes [19], inhibition of the bidirectional fluxes of spermine such as that provoked by MGBG can also compromise mitochondrial processes of physiological importance such as regulation of energy metabolism and replication of the mitochondrial genome. This, taken together with the documented activating effect of spermine on citrate synthase [41] and pyruvate dehydrogenase [42–45] and the strict correlation between the presence of spermine in the matrix and mitochondrial DNA replication [46], strongly support the hypothesis that prevention of mitochondrial spermine flux at least in part underlies the cytotoxic effects of this drug.

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