Tissue-specific modulation of the mitochondrial calcium uniporter by magnesium ions

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This paper analyzes the kinetics of the Ca^{2+} uniporter of mitochondria from rat heart, kidney and liver operating in a range of Ca^{2+} concentrations near the steady-state value (1–4 μM). Heart mitochondria exhibit the lowest activity, and physiological Mg^{2+} concentrations inhibit the mitochondrial Ca^{2+} uniporter by approx. 50% in heart and kidney, and by 20% in liver. At physiological Ca^{2+} and Mg^{2+} concentrations the external free Ca^{2+} maintained by respiring mitochondria in vitro is higher in heart and kidney with respect to liver mitochondria. This behaviour could represent an adaptation of different mitochondria to their specific intracellular environment.

Mitochondrial calcium uptake *Magnesium effect* *Heart* *Kidney* *Liver* *Mitochondria*

1. INTRODUCTION

Calcium transport is a basic function of mitochondria from a wide variety of mammalian tissues [1,2]. It is well established that mitochondria possess two pathways for Ca^{2+} transport: (i) the Ca^{2+} uniporter, allowing passive Ca^{2+} equilibration along $\Delta\tilde{\mu}$ Ca; in the presence of an inside-negative membrane potential, this pathway mediates Ca^{2+} uptake, and can be reversed to catalyze Ca^{2+} efflux upon collapse of the membrane potential [3]; (ii) the putative Ca^{2+} antiporter, catalyzing Ca^{2+} efflux against $\Delta\tilde{\mu}$ Ca; this pathway, which keeps the Ca^{2+} accumulation ratio away from electrochemical equilibrium [4–6], does not operate at $\Delta\psi \leqslant 120$ mV [4,5], while its activity increases linearly with increase of $\Delta\psi$ above 120 mV [7].

Mg^{2+} is known to affect the kinetics of the Ca^{2+} uniporter [8–10] profoundly, inducing a clear sigmoidicity in the curve relating initial rate of Ca^{2+} uptake to external free Ca^{2+} concentration [11,12], and shifting the steady-state extramitochondrial free Ca^{2+} maintained by energized liver mitochondria to higher levels [5,13]. Here, we have characterized the effect of Mg^{2+} on the kinetics of the Ca^{2+} uniporter of rat liver, kidney and heart mitochondria operating in a range of Ca^{2+} concentrations close to the aerobic set point value (1–4 μM). We show that the Ca^{2+} uniporter of kidney and heart mitochondria is far more sensitive to Mg^{2+} inhibition than the Ca^{2+} uniporter of liver mitochondria. As a consequence, at physiological Mg^{2+} and Ca^{2+} concentrations, mitochondria from kidney and heart maintain steady-state extramitochondrial free Ca^{2+} at a higher level than liver mitochondria. This behaviour could represent an adaptation of different mitochondria to their specific intracellular environment.

Abbreviations: $\Delta\tilde{\mu}$Ca, Ca^{2+} electrochemical gradient; $\Delta\psi$, membrane potential; pCa_o and pMg_o, $-\log [Ca^{2+}]$ and $-\log [Mg^{2+}]$ outside the mitochondria

2. MATERIALS AND METHODS

2.1. *Preparation of mitochondria*

Rat liver and kidney mitochondria were prepared by standard centrifugation procedures in 0.25 M sucrose, 10 mM Tris-HCl, pH 7.4, 0.1 mM

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EGTA, the last washing being carried out in an EGTA-free medium. Rat heart mitochondria were prepared by a modification of the method in [14] in the same medium described above. Homogenization of the minced tissue was carried out with a Polytron PT-10 homogenizer (rheostat in position 4) by two cycles of 10 s with a pause of 30 s to allow cooling. After low-speed centrifugation of the homogenate, the supernatant was filtered and stored in an ice-water bath, while the pellet was resuspended in the same medium and homogenized again exactly as described above. After low-speed centrifugation the supernatant was filtered, pooled with the first supernatant and centrifuged for 10 min at 10000 × *g*. The pellet was washed once and resuspended in an EGTA-free medium. We found that this procedure increases the yield without altering the quality of the mitochondria, as confirmed by the respiratory control ratio of the mitochondria obtained from the first and the second homogenization.

