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Short communication

The effect of methylglyoxal-bis(guanylhydrazone) on mitochondrial Ca^{2+} fluxes

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Abstract

Methylglyoxal-bis(guanylhydrazone) (MGBG) induces a dose-dependent inhibition of the electrophoretic Ca^{2+} uptake by rat liver mitochondria (RLM) without affecting the electrical membrane potential. MGBG is also able to inhibit the electroneutral Ca^{2+} release from mitochondria. These effects result in a progressive increase of Ca^{2+} level in suspending medium indicating that Ca^{2+} uptake is inhibited at higher extent than Ca^{2+} efflux. Spermine instead, induces a lowering of external Ca^{2+} concentration. This action is reversed by MGBG which again raises the external Ca^{2+} concentration then in the absence of spermine, though at a lower extent. The mechanism of MGBG effects and their implications on energy metabolism are discussed.

Keywords: MGBG; Ca^{2+}; Mitochondria

1. Introduction

The interest in the polycation MGBG arose when it was recognized as an antitumor agent [1]. Due to its high toxicity it was subsequently discarded from clinical use until, recently, the observation that a special administration schedule attenuates its toxicity while maintaining its antiproliferative activity, re-proposed it as therapeutic drug [2]. The strong cytotoxic effects exhibited by MGBG have been mainly attributed to an inhibition on mitochondrial phosphorylation [3–6]. At concentrations ranging from 0.5 to 1 mM, MGBG protects liver mitochondria against the membrane permeability transition (MPT) induced by toxic Ca^{2+} concentration plus phosphate or other oxidant agents as oxaloacetate, acetoacetate or *tert*-butylhydroperoxide. This appears to be due to a competition between MGBG and Ca^{2+} for the same binding site on the external surface of inner membrane, thus restricting the rate of Ca^{2+} influx into mitochondria [7]. To test this assumption in the present paper the action of MGBG on the rate of both Ca^{2+} influx and efflux in RLM has been studied.

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Abbreviations: MGBG, methylglyoxal-bis(guanylhydrazone); MPT, membrane permeability transition; RLM, rat liver mitochondria.

2. Methods

2.1. Isolation of mitochondria

RLM were isolated by conventional differential centrifugation in a buffer containing 250 mM sucrose and 5 mM Hepes (pH 7.4), 1 mM EDTA. EDTA was omitted from the final washing solution. Protein content was measured by the biuret method with bovine serum albumin as a standard.

2.2. Conditions of incubation

Mitochondria (1 mg protein/mL) were incubated in a water-jacketed cell at 20°. The standard medium contained: 200 mM sucrose, 10 mM Hepes (pH 7.4), 10 μM $CaCl_2$, 5 mM succinate, 1.25 μM rotenone, 1 mM Na-phosphate, 3 mM ATP and 0.3 mM $MgCl_2$. Variations and/or other additions are given with the individual experiments presented.

2.3. Ca^{2+} fluxes measurement

Ca^{2+} fluxes were measured by the Ionetics Calcium STAT electrode. The Ca^{2+}-electrode signal was standardized against known values of free Ca^{2+} concentrations using Ca^{2+}/nitrilotriacetate buffers [8].

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2.4. Membrane potential measurement

Membrane potential was calculated on the basis of the movements of the lipid-soluble cation tetraphenylphosphonium (TPP^+) through the inner membrane. A specific electrode for TPP^+ was prepared according to Kamo *et al.* [9].

3. Results and discussion

As previously reported [10], MGBG is most likely taken up by isolated RLM by a mechanism similar to that of spermine [11]. This uptake is responsible for the dose-dependent inhibition of Ca^{2+} uptake by RLM incubated in the presence of protective agents as ATP and Mg^{2+} (Fig. 1A). As shown in the inset of the figure such an inhibition is not due to a decrease of transmembrane potential ($\Delta\Psi$), the driving force for the electrophoretic uptake of Ca^{2+}. Therefore, it may be assumed that MGBG inhibits Ca^{2+} influx by interreacting with either the uniporter responsible for the transmembrane transport of Ca^{2+}, or the binding sites of the cation on the external surface of inner mitochondrial membrane.

