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Effect of Mersalyl on Mitochondrial Mg^{++} Flux

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Abstract

The mercurial mersalyl has little effect either on rapid Mg^{++} binding by isolated rat liver mitochondria or on the total Mg^{++} content of these organelles measured after 0.75 min of incubation at 20°C. The data do not support the previous suggestion that the increased permeability to K^+ of mitochondria treated with mersalyl results from release of endogenous Mg^{++}. An increased pH-dependence of unidirectional Mg^{++} flux into respiring rat liver mitochondria is suggested to arise indirectly from inhibition by mersalyl of pH shifts associated with exchanges of endogenous phosphate. In addition, mersalyl appears to have a stimulatory effect on Mg^{++} influx. Mersalyl also increases the average rate of unidirectional efflux of endogenous Mg^{++}. The stimulatory effects of mersalyl on Mg^{++} flux are similar to, although quantitatively less than, the previously reported effects of mersalyl on mitochondrial K^+ flux.

Introduction

Net uptake of Mg^{++} by heart and liver mitochondria is sensitive to inhibitors of respiration [1–3]. Net efflux of Mg^{++} from mitochondria is also dependent on respiration [3–6]. Addition of either inorganic phosphate or the thiol-oxidizing reagent diamide stimulates net Mg^{++} efflux [3–6]. The sulfhydryl reagent NEM[1], which inhibits the mitochondrial phosphate/hydroxyl translocator [7], blocks the stimulation of net Mg^{++} efflux by P_i or diamide [5,6]. Indirect evidence based on changes in fluorescence of chlorotetracycline has been interpreted as indicating that *p*-hydroxymethylmercuribenzoate causes release of endogenous Mg^{++} [8]. Depletion of mitochondrial ADP following treatment with the mercurial mersalyl is reported to accompany enhanced net Ca^{++} efflux from mitochondria [9]. It is suggested by Harris et al. that loss of

[1]Abbreviation used: NEM, *N*-ethylmaleimide.

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Mg^{++} with ADP may result from reaction of mercurials with membrane thiol groups.

Stimulation of net K^+ transport across mitochondrial membranes by mersalyl and other mercurials has been reported [10–12]. Southard et al. have proposed that mercurials cause release or induction within the mitochondrial membrane of an ionophore which mediates K^+/H^+ exchange. Alternatively it has been suggested that mercurials affect K^+ permeability by blocking thiol groups necessary for tight binding of Mg^{++} to the inner mitochondrial membrane [8]. Increased permeability to K^+ in the presence of EDTA or the ionophore A23187 has similarly been attributed to depletion of endogenous Mg^{++} [13, 14].

Unidirectional fluxes of Mg^{++} and K^+ across the limiting membranes of isolated rat liver mitochondria have been measured by means of the radioisotopes ^{28}Mg and ^{42}K. The mechanisms mediating unidirectional Mg^{++} and K^+ fluxes are similar in many respects. Rates of both influx and efflux of Mg^{++} as well as K^+ are dependent on respiration [15–18]. In each case, plots of the reciprocal of the cation influx rate against the reciprocal of the external cation concentration are linear [16,18,19]. This pattern is distinct from the sigmoidal concentration-dependence observed under some conditions for unidirectional and net Ca^{++} fluxes into mitochondria [20–23]. The K^+ analog Tl^+ competitively inhibits influx of Mg^{++} and K^+, while Mg^{++} competitively inhibits K^+ influx [16, 18, 19]. K^+ and Mg^{++} influx rates increase with increasing external pH [18, 24]. An increased pH dependence of K^+ and Mg^{++} influx rates in the presence of NEM has been attributed to prevention by NEM of transmembrane pH shifts associated with P_i/OH^- exchange. In the presence of NEM, a linear dependence of K^+ and Mg^{++} influx rates on external hydroxyl ion concentration is observed. The pH dependence of K^+ and Mg^{++} influx rates is suggested to be consistent with a proposed nonelectrogenic mechanism involving cotransport of cations with OH^- into the mitochondria [18, 24].