2.2. *Assays*

Ca^{2+} movements were monitored with a Ca^{2+}-selective electrode (W. Möller, Zürich) exactly as described in [7]. The initial rate of Ca^{2+} influx was driven by the K^+ diffusion potential built up by the addition of excess valinomycin to non-respiring mitochondria [11,15]. In all experiments it was ascertained that the rate-limiting reaction was constituted by Ca^{2+} and not by K^+ transport. Oxygen consumption was monitored with a Clark oxygen electrode (Yellow Springs Instruments, Yellow Springs, OH) in a magnetically stirred, thermoequilibrated vessel. Mitochondrial protein was assayed with the biuret method, with bovine serum albumin as a standard. All chemicals were analytical grade.

3. RESULTS

Fig.1 analyzes the dependence of the initial rate of Ca^{2+} uptake on the external free Ca^{2+} concentration in heart, kidney and liver mitochondria in a range of Ca^{2+} concentrations near the steady-state value. Linear relationships are obtained, and the slopes are (nmol Ca^{2+} · mg protein^{-1} · min^{-1} · μM^{-1} Ca^{2+}): 1.61 in heart, 3.26 in kidney, and 3.43 in liver mitochondria in the absence of added Mg^{2+}, and 0.73 in heart, 1.35 in kidney, and 2.73 in liver mitochondria in the presence of 1.5 mM Mg^{2+}. Thus, physiological concentrations of Mg^{2+}

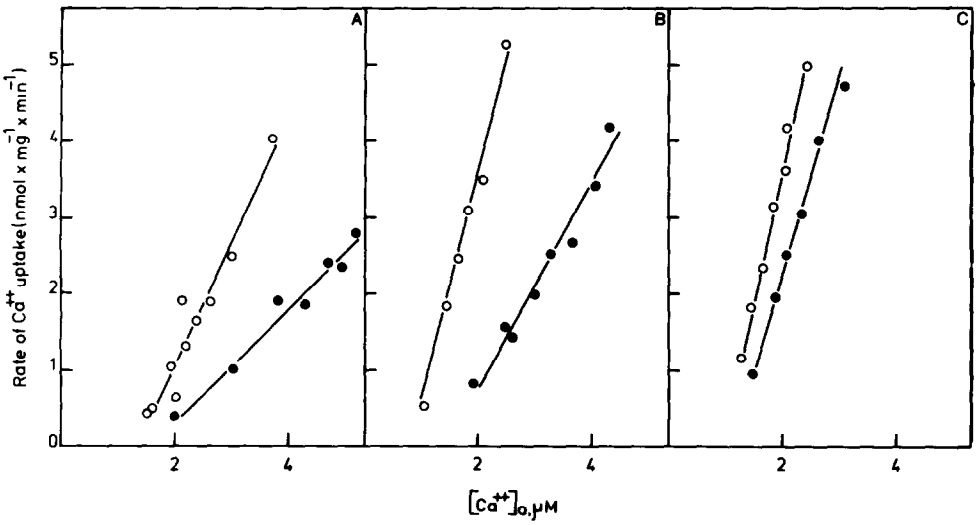

Fig.1. Rate of Ca^{2+} influx as a function of external free Ca^{2+} concentration. Effect of Mg^{2+}. The incubation medium contained 0.14 M sucrose, 40 mM choline/Cl, 10 mM Tris/Mops, pH 7.0, 0.5 mM P_i/Tris, 1 mg/ml BSA, 2 μM rotenone, 1 μg/ml oligomycin, 2 μg/ml valinomycin, 1 μg/ml antimycin A. (○—○) No added Mg^{2+}; (●—●) 1.5 mM Mg^{2+}. The indicated free Ca^{2+} concentrations were obtained by back titration with EGTA of contaminating Ca^{2+} (5 μM). Final volume 2 ml, 30°C. The experiment was started by the addition of 2 mg mitochondrial protein, and values on the ordinate refer to initial rate of Ca^{2+} uptake. (A) Heart, (B) kidney, (C) liver mitochondria.

inhibit the mitochondrial Ca^{2+} uniporter by 55% in heart, by 58% in kidney and by only 20.5% in liver mitochondria. The experimental slopes, obtained by linear regression analysis of the data, do not extrapolate to zero. One possible explanation is the simultaneous operation of the independent efflux pathway, which could lead to a slight underestimation of the actual initial rate of Ca^{2+} uptake. It must however be considered that the absolute values of extramitochondrial free Ca^{2+} are based on the electrode calibration with Ca^{2+} buffers [5,7], and that a minor inaccuracy in the affinity constants taken for Ca^{2+} and Mg^{2+} could be responsible for this observation.

