Mn^{2+} prevents the Ca^{2+}-induced inhibition of ATP synthesis in brain mitochondria

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Uptake of Ca^{2+} by rat brain mitochondria causes an inhibition of respiratory stimulation by ADP, and the inhibition is relieved upon Na^{+}-induced release of Ca^{2+} from the mitochondria, in accordance with earlier reports. We show that simultaneous uptake of Ca^{2+} and Mn^{2+} results in no inhibition of ADP-stimulated respiration, indicating that Mn^{2+} prevents the Ca^{2+}-induced inhibition of ATP synthesis, without preventing Ca^{2+} accumulation in the mitochondria. The results are discussed in relation to a possible involvement of the mitochondrial ATPase-inhibitor protein in the observed effects of Ca^{2+} and Mn^{2+}.

ATP synthesis *ADP-stimulated respiration* *Brain mitochondria* *Ca^{2+}-induced inhibition* *ATPase-inhibitor protein* *Mn^{2+} antagonism*

1. INTRODUCTION

Evidence has accumulated which indicates that Ca^{2+} uptake causes an inhibition of mitochondrial ATP synthesis as revealed by measurements of phosphate uptake [1,2], ATPase activity [3], and ADP-stimulated respiration [4–6]. In liver mitochondria, ATP synthesis is restored after terminated Ca^{2+} uptake [1,2], whereas in mitochondria from other tissues, including Ehrlich ascites tumor [4,5], heart [3], and brain [6,7], the inhibition of ATP synthesis persists and is not reversed unless Ca^{2+} is released from the mitochondria [6,7] Ca^{2+} accumulated in the mitochondria may inhibit ATP synthesis by inhibiting the ADP–ATP translocator [4], by preventing the release of the ATPase-inhibitor protein that becomes associated with the ATP synthase during active Ca^{2+} uptake [3], or by competing with intramitochondrial Mg^{2+} for the binding of ADP necessary for the ATP synthase to operate [6], but none of these explanations has so far been proven.

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Mn^{2+} efficiently antagonizes several adverse effects of Ca^{2+} on mitochondria, including the inhibition of NAD^{+}-linked oxidations [8,9], the uncoupling of oxidative phosphorylation [8–10] and the swelling of mitochondria [11]. It has also been shown that mitochondria actively take up Mn^{2+} in a manner similar to the Ca^{2+} uptake [12], and that, when added together, the two cations are taken up in a cooperative manner, Ca^{2+} accelerating the uptake of Mn^{2+}, and Mn^{2+} retarding the uptake of Ca^{2+} [13–17]. Thus it was of interest to investigate the effect of Mn^{2+} on ATP synthesis and its inhibition by Ca^{2+}. As reported here, Mn^{2+} prevents the Ca^{2+}-induced inhibition of ATP synthesis in brain mitochondria, without preventing the uptake of Ca^{2+}; i.e., the inhibitory effect of Ca^{2+} on ATP synthesis can be overcome without releasing the accumulated Ca^{2+} from the mitochondria.

2. EXPERIMENTAL

Brain mitochondria were prepared from male Sprague-Dawley rats, by a modification of the method in [18] as described [19]; for a detailed description of the procedure, see [20]. Respiration was measured with an oxygen electrode, in a reac-

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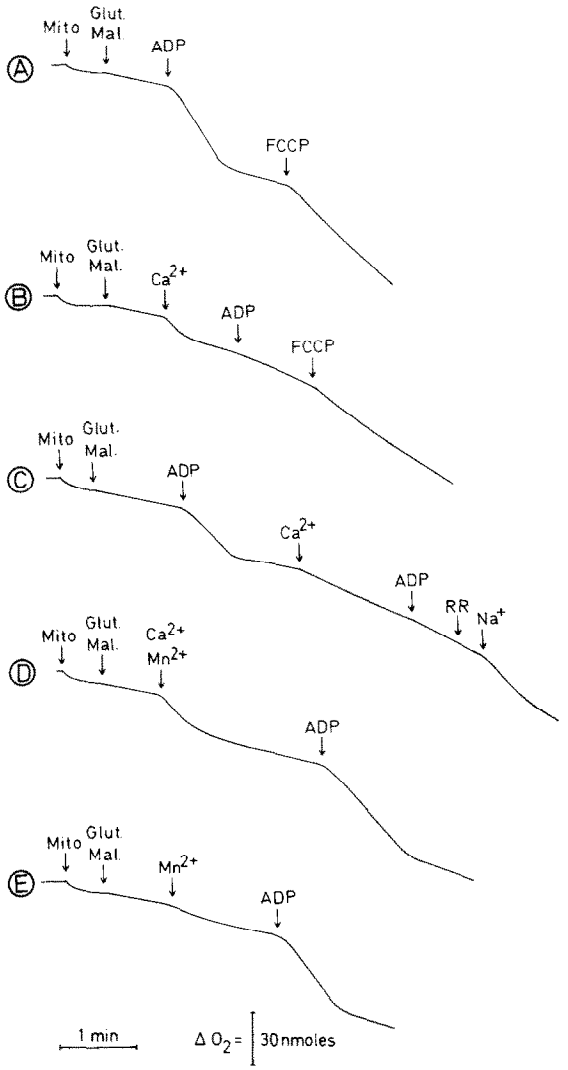