It is well known that Ca^{2+} accumulated in mitochondria matrix space can be released into the medium in an independent process. It has been therefore proposed that liver mitochondria achieve a steady state in which Ca^{2+} is continuously taken up by Ca^{2+} uniporter and released by an electroneutral efflux pathway most likely in exchange

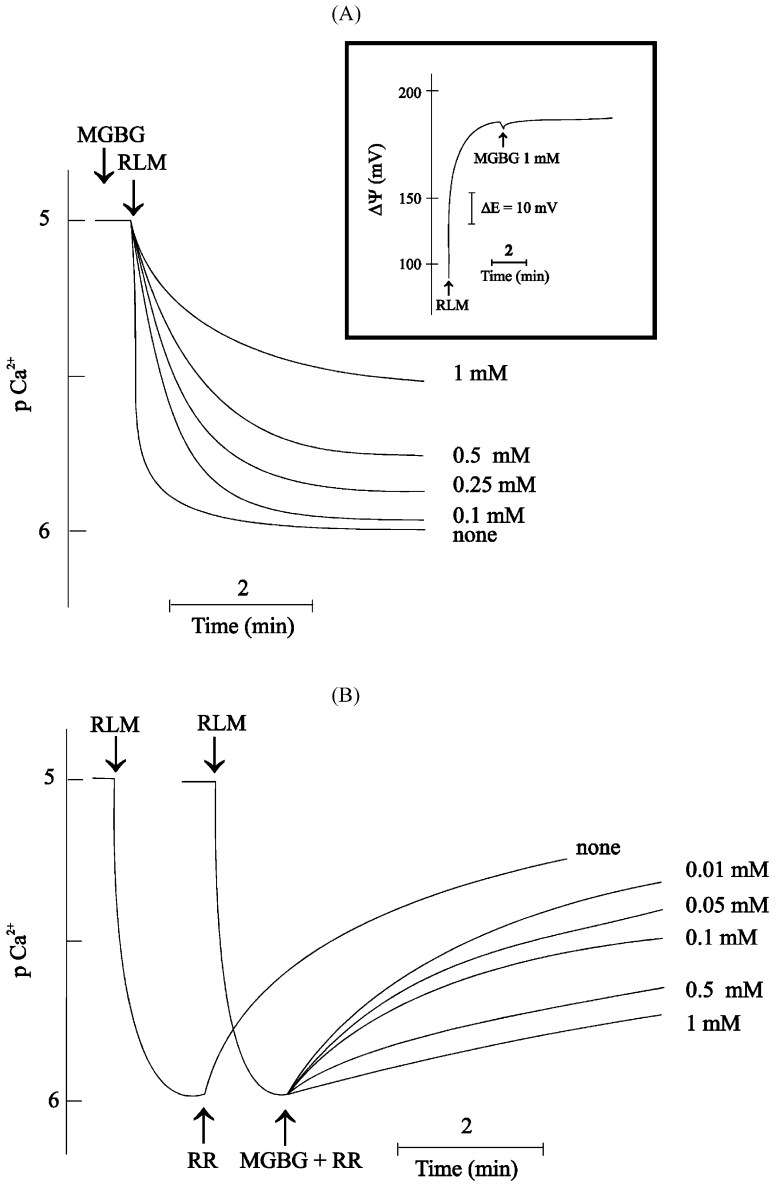

Fig. 1. Effect of MGBG on Ca^{2+} accumulation (A) and on ruthenium red-insensitive Ca^{2+} efflux (B). RLM were incubated as described in Section 2. MGBG was added at concentration indicated in the figure, and ruthenium red (RR) at 0.5 μM concentration. The inset shows the effect of MGBG on $\Delta\Psi$ of RLM incubated as mentioned above.

