Modulation of Oxidative Phosphorylation by \( \text{Mg}^{2+} \) in Rat Heart Mitochondria

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The effect of varying the \( \text{Mg}^{2+} \) concentration on the 2-oxoglutarate dehydrogenase (2-OGDH) activity and the rate of oxidative phosphorylation of rat heart mitochondria was studied. The ionophore A23187 was used to modify the mitochondrial free \( \text{Mg}^{2+} \) concentration. Half-maximal stimulation \( (K_{0.5}) \) of ATP synthesis by \( \text{Mg}^{2+} \) was obtained with 0.13 ± 0.02 mM \( (n = 7) \) with succinate (+rotenone) and 0.48 ± 0.13 mM \( (n = 6) \) with 2-oxoglutarate (2-OG) as substrates. Similar \( K_{0.5} \) values were found for NAD(P)H formation, generation of membrane potential, and state 4 respiration with 2-OG. In the presence of ADP, an increase in \( P_i \) concentration promoted a decrease in the \( K_{0.5} \) values of ATP synthesis, membrane potential formation and state 4 respiration for \( \text{Mg}^{2+} \) with 2-OG, but not with succinate. These results indicate that 2-OGDH is the main step of oxidative phosphorylation modulated by \( \text{Mg}^{2+} \) when 2-OG is the oxidizable substrate; with succinate, the ATP synthase is the \( \text{Mg}^{2+} \)-sensitive step. Replacement of \( P_i \) by acetate, which promotes changes in intramitochondrial pH abolished \( \text{Mg}^{2+} \) activation of 2-OGDH. Thus, the modulation of the 2-OGDH activity by \( \text{Mg}^{2+} \) has an essential requirement for \( \text{P}_i \) (and ADP) in intact mitochondria which is not associated to variations in matrix pH.

The notion that the cytosolic concentration of free \( \text{Mg}^{2+} \) \([\text{Mg}^{2+}]_c\) had a constant value around 1 mM under different conditions has changed in recent years. By using permeant fluorescent dyes and nuclear magnetic resonance, tissue-dependent variations in \([\text{Mg}^{2+}]_c\) in the range of 0.4 to 0.8 mM have been observed, in response to several hormones and agonists. For instance, norepinephrine induced a net release of cellular \( \text{Mg}^{2+} \) (1), while vasopressin induced \( \text{Mg}^{2+} \) accumulation in isolated hepatocytes (2). Likewise, increments of 50% in \([\text{Mg}^{2+}]_c\) have been determined, after stimulation with the muscarinic agonist carbachol, and a 10% increase was observed, after addition of forskolin in rat sublingual mucous acini (3). In acinar pancreatic cells, addition of acetylcholine or cholceystokinin-octapeptide promoted a significant diminution in \([\text{Mg}^{2+}]_c\) (4). Arginine-vasopressin and endothelin-1 induced an increment in \([\text{Mg}^{2+}]_c\) in muscle cells, probably through a \( \text{Ca}^{2+} \)-mediated mechanism (5). Depletion of inositol 1,4,5-trisphosphate-sensitive \( \text{Ca}^{2+} \) stores, induced by \( \alpha \)-adrenergic agonists, activated the uptake of \( \text{Mg}^{2+} \) by these organelles (6).

Extracellular ATP stimulated the release of 10% of cellular \( \text{Mg}^{2+} \) in ascertes cells (7); it was proposed that cAMP promoted \( \text{Mg}^{2+} \) release through the activation of a plasma membrane Na'/\( \text{Mg}^{2+} \) antporter (8). However, a report indicating that addition of cAMP also induced a net release of 20–25% of total \( \text{Mg}^{2+} \) in rat liver mitochondria (9) was not confirmed (10). In beef heart mitochondria, the transition from basal (state 4) to active (state 3) respiration led to a small, but significant elevation in the mitochondrial matrix free \( \text{Mg}^{2+} \) concentration \([\text{Mg}^{2+}]_m\) from 0.5 mM to 0.6–0.7 mM. This increase in \([\text{Mg}^{2+}]_m\) persisted during ATP synthesis, until added ADP was exhausted; at this time \([\text{Mg}^{2+}]_m\) returned to basal levels. These variations in \([\text{Mg}^{2+}]_m\) were inhibited by oligomycin (11). An elevation in \([\text{Mg}^{2+}]_m\) from 44 \( \mu \)M to 1.69 mM also induced a stimulation in the rate of citrulline synthesis in rat liver mitochondria (12). Modulation of mitochondrial glutaminase by 0–2 mM \( \text{Mg}^{2+} \) has also been observed (13).