Fig. 1. Oxygen-electrode recordings of rat brain mitochondria respiring in a reaction medium consisting of 150 mM KCl and 10 mM K-phosphate buffer (pH 7.4). The reaction medium was passed through a chelating Sepharose 6B (Pharmacia Fine Chemicals, Uppsala) column prior to use, to reduce the content of Ca^{2+}. When indicated, 0.8 mg mitochondrial protein, (Mito) 5.8 mM K-glutamate + 5.8 mM K-malate (Glut. Mal.) 280 μM ADP, 1.2 μM carbonylcyanide-4-trifluoromethoxy phenylhydrazone (FCCP), 116 μM $CaCl_2$, 0.6 μM ruthenium red (RR), 12 mM NaCl, 116 μM $MnCl_2$ were added. Reaction chamber volume was 0.86 ml and temp. 23°C.

tion mixture specified in fig. 1. In some experiments, Ca^{2+} uptake was monitored simultaneously, by the use of a Ca^{2+} electrode [21]. In other experiments, Ca^{2+} uptake was followed spectrophotometrically, with Arsenazo III as an indicator of extramitochondrial Ca^{2+} [22]. Ca^{2+} uptake was also estimated by measuring the disappearance of $^{45}Ca^{2+}$ from the medium, using a Millipore-filtration technique [17]. The conditions for these experiments are described in fig. 2. Mitochondrial protein was determined as in [23].

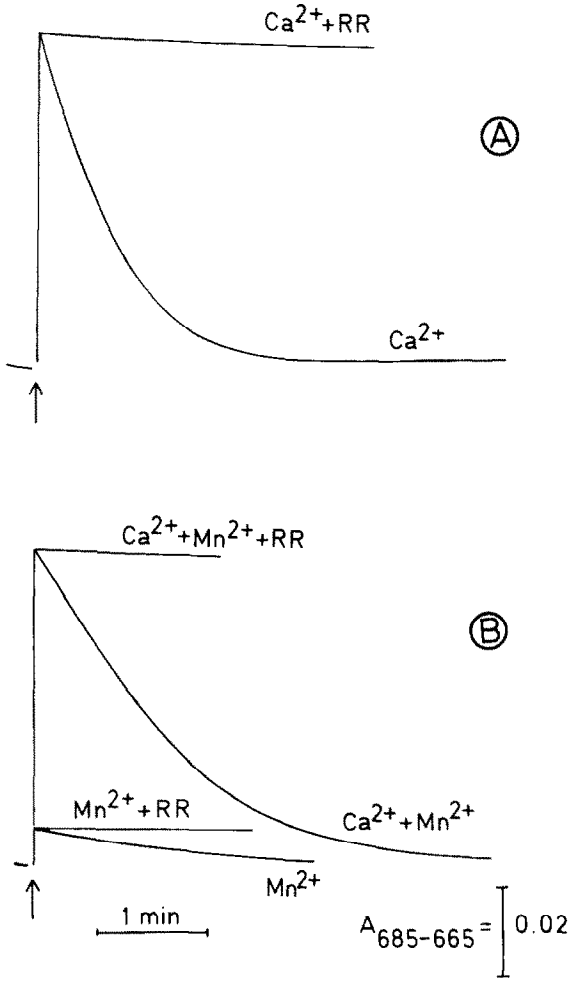

Fig. 2. Ca^{2+} and Mn^{2+} uptake by brain mitochondria was monitored continuously at room temperature using an Aminco DW-2 dual-wavelength spectrophotometer set at 685–665 nm. The incubation mixture contained 10 mM K-phosphate buffer (pH 7.4), 150 mM KCl, 1.5 mM Tris–succinate, 1.5 μM rotenone, 100 μM arsenazo III, 0.8 mg mitochondria and, when indicated, 0.7 μM ruthenium red (RR). At the arrows, 30 nmol Ca^{2+} and/or 30 nmol Mn^{2+} were added as indicated.