Fig.2 shows that the pattern of uniporter inhibition by increasing Mg^{2+} concentrations is markedly different in mitochondria from different tissues, 50% inhibition being obtained at 1 mM Mg^{2+} in heart and kidney mitochondria, and at 2 mM Mg^{2+} in liver mitochondria. It is noteworthy that at 1 mM Mg^{2+} the uniporter of liver mitochondria is inhibited by 10% only.

Fig.3 analyzes the effect of increasing Mg^{2+} concentrations on the steady-state external free Ca^{2+} maintained by respiring heart, kidney and liver mitochondria in vitro. In keeping with the data obtained from the initial rate experiments, the shift induced by Mg^{2+} is more pronounced in heart and

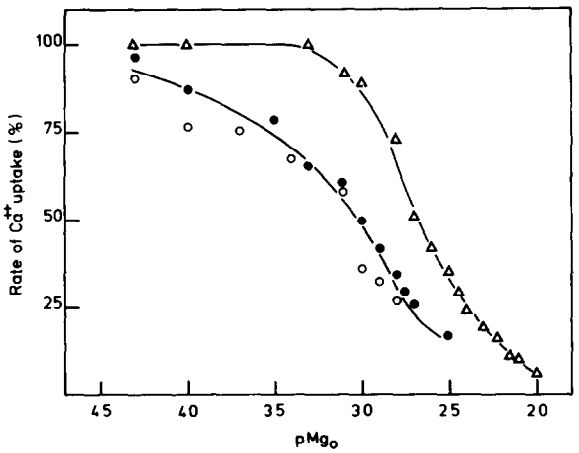

Fig.2. Rate of Ca^{2+} influx as a function of pMg_o. Experimental conditions as in fig.1 with 7.5 μM free Ca^{2+} and Mg^{2+} as indicated. Values on the ordinate refer to initial rate of Ca^{2+} uptake in %, where 100% is the rate in the absence of added Mg^{2+}. (○—○) Heart, (●—●) kidney, (△—△) liver mitochondria.

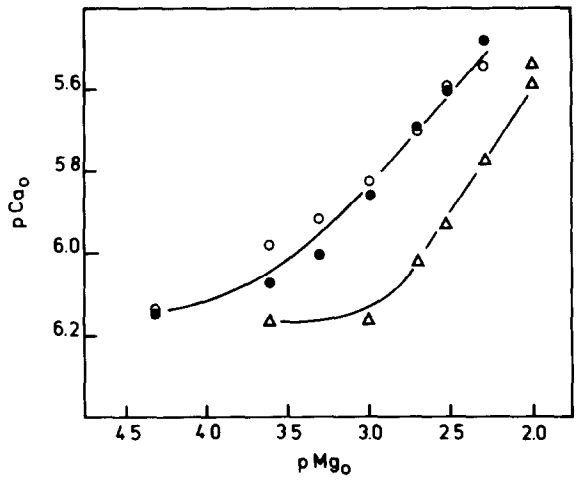

Fig.3. Effect of Mg^{2+} on steady-state pCa_o. The incubation medium was the same as in fig.1, plus 5 mM succinate/Tris. Valinomycin and antimycin were omitted, 3 μM free Ca^{2+} and Mg^{2+} as indicated. Final volume 2 ml, 30°C. The experiment was started by the addition of 2 mg mitochondrial protein, and values on the ordinate refer to pCa_o at steady state. (○—○) Heart, (●—●) kidney, (△—△) liver mitochondria.

kidney as compared to liver mitochondria. In the absence of Mg^{2+}, all types of mitochondria maintain a pCa_o of 6.2 (0.63 μM). In the presence of 1 mM Mg^{2+}, this value is almost unchanged in the case of liver mitochondria, while it is shifted to a pCa_o of 5.8 (1.6 μM) in the case of heart and kidney mitochondria. The rate of Ca^{2+} uptake is higher in kidney than in heart mitochondria, both in the absence and in the presence of Mg^{2+} (fig.1). Thus, the maintenance of the same steady-state pCa_o under aerobic conditions at increasing $[Mg^{2+}]_o$ is surprising. This apparent inconsistency is due to the fact that the rate of Ca^{2+} efflux on the independent pathway is higher in kidney than in heart mitochondria (M. Favaron, unpublished).