All of these reports describing an active movement of \( \text{Mg}^{2+} \) in cells and mitochondria of different tissues and in response to different agonists suggest that \( \text{Mg}^{2+} \) may play a role as a second messenger in the cell. In this work, we show that variations of external \( \text{Mg}^{2+} \), and hence in \([\text{Mg}^{2+}]_m\), can modulate the activities of the 2-ODGH and the ATP synthase and, in consequence, \( \text{Mg}^{2+} \) may affect the rate of oxidative phosphorylation in isolated rat heart mitochondria.

MATERIALS AND METHODS

Rat heart mitochondria were isolated from male Wistar rats of 250–300-g weight according to a previously described method using the protease type XXVII (Nagarse) from Sigma (14).

Dye Loading—Heart mitochondria were loaded with Mag-Fura-2 or BCECF (Molecular Probes) by incubating 30–40 mg of mitochondrial protein in 2 ml of a medium composed of 250 mM sucrose, 10 mM MOPS, 0.5 mM EGTA, 1 mM MgCl\(_2\), 1 mM ADP, 0.2% fatty acid-free bovine serum albumin, pH 7.4, and 5 \( \mu \)M Mag-Fura-2/AM or BCECF/AM at 25 °C for 20 min. At the end of this incubation period, mitochondria were diluted 10–15 times with ice-cold SHE medium + 0.2% bovine serum albumin, centrifuged, resuspended in 1 ml of SHE medium, and kept on ice until use. Mitochondria loaded by following this procedure showed higher respiratory control values than non-loaded mitochondria, 8.6 and 4.3 \( (n = 2) \), respectively, with 10 mM 2-oxoglutarate as a substrate.

Determination of \([\text{Mg}^{2+}]_m\)—Mag-Fura-2-loaded mitochondria (0.5 mg protein/ml) were incubated in 120 mM KCl, 20 mM MOPS, 0.5 mM EGTA (KME medium), 5 mM succinate, and 2 \( \mu \)M rotenone, at pH 7.25 and 30 °C. To avoid interference by matrix NAD(P)H fluorescence, 2-oxoglutarate was not used as an oxidizable substrate for determinations of \([\text{Mg}^{2+}]_m\). Fluorescence changes were monitored under smooth stirring and gassing with 100% O\(_2\) in an Aminco Bowman Series 2 spectrofluorometer. Excitation wavelengths were 340 and 388 nm and emission was collected at 483 nm. \([\text{Mg}^{2+}]_m\) was determined from the fluorescence ratio signal \( (R, 398/340 \text{ nm}) \). \( R_{\text{max}} \) and \( R_{\text{min}} \) were obtained at the end of each experiment. \( R_{\text{max}} \) was generated by addition of 800 pmol of A23187/mg protein and sufficient EDTA-Tris, pH 8.0, to chelate all the

\[ \text{Mg}^{2+} \]
Fig. 1. Mitochondrial Mg2+ gradient in the presence and in the absence of A23187. Mag-Fura-2-loaded mitochondria (0.5 mg/ml) were incubated as described under “Materials and Methods” in the presence of 10 mM NaCl, 5 mM P2, and the indicated Mg2+ concentrations; the matrix content of Mag-Fura-2 was estimated to be 150 ± 30 pmol/mg of protein (n = 5). Calibration of the fluorescence ratio signal was performed and [Mg2+]i was calculated as described under “Materials and Methods.” The bandwidths were 4 nm for both excitation and emission wavelengths, while the sensitivity was set at 500 V. The results represent the mean ± S.D. from three different mitochondrial preparations. The inset shows the determination of intramitochondrial ATP and ADP of five different mitochondrial preparations incubated for 10 min with the indicated concentrations of MgCl2 and in the presence or in the absence of 800 pmol of A23187/mg of protein. **p < 0.05, *p < 0.01; p < 0.005 (Student’s t test for paired samples).