3. RESULTS

Fig. 1 shows oxygen-electrode recordings of rat brain mitochondria respiring with glutamate + malate as substrate under various conditions: (A) control experiment, illustrating the stimulation of respiration by 280 μM ADP and its reversal after the exhaustion of added ADP; (B) addition of 116 μM Ca^{2+} resulted in a transient stimulation of respiration, due to the uptake of Ca^{2+}. Subsequent addition of ADP resulted in virtually no increase in the rate of oxygen uptake, in accordance with [4–7]. Addition of the uncoupler FCCP stimulated respiration, showing that the effect of Ca^{2+} was due primarily to an inhibition of ATP synthesis rather than an inhibition of the respiration per se. Trace (C) is essentially a repeat of (B) but using smaller amounts of mitochondria and ADP in order to allow longer recording. After an initial ADP cycle, Ca^{2+} was added, followed by a second addition of ADP. The latter gave as expected a strongly diminished stimulation of respiration as compared with the first addition of ADP. Subsequent addition of ruthenium red and Na^+, resulting in an efflux of Ca^{2+} [24], restored the ADP response, in agreement with [6,7]. In (D), 116 μM Ca^{2+} + 116 μM Mn^{2+} were added simultaneously to mitochondria respiring in the presence of glutamate + malate. This resulted in a transient stimulation of respiration, the extent of which was about twice that in trace B, consistent with the earlier findings [13–17] that both cations are taken up by the mitochondria. Subsequent addition of ADP resulted in a normal, uninhibited stimulation of respiration, in contrast to the inhibition observed in (B) where Ca^{2+} alone was added prior to the addition of ADP. When, in (E) Mn^{2+} was added alone prior to the addition of ADP, it caused a relatively weak stimulation of respiration (consistent with the findings [13–17] that, under these conditions, Mn^{2+} is taken up relatively slowly), and the subsequent addition of ADP resulted in a respiratory stimulation similar to that of the control (A). As found in complementary experiments, the effect of Mn^{2+} in preventing the Ca^{2+}-induced inhibition of the ADP response was optimal when Mn^{2+} was added simultaneously and in equal concentration with Ca^{2+}. Mg^{2+} at $\leq$3.3 mM, found to mimic the effect of Mn^{2+} in retarding Ca^{2+} uptake but without itself being taken up by the mitochondria [17], had no noticeable effect in preventing the Ca^{2+}-induced inhibition of ADP response in this system. Results similar to those in fig. 1 were obtained with succinate as substrate.

That Ca^{2+} indeed was taken up by the brain mitochondria in the presence of Mn^{2+} (similar to what was earlier found with liver mitochondria [13–17]) was shown by using arsenazo III as an indicator of extramitochondrial Ca^{2+} (fig. 2). Ca^{2+} uptake was virtually complete both in the absence (fig. 2A) and in the presence (fig. 2B) of an equimolar amount of Mn^{2+}, although, as expected, the Ca^{2+} uptake was somewhat slower in the latter case than in the former. Mn^{2+} alone gave little response with the arsenazo III assay (fig. 2B). Ruthenium red inhibited the uptake of both cations. The uptake of Ca^{2+} in the presence of Mn^{2+} was further ascertained in two ways: by using a Ca^{2+} electrode in conjunction with the oxygen-electrode measurements; and by measuring $^{45}Ca^{2+}$ in the Millipore-filtered suspending medium after terminated Ca^{2+} uptake as monitored by the arsenazo III assay (not shown).

4. CONCLUSIONS AND COMMENTS

It is evident from the above data that the Ca^{2+}-induced inhibition of ATP synthesis in brain mitochondria is prevented by Mn^{2+}, without inhibition of the Ca^{2+} uptake itself. It is obvious, therefore, that the accumulated Ca^{2+} per se is not inhibitory, provided that Mn^{2+} also is accumulated by the mitochondria. It is not possible at present to decide whether the Ca^{2+} effect is due to an inhibition of the ADP–ATP translocator [4], to a competition with intramitochondrial Mg^{2+} [6], or to an inhibition of the release of the ATPase-inhibitor protein from ATP synthase [3], possibilities considered in the past. Whatever the explanation, however, it seems to be clear that Mn^{2+} efficiency prevents this effect. The fact that ATP synthesis is not permanently inhibited by Ca^{2+} in liver mitochondria seems to argue against the two former explanations, since it is probable that an active ADP–ATP translocator [25] and the availability of intramitochondrial Mg^{2+} [26] are indispensable requisites for ATP synthesis in liver mitochondria as well. A more likely explanation is that Ca^{2+}, possibly in combination with ATP [27],

inhibits the release of the ATPase-inhibitor protein, and that this effect is less pronounced in liver than in tumor cells, heart and brain [28]. In fact, there is evidence that accumulated Ca^{2+} may prevent the substrate-induced release of the inhibitor from heart-mitochondrial ATPase [3]. A possible explanation for the Mn^{2+} effect described here would therefore be that Mn^{2+} counteracts the Ca^{2+}-induced inhibition of the release of ATPase-inhibitor protein from the ATP synthase. We are exploring this possibility.

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