4. DISCUSSION

In energized mitochondria at steady state, Ca^{2+} is taken up by the uniporter and released by the putative antiporter, the rate of cycling being low [16] due to the low operation rate of the efflux pathway. While the uniporter activity is sharply dependent on extramitochondrial free Ca^{2+} concentration [11,12,14,17,18], the activity of the efflux pathway in the presence of physiological con-

centrations of P_i is largely independent of the amount of Ca^{2+} taken up [19]. Thus, if the extramitochondrial free Ca^{2+} maintained at steady state by energized mitochondria in vitro is suddenly raised by one order of magnitude (e.g., from 10^{-6} to 10^{-5} M), it takes only a few seconds to regain the steady state, due to the very fast Ca^{2+} uptake on the uniporter. If, on the other hand, the change of extramitochondrial free Ca^{2+} is in the opposite direction (e.g., from 10^{-6} to 10^{-7} M), the time required to regain the steady state is longer, due to the slow kinetics of the efflux pathway. Although the pCa_o maintained by respiring mitochondria in vitro corresponds to a free Ca^{2+} concentration which is higher than the resting level of most cell types tested so far [20], a large number of physiological stimuli raise intracellular free Ca^{2+} to levels at which mitochondrial buffering can be predicted to take place [20]. Thus, a major problem of mitochondria in vivo could be the prevention of excessive Ca^{2+} accumulation. It seems conceivable that cells subjected to high Ca^{2+} traffic (such as kidney cells) or to cyclic increase of intracellular free Ca^{2+} (such as cardiac cells) have developed this regulatory ability to a higher extent.

To our knowledge, this report is the first indication that a tissue-specific modulation can be exerted at the level of the uniporter. Our data indicate that: (i) in the absence of added Mg^{2+}, an increase of 1 μM free Ca^{2+} above the mitochondrial set point brings about an increase in Ca^{2+} uptake rate of (nmol Ca^{2+} · mg protein^{-1} · min^{-1}): 1.61 in heart, 3.26 in kidney and 3.43 in liver mitochondria. Thus, at physiological Ca^{2+} concentrations, the uniporter of heart mitochondria exhibits the lowest activity; (ii) in the presence of physiological Mg^{2+} concentrations, the mitochondrial set point for Ca^{2+} buffering is higher in heart and kidney than in liver mitochondria. If it is permissible to extrapolate these data to mitochondria in vivo, it could be predicted that mitochondrial Ca^{2+} uptake will not take place until cytosolic free Ca^{2+} is raised to 1.5–2 μM in heart and kidney, while it will already occur at 0.5–1 μM in liver cells; (iii) in the presence of 1.5 mM Mg^{2+}, an increase of 1 μM free Ca^{2+} above the mitochondrial set point brings about an increase in uptake rate of (nmol · mg protein^{-1} · min^{-1}): 0.73 in heart, 1.35 in kidney and 2.73 in liver mitochondria. Thus, the affinity for Mg^{2+} of the putative regulatory site(s) of the mitochondrial uniporter is higher in heart and kidney with respect to liver mitochondria. It must be observed that intracellular free Mg^{2+} concentrations measured so far by different techniques are rather close to those employed here, ranging from 0.6 mM in frog skeletal muscle [21] to 1 mM in rat liver, kidney, and brain [22], pig and mouse lymphocytes [23] and Ehrlich and Yoshida tumour cells [24], and to 2.5 mM in intact perfused guinea pig heart [25]. Taken together, these data support the view that mitochondria from different tissues have adapted their Ca^{2+} transport systems to their specific intracellular environment.

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