Mg2+ present in the incubation medium; 0.005% (v/v) Triton X-100 was added to ensure complete Mg2+ equilibration across the membrane. Rmax was obtained after further addition of 70 mM MgCl2. Calculation of [Mg2+]i was made using the following equation (15):

\[
[Mg^{2+}] = K_{d[Mg^{2+}]} \frac{(R - R_{max})}{S_i - S_f},
\]

where \(K_{d[Mg^{2+}]}\) is the dissociation constant for the Mg2+-dye complex in the mitochondrial matrix and \(S_i\) and \(S_f\) are the dye fluorescence intensities at 398 nm with zero and excess Mg2+, respectively. The \(K_{d[Mg^{2+}]}\) value was determined experimentally to be 1.52 ± 0.18 pm (n = 5).

**pH Determination**—BCECF-loaded mitochondria (0.5 mg of protein/ml) were incubated in KME medium containing 0.5 mM 2-oxoglutarate, 10 mM NaCl, 600 μM ADP, 3.5 μM oligomycin, 800 pmol of A23187/mg of protein and different concentrations of Mg2+, or protein. For pH calculations, a calibration plot was generated incubating 0.5 mg of protein/ml in the medium mentioned above, at the desired pH, in the presence of 2 μM carbonyl cyanide m-chlorophenylhydrazone, 200 pmol of nigericin/mg of protein and 0.005% Triton X-100 to equilibrate all ion gradients. Excitation wavelengths were 450 and 500 nm; fluorescence was collected at 530 nm. The plot of pH values versus fluorescence ratio signal gives a straight line between pH 6.8 and 7.8.

**ATP Synthesis**—Mitochondria (1 mg of protein/ml) were incubated in KME medium containing 0.5 mM 2-oxoglutarate or 5 mM succinate (+1 μM rotenone), 10 mM NaCl, 10 mM glucose, 30 units of hexokinase, and 5 mM 32Pi (specific activity, 1–1.5 × 10⁶ cpm/ml, Cerenkov radiation), at 30 °C. After 5 min, 1.2 mM ADP was added, and the reaction was stopped 30 s later by addition of 200 μl of 30% (v/v) cold trichloroacetic acid. Excess 32Pi was extracted as described previously using acetone.

**Activity of 2-OGDH**—Mitochondria (1 mg of protein/ml) were suspended in KME medium containing 0.5 mM 2-oxoglutarate, 10 mM NaCl, 600 μM ADP, pH 7.25, and different concentrations of Mg2+ and P2 at 30 °C. Matrix NAD(P)H formed was determined following mitochondrial incubation with the excitation wavelength at 340 nm. To obtain the fluorescence minimum, mitochondria were incubated in the absence of added substrates until endogenous substrates were depleted (approximately 5–8 min) (NADP(H)/H+ = 0%); the fluorescence maximum was reached by adding 5 μM rotenone for complete reduction of NADP(H) (NADP(H)/H+ = 100%) at the end of each experiment.

Membrane Potential (Δϕ)—Mitochondria (1 mg protein/ml) were suspended in KME medium containing 0.5 mM 2-oxoglutarate, 10 mM NaCl, 5 μM safranine O, at 30 °C. Absorbance was recorded at 554 ± 520 nm (17, 18), using a dual-beam SLM Aminco DW2000 spectrophotometer. Zero Δϕ was reached by addition of 1 μM carbonyl cyanide m-chlorophenylhydrazone and 2 μM rotenone at the end of each experiment.

The membrane potential was also quantitatively measured using the distribution of [³H]TPP. Mitochondria (1.5 mg protein/ml) were suspended in 500 μl of KME medium containing 5 mM P2, 10 mM NaCl, 0.8 μM [³H]TPP (specific activity, 4–5 × 10⁶ cpm/ml) at 30 °C and different concentrations of Mg2+. After 5 min, 800 pmol of A23187/mg of protein were added; 3 min later 1 mM 2-oxoglutarate was added, and the incubation was continued for another 3 min. Then, mitochondria were centrifuged at 14,000 rpm for 1 min in a microcentrifuge. Aliquots from the pellet and supernatant were taken to measure the [³H]TPP distribution; the membrane potential was determined as described previously (19).

**Oximetry Assays**—Mitochondrial respiration was measured utilizing an oxygen Clark-type electrode. Mitochondria (0.6 mg of protein/ml) were incubated in KME medium containing 1 mM 2-oxoglutarate, 10 mM NaCl, 1 or 5 mM P2, and 800 pmol of A23187/mg of protein. After 5 min, 600 μM ADP was added, and the change in the rate of respiration was measured.