Mersalyl, like NEM, blocks the mitochondrial phosphate/hydroxyl translocator and additionally inhibits phosphate/dicarboxylate exchange [7]. A stimulatory effect of mersalyl on unidirectional K^+ influx and efflux rates has been explained on the basis of inhibition of conversion of a respiration-induced ΔpH to $\Delta\psi$ via phosphate and dicarboxylate exchanges [16, 17]. As in the presence of NEM, a linear dependence of K^+ influx on external OH^- is observed in the presence of mersalyl [25]. However, while NEM decreases rates of K^+ influx at neutral or slightly acidic pH and stimulates K^+ influx at alkaline pH, mersalyl stimulates K^+ influx over the entire pH range studied from 6.8 to 8.0. On this basis it has been suggested that mersalyl may have a stimulatory effect on the mechanism of K^+ influx which is in addition to its indirect effect related to inhibition of proton-linked phosphate exchange [25].

The present studies have examined the effect of mersalyl on unidirectional fluxes of Mg^{++} into and out of respiring rat liver mitochondria.

Materials and Methods

Rat liver mitochondria were isolated by standard procedures as in previous studies [18]. Mitochondrial protein was assayed by the biuret procedure [26]. Mitochondria were incubated at 20°C in media containing 200 mM sucrose, 30 mM Tris, 7.5 mM succinate, the measured concentrations of Mg^{++} indicated in the figure legends, and the radioisotopes ^{28}Mg (approx. 0.15 μCi/ml), $^{3}H_2O$ (approx. 2.5 μCi/ml), and in some experiments (^{14}C) sucrose (approx. 0.4 μCi/ml). The pH was adjusted to the values listed in the figure legends with HCl. Mitochondrial samples were separated from incubation media by rapid centrifugation through silicone [27]. ^{28}Mg was assayed by liquid scintillation counting of the Cerenkov radiation in aqueous dilutions of acidified mitochondrial samples and supernatants, and the counts were corrected for decay. Following decay of the ^{28}Mg, total Mg^{++} levels were assayed by atomic absorption, and ^{14}C and/or ^{3}H counts were determined using a standard liquid scintillation counting cocktail.

$^{3}H_2O$ and (^{14}C) sucrose distribution spaces and Mg^{++} levels were calculated from the data as in previous studies [28]. The mitochondrial content of labeled Mg^{++} was calculated from the ^{28}Mg counts associated with the sedimented mitochondria and the initial (0.75 min of incubation) supernatant specific activity. Unidirectional Mg^{++} influx rates were calculated as the difference in labeled Mg^{++} associated with the mitochondria between samples taken after 0.75 and 8 min of incubation. Net Mg^{++} fluxes were calculated as the change in total mitochondrial Mg^{++} over the same time period. The values of labeled and total Mg^{++} used for the Mg^{++} flux calculations were not corrected for contaminating external Mg^{++}, which was estimated from measured (^{14}C) sucrose spaces to be constant during the time course of the measurements. During 8 min incubations, the uptake of labeled Mg^{++} is an essentially linear function of incubation time [18]. Unidirectional Mg^{++} efflux rates were calculated as the difference between influx and net flux rates.

^{28}Mg was obtained from Brookhaven National Laboratory. Other isotopes were obtained from New England Nuclear. The silicone used (SF-1154) was a generous gift of the General Electric Company.