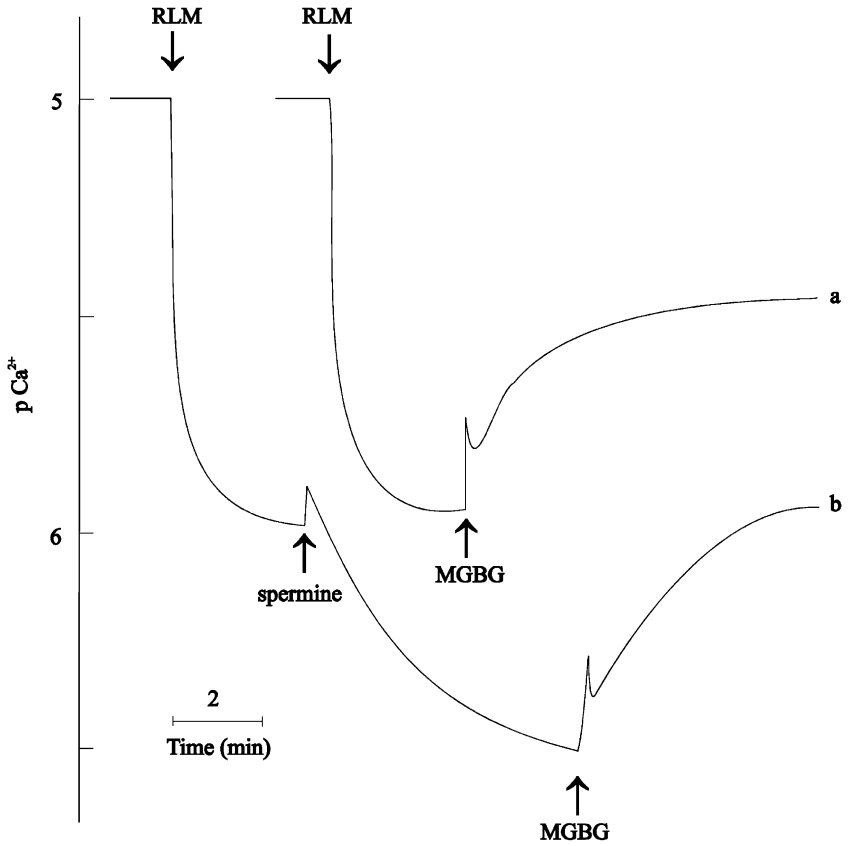

Fig. 2. Effect of MGBG on external Ca^{2+} steady state concentration. A comparison with spermine. RLM were incubated as described in Section 2. Where indicated 1 mM MGBG and 0.3 mM spermine were added.

with H^+ [12]. Owing to this energy dissipating cycle, Ca^{2+} efflux becomes evident when Ca^{2+} uptake is blocked by ruthenium red, an inhibitor of the uniporter [13].

As shown in Fig. 1B, when added to Ca^{2+}-loaded mitochondria, 1 mM MGBG strongly inhibits the efflux of the cation being made evident by ruthenium red addition. Due to the different experimental conditions used for measuring the rate of Ca^{2+} uptake and efflux, a comparison of the two rates is not allowed. However, as it appears in the results reported in Fig. 2 (curve a), addition of 1 mM MGBG to RLM immediately after the uptake of Ca^{2+}, results in a progressive increase of Ca^{2+} level in the suspending medium, indicating that MGBG inhibits Ca^{2+} uptake at the higher extent than Ca^{2+} efflux. Since steady state conditions are, of course, attained when the rates of uptake and efflux are equal [14] and considering that the rate of the uptake increases proportionally to the external Ca^{2+} concentration [15], it may be assumed that MGBG shifts the steady state to higher Ca^{2+} concentrations (from 1 to 3 μM). Fig. 2 also shows that an opposite result is obtained, as previously observed by Nicchitta and Williamson [16], if spermine is added at the place of MGBG. In this case, the steady state is shifted to a lower, and hence near to the physiological, Ca^{2+} concentration, about 0.2–0.3 μM (curve b). A subsequent addition of MGBG relocates the steady state to higher Ca^{2+} concentration, though at lower level then in the absence of spermine (from 0.3 to 1 μM) (curve b).

It has been proposed that spermine presents its effect by inducing an allosteric increase in affinity of Ca^{2+} for its uniporter [17–19] and a decrease in the cooperativity of uptake [18], with the result of an uptake inhibition, and by an inhibitory effect on the electroneutral efflux [17,19] of larger extent than the uptake. Spermine can bind to two specific binding sites, named S_1 and S_2, S_1 having a higher affinity and a lower capacity than S_2 [20]. However, at the concentration used to change the Ca^{2+} steady state, 0.3 mM, spermine binds only to S_1 site [20]. This interaction is responsible of the observed effects and demonstrates that S_1 site is strictly close to both the Ca^{2+} transport systems. It has been suggested that the binding site of MGBG is not the same as S_1 spermine binding site but these sites are strictly close on the same protein [21].

The opposite effect observed with MGBG could be the result of an allosteric mechanism as that of spermine which however exhibits a different inhibition on both the Ca^{2+} transporter.

The proximity of MGBG site to that of spermine would induce, upon MGBG binding, a perturbation capable of displacing most of bound spermine from S_1 and S_2 sites [21] with the result of reversing external Ca^{2+} steady state (Fig. 2). This conclusion is supported by the observation

that spermidine, which exhibit a lower binding capacity than spermine to S_1 site [22], is less effective than spermine in inducing the lowering of Ca^{2+} steady state [23], while putrescine, which binds only to S_2 site [22] is completely ineffective [23].

The substantial slowing down of Ca^{2+} cycling induced by MGBG should result in a lower dissipation of the proton-motive energy, as well as in reduced amount of matrix Ca^{2+} concentration. Both these effects, particularly the latter, may rationalize the protective action of MGBG on MPT induced by high Ca^{2+} concentrations and phosphate [7]. Although this protection can have beneficial effects on membrane structure and function, the recent observed inhibition by MGBG on the bidirectional fluxes of spermine across the inner membrane can compromise the regulation of energy metabolism and other processes of physiological importance [21].

The results reported in Fig. 2 demonstrating that MGBG induces a decrease in the matrix Ca^{2+} concentration during the steady state, further support the proposal about a deficiency in energy metabolism achieved in the presence of the drug. In this condition the activity of all the Ca^{2+}-dependent mitochondrial dehydrogenases (pyruvate dehydrogenase, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase) can result seriously affected.

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