**Matrix ATP and ADP Content**—Mitochondria (2.5 mg of protein/ml) were incubated in KME medium plus 5 mM succinate and 2 μM rotenone at 30 °C for 10 min under orbital shaking. Then, 3% (v/v) cold perchloric acid, 25 mM EDTA was added, the suspension was centrifuged, and the supernatant neutralized for enzymatic determination of ATP and ADP. Essentially identical results were obtained when mitochondria were previously sedimented in a microcentrifuge at 6–10 °C and further denaturalized by the addition of perchloric acid.

**RESULTS**

The increase in the external Mg2+ concentration induced a proportional, but small elevation in [Mg2+]i in rat heart mitochondria (Fig. 1). This Mg2+ gradient ([Mg2+]i/[Mg2+]ex) showed a slope of 0.066, in the range 0–3 mM externally added Mg2+, indicating that Mg2+ does not easily equilibrate across the mitochondrial inner membrane, probably due to a slow Mg2+ influx, or to an active Mg2+ efflux. Similar results were previously reported for rat liver mitochondria (12). To accelerate the equilibration of Mg2+, the divalent cation ionophore A23187 was added. Fig. 1 shows that the ionophore modifies the steady-state concentration of matrix Mg2+, although equil-

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Fig. 2. Activation of ATP synthesis by Mg$^{2+}$. Mitochondria were incubated as described under "Materials and Methods" with the indicated Mg$^{2+}$ concentrations, in the presence of 800 pmol of A23187/mg of protein; 1200 μM ADP was added to initiate the reaction. The plots are representative, $K_{0.5}$ values in millimoles are mean ± S.D. from seven (for 5 mM succinate, succ) and six (for 0.5 mM 2-OG) different preparations. The solid lines represent the best fit lines derived from the Hill equation. The $V_{max}$ values obtained from such a fitting are in nanomoles/(mg × min). The rate of ATP synthesis attained in the absence of added Mg$^{2+}$ was 250 nmol/(min × mg) with succinate and 100 nmol/(min × mg) with 2-OG, and it was subtracted from the rates obtained in the presence of Mg$^{2+}$. The inset shows the determination of substrate level phosphorylation under identical experimental conditions to those used for the ATP synthesis assay, but in the presence of a saturating concentration of oligomycin (3 μM).

The rate of oxidative phosphorylation, assayed in the presence of 3 mM Mg$^{2+}$ and with or without A23187, prompted us to determine the Mg$^{2+}$ dependence of hexokinase. Under the conditions of ATP synthesis (see Fig. 2), $K_{0.5}$ values for Mn$^{2+}$ were 0.60 ± 0.047 mM (n = 3) with succinate and 0.92 ± 0.052 mM (n = 3) with 2-OG as a substrate.

Since succinyl-CoA synthase also requires Mg$^{2+}$, its contribution to the uptake of $^{32}$P, was assayed. In the inset of Fig. 2, it is shown that substrate level phosphorylation by the Krebs cycle accounted for up to 40–50% of total ATP synthesis during oxidative phosphorylation with 2-OG as an oxidizable substrate. As substrate-level phosphorylation and oxidative phosphorylation with 2-OG showed different sensitivities to Mg$^{2+}$, an effect of Mg$^{2+}$ on sites different from succinyl-CoA synthase seemed likely.

To discard the participation of contaminating ATPases, in the ATP synthesis assays, hexokinase + glucose was used to capture ATP generated by oxidative phosphorylation. This prompted us to determine the Mg$^{2+}$ dependence of hexokinase. Under the conditions of ATP synthesis (see Fig. 2), $K_{0.5}$ values of hexokinase for ATP-Mg were 92 ± 6 μM in the presence of 100 μM ATP, and 31 μM in the presence of 100 μM ATP. These two concentrations of added ATP represent the maximal level of ATP synthesis (for 1 mg of protein/ml in 30 s at 30 °C) during oxidative phosphorylation with succinate and 2-OG, respectively. The sensitivity of hexokinase to Mg$^{2+}$ revealed that this enzyme is not involved in the lower sensitivity of oxidative phosphorylation to Mg$^{2+}$ with 2-OG as a substrate (see Fig. 2). However, in the presence of succinate, the sensitivity of oxidative phosphorylation to Mg$^{2+}$ might result from a mixed response of both hexokinase and ATP synthase to Mg$^{2+}$. However, an essentially identical sensitivity of oxidative phosphorylation to Mg$^{2+}$ was observed in the absence of hex-
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okase (\(K_{0.5} = 0.13 \pm 0.014 \text{ mm}, n = 3\)), with succinate (+rotenone).