Results

The effect of 150 μM mersalyl on Mg^{++} influx and efflux rates is shown in Table I, column A. Mersalyl stimulates Mg^{++} influx at pH 8. A slight

Table I. Effect of Mersalyl on Mg^{++} Flux and Mitochondrial Mg^{++} Content[a]

Conditions	pH	A (μmoles Mg^{++}/g · min)		B (initial μmoles Mg^{++}/g)	
		Influx	Efflux	Labeled Mg^{++}	Total Mg^{++}
Control	7	0.43 ± 0.13	0.25 ± 0.14	4.5 ± 0.4	23.9 ± 1.2
Control	8	0.56 ± 0.15	0.35 ± 0.16	7.1 ± 0.3	27.8 ± 1.8
+ Mersalyl	7	0.44 ± 0.11	0.43 ± 0.17	5.0 ± 0.4	26.0 ± 0.5
+ Mersalyl	8	1.34 ± 0.07	0.52 ± 0.10	8.4 ± 0.5	29.6 ± 1.1

[a]Data are pooled from two experiments in which the Mg^{++} concentration in the medium was 0.5 mM and the mitochondrial protein concentrations were 3.5 and 4.5 mg/ml. All values are means of four determinations ± standard deviations. In A, unidirectional Mg^{++} influx and efflux rates are listed in units of μmoles per gram of protein per minute. In B, the mitochondrial contents of labeled and total Mg^{++} are depicted in units of μmoles per gram of protein. The values are corrected for contaminating external Mg^{++}, estimated as the product of the distribution space of (^{14}C) sucrose sedimenting with the mitochondria and the supernatant Mg^{++} concentration. Mersalyl when present was at 150 μM.

stimulation of Mg^{++} efflux is observed at both pH 7 and pH 8, although the standard deviations about mean values overlap. The data in Table I, column B, show that mersalyl has little effect on the initial (0.75 min of incubation) content of labeled or total Mg^{++}. The slight increase in labeled Mg^{++} content in the presence of mersalyl at pH 8 can largely be accounted for by the stimulated influx of Mg^{++} during the time period of initial sampling. Extrapolating back to zero time, the amount of labeled Mg^{++} associated with the mitochondria does not significantly exceed control values. The rapid ^{28}Mg binding, which is complete in less than the sampling time, is not sensitive to

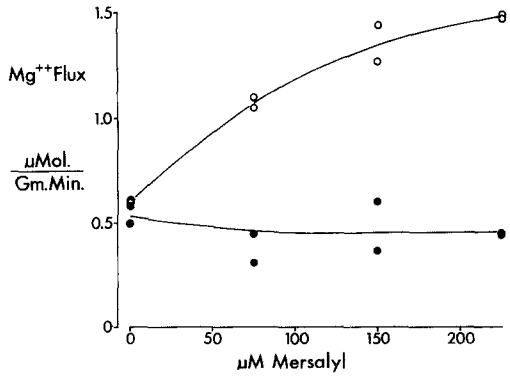

Fig. 1. Effect of varied concentrations of mersalyl on Mg^{++} influx. Conditions were the same as indicated in the legend to Table I. The Mg^{++} influx rate, in units of μmoles per gram of protein per minute, is plotted against the concentration (μM) of mersalyl in the medium. Symbols: ●, medium at pH 7.0; O, medium at pH 8.0.

metabolic inhibitors and was previously suggested to correspond to a passive adsorption process. The slower uptake of Mg^{++} which proceeds during the 8 min incubations is dependent on respiration and is assumed to represent the energy-linked flux of Mg^{++} into the mitochondria [28]. The data of Table I, column B, indicate that the rapid ^{28}Mg binding is pH-dependent, as is the rapid binding of labeled K^+ [24].

The concentration dependence of the effect of mersalyl on Mg^{++} influx is shown in Fig. 1. The results show little effect of mersalyl on Mg^{++} influx from a pH 7 medium. Nearly maximal stimulation of Mg^{++} influx at pH 8 is caused by 150 μM mersalyl (33 μmoles/g protein). This concentration of mersalyl is comparable to that found to cause nearly maximal stimulation of K^+ influx at pH 8 [25] and is higher than the approximately 10 μmoles of mersalyl per gram of protein (52 μM) reported to cause nearly complete inhibition of the mitochondrial phosphate/hydroxyl transporter [7].