The change in the magnitude of the membrane potential, as estimated from the distribution of TPP\(^+\), was initially used to monitor indirectly variations in 2-OGDH activity in mito-

chondria incubated with limiting concentrations of 2-OG (Fig. 3A). A membrane potential (\(\Delta\psi\)) of 130 mV in the absence of added ADP and Mg\(^{2+}\) and in the presence of A23187 and Pi, was determined. This value increased to 140 mV by the increase in

\[ [\text{Mg}^{2+}]_{\text{ex}} \]

(Fig. 3A, circles). With 600 \(\mu\)M ADP, steady state \(\Delta\psi\) diminished to 112 mV by increasing \([\text{Mg}^{2+}]_{\text{ex}}\) (Fig. 3A, squares), due to stimulation of ATP synthesis. Although under these last conditions the oxidative system was activated by

Mg\(^{2+}\), the diminution in \(\Delta\psi\) indicated that, at Mg\(^{2+}\) concentrations of 0.15 mM, activation of the phosphorylating system of the pathway by Mg\(^{2+}\) was predominant. At higher Mg\(^{2+}\) concentrations (>1.5 mM), activation of the oxidative system prevailed over that of the phosphorylating system, resulting in \(\Delta\psi\) values larger than those obtained at zero Mg\(^{2+}\) (data not shown).

The enhancement of \(\Delta\psi\) up to 162 mV by increasing \([\text{Mg}^{2+}]_{\text{ex}}\), in the presence of ADP + Pi + oligomycin (Fig. 3A, triangles), which was larger than that reached at the same concentration of \([\text{Mg}^{2+}]_{\text{ex}}\) in the absence of ADP, indicated that ADP was a modulator of the Mg\(^{2+}\) activation. In the absence of added Mg\(^{2+}\), removal of Pi markedly diminished \(\Delta\psi\) (Fig. 3A, diamonds). In the absence of Pi, \(\Delta\psi\) increased when \([\text{Mg}^{2+}]_{\text{ex}}\) was increased, but only to 105 mV. This latter observation prompted us to determine the effect of different concentrations of Pi on the activity of 2-OGDH.

In comparison to the \([\text{H}]\text{TPP}^+\) method, the absorbance difference of safranin O (Fig. 3B) allows for continuous monitoring of \(\Delta\psi\) and a large number of experiments with the same mitochondrial preparation. Using the safranin O signal, the increase in Pi concentration (in the presence of ADP + oligomycin) potentiated the activating effect of Mg\(^{2+}\) on the steady state value of \(\Delta\psi\) (Fig. 3B). Thus, the half-maximal stimulation of \(\Delta\psi\) by Mg\(^{2+}\) was decreased and the maximal value of \(\Delta\psi\) was elevated by increasing Pi concentrations. This effect of Pi was not apparent in the absence of added ADP. Similar results to those of Fig. 3B were obtained by measuring the \([\text{H}]\text{TPP}^+\) distribution under the same conditions (data not shown).

The activity of 2-OGDH was also measured, following the level of reduction of matrix pyridine nucleotides. In the absence of added Pi, the increase in \([\text{Mg}^{2+}]_{\text{ex}}\) did not promote the generation of NAD(P)H (Fig. 4A). However, an increase in Pi concentration induced both a decrease in the \(K_{0.5}\) value for Mg\(^{2+}\) and an increase in the level of NAD(P)H reduction. The rate of respiration measured in the presence of ADP + oligomycin (state 4) was also stimulated by increasing \([\text{Mg}^{2+}]_{\text{ex}}\) (Fig. 4B). Again, the presence of increasing Pi concentrations potentiated the stimulation by Mg\(^{2+}\), through a diminution in the \(K_{0.5}\) value for Mg\(^{2+}\) and an increase in the maximal rate of respiration. Thus, the data of Figs. 3 and 4 indicate that Pi, in the presence of ADP, potentiates the activating effect of Mg\(^{2+}\) on 2-OGDH activity.

The study of the effect of different Pi concentrations on the Mg\(^{2+}\) sensitivity of ATP synthesis and state 3 respiration supported by succinate (+rotenone) revealed a negligible effect on the \(K_{0.5}\) value for Mg\(^{2+}\), indicating that the effect of Pi was exerted only at the Krebs cycle level. Lack of Mg\(^{2+}\) activation on state 4 respiration with succinate (+rotenone) as substrate and oligomycin (data not shown), discarded the possibility that Mg\(^{2+}\) activated the respiratory chain.

Matrix acidification brought about by the Pi uptake might be involved in Mg\(^{2+}\) activation of 2-OGDH. The activating effect of 5 mM Pi on the stimulation of matrix NAD(P)H formation by Mg\(^{2+}\) was not reproduced by addition of 10 or 20 mM acetate (data not shown); the final steady-state pH values in BCECF-loaded mitochondria incubated with 10 mM acetate or 5 mM Pi, in the presence of ADP, oligomycin, and A23187, were 6.91 and 6.88 with no added Mg\(^{2+}\), and 7.11 and 7.16 with 1 mM Mg\(^{2+}\), respectively. These results indicate that matrix acidification is not the mechanism involved in the Pi potentiating effect.
The ATP synthase prevailed over Mg\(^{2+}\) exchanges with matrix ATP. As ATP has a higher affinity for free ADP and ATP (25). During ATP synthesis, external ADP and ATP might also perturb matrix Mg\(^{2+}\) adenine nucleotide translocase activity through the diminution of the internal substrate. Therefore, the effect of increasing [Mg\(^{2+}\)]\(_{\text{int}}\) on the rate of oxidative phosphorylation is not readily apparent. As the elevation of external Mg\(^{2+}\), and hence [Mg\(^{2+}\)]\(_{\text{int}}\), resulted in higher rates of ATP synthesis with succinate as substrate, it can be assumed that Mg\(^{2+}\) activation of the ATP synthase prevailed over Mg\(^{2+}\) inhibition of the adenine nucleotide translocase. Stimulation of the rate of oxidative arsenylation, an analogous process to oxidative phosphorylation, but without the participation of adenine nucleotide translocase (16), by Mg\(^{2+}\) using succinate (data not shown), supported the interpretation of an activating effect of Mg\(^{2+}\) on the ATP synthase.

In addition to a direct interaction of Mg\(^{2+}\) with the oxidative phosphorylation enzymes, Mg\(^{2+}\) might also perturb matrix Ca\(^{2+}\) homeostasis, and hence, affect the rate of ATP synthesis (16, 19, 26) (reviewed in Moreno-Sánchez and Torres-Márquez (27)). For instance, Mg\(^{2+}\) might compete with Ca\(^{2+}\) for the same binding sites in 2-OGDH. A decreased Ca\(^{2+}\) sensitivity by increasing Mg\(^{2+}\) has been observed for the NAD\(^{-}\)-isocitrate dehydrogenase (28), whereas an enhanced Ca\(^{2+}\) sensitivity was described for the pyruvate dehydrogenase phosphatase (29). Although the sensitivity of 2-OGDH to Ca\(^{2+}\), at different Mg\(^{2+}\) concentrations, has not yet been determined, Panov and Scarpa (30) reported that 2-OGDH can be activated synergistically by both Mg\(^{2+}\) and Ca\(^{2+}\), implying the existence of different binding sites.

Panov and Scarpa (30) also determined a dissociation constant (K\(_D\)) for Mg\(^{2+}\) of 25 \(\mu\)M in the isolated 2-OGDH, with saturating concentrations of thiamine pyrophosphate, coenzyme A, and NAD\(^{+}\). Although such a K\(_D\) value for Mg\(^{2+}\) is lower than the K\(_D\) value obtained in this study (0.48 \(\mu\)M, see Fig. 2), it can be argued that the matrix concentrations of the 2-OGDH coenzymes in intact heart mitochondria may be limiting, and that 2-OGDH activity is not the only controlling step of the pathway (25). The value of the K\(_D\) or K\(_{a}\) for Mg\(^{2+}\) may establish the physiological relevance of variations in [Mg\(^{2+}\)]\(_{\text{int}}\). Thus, a K\(_{a}\) value of 0.48 \(\mu\)M would appear as more physiologically relevant for modulating 2-OGDH activity and the rate of oxidative phosphorylation, since this concentration is in the range of [Mg\(^{2+}\)]\(_{\text{int}}\) in intact mitochondria (31, 32).