The pH dependence of the effect of mersalyl on Mg^{++} influx is examined in greater detail in Fig. 2. A linear dependence of the Mg^{++} influx rate on the external Mg^{++} concentration is observed in the presence of mersalyl. Consistent with the data of Table I and Fig. 1, Mg^{++} influx rates near neutrality in the presence of mersalyl are similar to or slightly lower than control values, while stimulation is observed at alkaline pH.

The effect of mersalyl on the dependence of Mg^{++} influx from a pH 8 medium on the external Mg^{++} concentration is depicted in Fig. 3. Lineweaver–Burk plots remain approximately linear in the presence of mersalyl. Such plots in the presence and absence of mersalyl intersect to the left of the vertical axis. Thus the V_{max} of Mg^{++} influx increases in the presence of mersalyl. In six experiments equivalent to that depicted in Fig. 3, values of apparent K_m calculated for control and mersalyl-treated mitochondria were 0.60 ± 0.19 and 0.65 ± 0.20 mM Mg^{++} respectively (means ± standard deviations). Corresponding values of V_{max} for control and mersalyl-treated

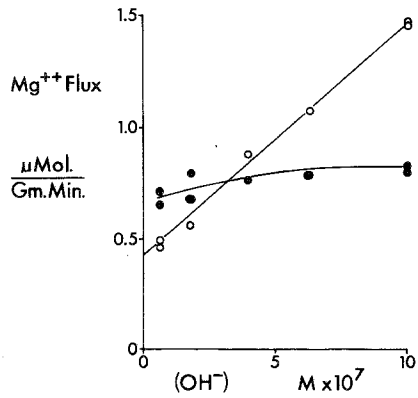

Fig. 2. The pH dependence of Mg^{++} influx in the presence and absence of mersalyl. The Mg^{++} concentration in the medium was 0.9 mM and the mitochondrial protein concentration was 7.0 mg/ml. The Mg^{++} influx rate, in units of μmoles per gram of protein per minute, is plotted against the molar hydroxyl ion concentration in the medium. Symbols: ●, control samples; ○, medium included 150 μM mersalyl. The lines are arbitrarily drawn.

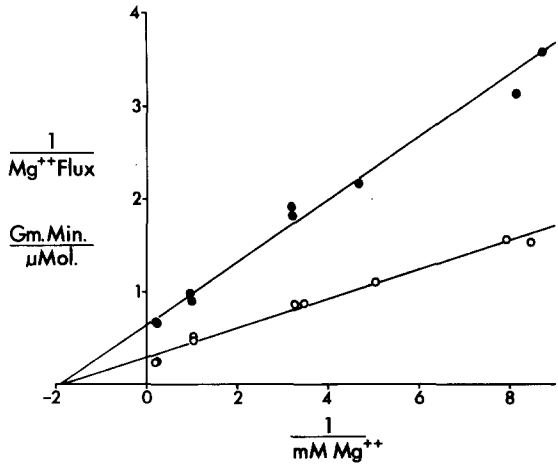

Fig. 3. Effect of mersalyl on the dependence of Mg^{++} influx on Mg^{++} concentration. The pH of the medium was 8.0 and the mitochondrial protein concentration was 5.6 mg/ml. The reciprocal of the Mg^{++} influx rate, in units of grams protein · min · μmole^{-1}, is plotted against the reciprocal of the mM Mg^{++} concentration in the medium. The lines drawn were calculated by the method of least squares. Symbols: ●, control samples; O, medium included 150 μM mersalyl.

mitochondria were 1.4 ± 0.3 and 3.8 ± 0.9 μmoles Mg^{++} per gram of protein per minute.