It should be noted, however, that the estimated K\(_{a}\) values for Mg\(^{2+}\) refers to the external Mg\(^{2+}\) concentrations, which were not fully equilibrated with the mitochondrial matrix by A23187 (cf. Fig. 1). Thus, the K\(_{a}\) value of 0.48 \(\mu\)M for external Mg\(^{2+}\) corresponds to a [Mg\(^{2+}\)]\(_{\text{int}}\) of 140 \(\mu\)M, which is slightly below the physiological range. Higher K\(_{a}\) values for Mg\(^{2+}\) were determined at low P\(_i\) concentrations. For instance, a K\(_{a}\) value of 1 \(\mu\)M for Mg\(^{2+}\) was observed in NAD(P)H formation with 1 \(\mu\)M P\(_i\) (see Fig. 4A). Such a K\(_{a}\) value was diminished to 0.5 \(\mu\)M by increasing P\(_i\) concentration up to 3 \(\mu\)M P\(_i\). The corresponding [Mg\(^{2+}\)]\(_{\text{int}}\) for 1 \(\mu\)M external Mg\(^{2+}\) would be 350 \(\mu\)M, a value well within the physiological range. A variation in the cytosolic P\(_i\) concentration from 0.83 to 3.1 \(\mu\)M induced by epinephrine was established in rat heart (33). Therefore, physiological modulation of the 2-OGDH activity by Mg\(^{2+}\) may depend on the level of cytosolic (and matrix) P\(_i\).

Other possible sites of modulation by Mg\(^{2+}\) during oxidative phosphorylation supported by 2-OG oxidation were the succinyl-CoA synthase, the ATP synthase and hexokinase (in the experiments of 32P incorporation into ATP). However, the Mg\(^{2+}\) sensitivity of these three enzymes showed that their saturation by Mg\(^{2+}\) was fully achieved at concentrations (<0.2–0.3 \(\mu\)M) that stimulated oxidative phosphorylation by less than 40%. The lack of stimulation of state 4 respiration by Mg\(^{2+}\) in mitochondria that oxidized succinate, in the presence of oligomycin, discarded an effect of Mg\(^{2+}\) at the level of the respiratory chain. Thus, these results indicate that 2-OGDH is one (but not the only) of the main controlling steps of oxidative phosphorylation (see also Moreno-Sánchez et al. (26)), at non-saturating Mg\(^{2+}\) concentrations. In this respect, control of the rate of oxidative phosphorylation by changes in the spermine/Mg\(^{2+}\) rates, without a concomitant increase in [Ca\(^{2+}\)]\(_{\text{int}}\), has been shown in dog pancreas mitochondria (19).

Modulation of the 2-OGDH activity by adenine nucleotides is well established (23). A synergistic effect by Ca\(^{2+}\) and adenine nucleotides has been described (28). Mg\(^{2+}\) also activates 2-OGDH (30) (this work), but in contrast to other enzyme effectors, the mechanism of action is by enhancing the catalytic enzyme capacity (k\(_{\text{cat}}\)), rather than by increasing substrate affinity. Potentiation of the modulating effect of Mg\(^{2+}\) by P\(_i\), although clearly demonstrated in this work, is somewhat puzzling. There is a report describing an activation of purified...
2-OGDH by a high concentration of Pi (>10 mM), through the diminution of the $K_{0.5}$ for 2-OG (34). Moreover, the Pi potentiating effect could be through promoting changes in matrix pH, since modulation of 2-OGDH activity by pH has also been reported (35). However, substitution of acetate for Pi, to induce similar matrix pH values, did not restitute the Mg$^{2+}$ sensitivity of 2-OGDH. Thus, a direct interaction of Pi with the enzyme is likely to occur. From the present findings, the question that arises is to what extent and how Pi and Mg$^{2+}$ affect the interplay of the other well described effectors, such as Ca$^{2+}$ and adenine nucleotides, and the coenzymes NAD$^+$, thiamine pyrophosphate, and coenzyme A, on 2-OGDH activity.

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REFERENCES

1. Romani, A., and Scarpa, A. (1990) FEBS Lett. 269, 37–40
2. Romani, A., Marfella, C., and Scarpa, A. (1993) J. Biol. Chem. 268, 29737–29744
3. Zhang, G. H., and Melvin, J. E. (1992) J. Biol. Chem. 267, 20721–20727
4. Singh, J., and Wisdom, D. M. (1995) Arch. Biochem. Biophys. 321, 387–397
5. Okada, K., San-e, Y., and Toshikazu, S. (1992) FEBS Lett. 356, 1–5
6. Zhang, G. H., and Melvin, J. E. (1994) J. Biol. Chem. 269, 10352–10356
7. Wolf, F. I., Di Francesco, A., Covacci, V., Corda, D., and Cittadini, A. (1996) Biochem. J. 321, 194–200
8. Wolf, F. I., Di Francesco, A., Covacci, V., and Cittadini, A. (1994) Biochem. Biophys. Res. Commun. 202, 1299–1304
9. Romani, A., Dowell, E., and Scarpa, A. (1991) J. Biol. Chem. 266, 24376–24384
10. Altschuld, R. A., Jung, D. W., Phillips, B. M., Narayan, P., Castillo, L. C., Whitaker, T. E., Hensley, J., Hohl, C. M., and Brierley, G. P. (1994) Am. J. Physiol. 266, H1103–H1111
11. Jung, D. W., Apel, L., and Brierley, G. P. (1990) Biochemistry 29, 4121–4128
12. Rodriguez-Zavalas, J. S., Saavedra-Molina, A., and Moreno-Sánchez, R. (1997) Biochem. Mol. Biol. Int. 411, 179–188
13. Kovacar, Z., Day, S. H., Collett, V., Brosnan, J. T., and Brosnan, M. E. (1995) Biochem. J. 305, 837–841
14. Moreno-Sánchez, R., and Hansford, R. G. (1988) Biochem. J. 256, 403–412
15. Grynkiewicz, G., Poenie, M., and Tsien, R. Y. (1985) J. Biol. Chem. 260, 3440–3450
16. Moreno-Sánchez, R. (1985) J. Biol. Chem. 260, 12554–12560
17. Ákerman, K. E., and Saris, N. L. (1976) Biochem. Biophys. Acta 326, 624–629
18. Zanotti, A., and Azzone, G. F. (1980) Arch. Biochem. Biophys. 201, 255–265
19. Moreno-Sánchez, R., Rodríguez-Enríquez, S., Cuellar, A., and Corona, N. (1995) Arch. Biochem. Biophys. 319, 432–444
20. Debono, M., Molloy, R. M., Dorman, D. E., Paschal, J. W., Babcock, D. F., Deber, C. M., and Pfeiffer, D. R. (1981) Biochemistry 20, 6865–6872
21. Jung, D. W., Chapman, C. J., Baysal, K., Pfeiffer, D. R., Brierley, G. P. (1996) Arch. Biochem. Biophys. 332, 19–29
22. Erdahl, W. L., Chapman, C. J., Wang, E., Taylor, K. W., and Pfeiffer, D. R. (1996) Biochemistry 35, 13817–13825
23. Rutter, G. A., and Denton, R. M. (1988) Biochem. J. 252, 181–189
24. Kovacic, Z., Day, S. H., Collett, V., Brosnan, J. T., and Brosnan, M. E. (1995) Biochem. J. 305, 837–841
25. Moreno-Sánchez, R., Rodríguez-Zavalas, J. S., Saavedra-Molina, A., and Moreno-Sánchez, R. (1997) Biochem. Mol. Biol. Int. 411, 179–188
26. Rutter, G. A., and Denton, R. M. (1989) Biochem. J. 263, 445–452
27. Thomas, A. P., Diggle, T. A., and Denton, R. M. (1986) Biochem. J. 238, 83–91
28. Pfeiffer, D. R. (1996) Biochemistry 35, 427–432
29. Rutter, G. A., Oshadeston, N. J., McCormack, J. G., and Denton, R. M. (1990) Biochem. J. 271, 627–634
30. Murphy, E., Freudenrich, C. C., and Lieberman, M. (1991) Annu. Rev. Physiol. 53, 273–283
31. Headrick, J., Dobson, G., Williams, J., McKirdy, J., Jordan, L., and Willis, R. (1984) Am. J. Physiol. 267, H1074–H1084
32. Lawlis, V. B., and Roche, T. E. (1981) Biochemistry 20, 2512–2518
33. Smith, B. C., Clotfelter, L. A., Cheung, J. Y., and La Noue, K. F. (1992) Biochem. J. 284, 819–826
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