Discussion

Mersalyl treatment does not result in any decrease in the total Mg^{++} content of the mitochondria or in the amount of rapidly exchangeable adsorbed Mg^{++}. Although a very small decrease in Mg^{++} binding would not be detectable with the techniques used, the data do not support the proposal [8, 9] that mercurials induce release of Mg^{++} from the mitochondrial membrane. Thus the suggestion that increased permeability to other cations in the presence of mercurials results from decreased Mg^{++} binding [8, 9] is not supported by the data. It should be noted that, aside from the presence of 0.5 mM Mg^{++} in the medium, conditions in the experiments depicted in Table I were equivalent to those in previous studies [25] in which stimulation of K^+ influx by mersalyl was demonstrated.

The linear dependence of Mg^{++} influx on external OH^- in the presence of mersalyl is similar to that observed previously for K^+ influx in the presence of mersalyl and for K^+ and Mg^{++} influx in the presence of NEM [18, 24, 25].

While Mg^{++} influx rates at or near neutrality in the presence of mersalyl are close to or slightly below control values, mersalyl stimulates K^+ influx over the entire pH range studied from 6.8 to 8.0. Nevertheless, plots such as those in Fig. 2, when compared to those such as in Fig. 5 of Diwan et al. [18], indicate that the Mg^{++} influx rate, like the K^+ influx rate, when extrapolated to low OH^- concentration in the presence of mersalyl exceeds that in the presence of NEM under equivalent conditions. Thus some stimulatory effect of mersalyl on Mg^{++} influx beyond the indirect effect it shares with the other inhibitor of P_i/OH^- exchange, NEM, is apparent. Furthermore, the V_{max} of Mg^{++} influx estimated at pH 8 in the presence of mersalyl, 3.8 ± 0.9 μmole per gram of protein per minute, exceeds the V_{max} value of 2.3 ± 0.5 previously reported for mitochondria at pH 8 in the presence of sufficient NEM to block P_i/OH^- exchange [18]. The V_{max} of K^+ influx is also greater in the presence of mersalyl than in the presence of NEM [24, 25].

Since mersalyl increases the V_{max} of K^+ as well as Mg^{++} influx, while having little effect on the apparent K_m for K^+ or Mg^{++}, the stimulatory effect cannot be explained on the basis of any change in affinity of the transport mechanism(s) for these cations [16, 25]. Some increase in V_{max} can be accounted for by the increased pH dependence in the presence of mersalyl, since pH affects primarily the V_{max} of cation influx [18, 24]. Perhaps transmembrane pH shifts are more effectively blocked by mersalyl than by NEM, since mersalyl blocks both of the phosphate translocators. However, since the data suggest higher rates of cation influx at "zero" (extrapolated) OH^- concentration, it is difficult to explain the effect of mersalyl solely on the basis of increased pH dependence.

A slight stimulation of Mg^{++} efflux by mersalyl is suggested by the data, although the precision of the measurements prevents exact quantitation of the effect. Under equivalent conditions mersalyl approximately doubles the rate of K^+ efflux at pH 8 [25]. The process of Mg^{++} efflux is generally less sensitive to treatments which affect K^+ efflux, e.g., varied external pH and cation concentration [18, 24]. The lower variability of Mg^{++} efflux rates may reflect in part the internal concentration of free Mg^{++}, which is probably much less than the total endogenous Mg^{++} content.

While the mechanism by which mersalyl stimulates mitochondrial cation transport remains unclear, the effect of the mercurial on Mg^{++} flux appears to be similar to, although somewhat less than, its effect on K^+ flux. The mechanisms of transport of Mg^{++} and K^+ across mitochondrial membranes exhibit many similarities, and cross competition is observed. However, whether a common mechanism transports both cations is uncertain. One additional similarity has recently been noted. The oxidative phosphorylation inhibitor dicyclohexylcarbodiimide, which decreases the apparent affinity for K^+ of the mitochondrial K^+ transport mechanism, increases the

apparent K_m for Mg^{++} of the mechanism mediating respiration-dependent Mg^{++} influx [29, 30].

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