THE RESOLUTION OF CALCIUM FLUXES IN HEART AND LIVER MITOCHONDRIA USING THE LANTHANIDE SERIES

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1. Introduction

There is now considerable evidence that the release of Ca^{2+} from heart mitochondria induced by Na^+ [1] is due to the existence of a specific Na^+–Ca^{2+} antiporter [2–4]; such a carrier has been identified also in mitochondria of skeletal muscle, adrenal cortex, salivary gland, brown fat and brain [5–7]. However, there is no detectable Na^+–Ca^{2+} exchange in mitochondria from liver, kidney and some other tissues [1,2,5]. Such mitochondria, on the other hand, display significant Na^+-insensitive release of Ca^{2+} [5,8–11].

The question arises therefore whether the Na^+-sensitive and Na^+-insensitive effluxes observed, for example, in heart and liver mitochondria respectively, really reflect the existence of two distinct systems that are fundamentally different, or whether both mitochondrial types contain a similar carrier which is simply more reactive towards Na^+ in mitochondria exemplified by those in heart. A further question concerns the identity of the efflux systems with respect to the carrier that catalyses Ca^{2+} influx [2–5,8–13]. This paper attempts to resolve these questions by comparing the sensitivity to different lanthanides of the relevant Ca^{2+} fluxes.

2. Methods

2.1. *Preparation of mitochondria*

Rat heart and liver mitochondria were prepared, and their protein content was determined, as described in [2,14].

2.2. *Measurement of Ca^{2+} fluxes*

Ca^{2+} was measured with a Ca^{2+}-selective electrode as described in detail in [2]. Each incubation medium (3 ml, maintained at 25°C) contained 120 mM KCl, 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonate (Tris salt, pH 7.2), 3 mg of mitochondrial protein, 3 μg of rotenone, and 10 μM $CaCl_2$.

After a preincubation period of 7 min, the initial rates of Ca^{2+} influx (phases A and D in fig.1) were measured following addition of 5 mM succinate (K^+ salt) as respiratory substrate. The addition of ruthenium red, a specific inhibitor of influx [9,15] blocks uptake and causes a slow efflux of Ca^{2+} from

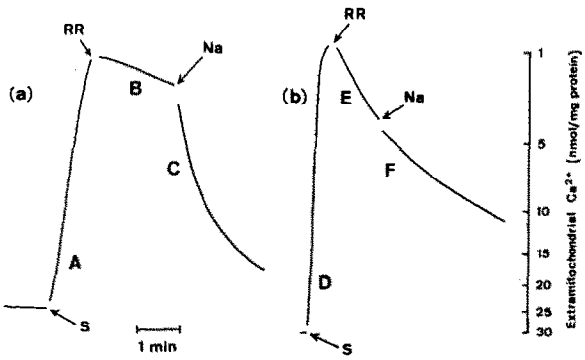

Fig.1. The uptake and release of Ca^{2+} by rat heart and liver mitochondria. Mitochondria from heart (a) and liver (b) were incubated as described in the methods section. Respiratory-dependant uptake of Ca^{2+} was started by the addition of 5 mM succinate (S), and inhibited with 1 nmol of ruthenium red · mg protein^{-1} (RR). 10 mM NaCl (Na) was then added. The letters A to F indicate the phases of uptake and release referred to in the text.

heart mitochondria (phase B, fig.1a) and a more rapid efflux from liver mitochondria (phase E, fig.1b). Na^+ markedly stimulates efflux from heart mitochondria (phase C, fig.1a), but not from liver mitochondria (phase F, fig.1b). The effects of lanthanides on the initial rates of phases A to F were investigated by adding the lanthanide 5 s before either succinate (for A and D), ruthenium red (for B and E) or Na^+ (for C and F). Stock solutions of the trivalent lanthanides (5 mM, nitrate salts) were prepared as described [16] to give minimal loss of the lanthanide due to adsorbance. The quantities of lanthanides needed for 50% inhibition of the rates of Ca^{2+} fluxes were obtained from plots (which were essentially linear [3,16–18]) of the quantity of lanthanide versus percentage inhibition over the range 45–55% inhibition.

3. Results and discussion

Figure 2a shows the sensitivity to lanthanides of the uptake of Ca^{2+} by heart and liver mitochondria. In both mitochondrial types, La^{3+} is the poorest inhibitor, and the potency of the lanthanides increases markedly (18–30-fold) with decreasing ionic radius to Dy^{3+}. The best inhibitor with liver mitochondria is Dy^{3+}, which has the same ionic radius as Ca^{2+} (0.99 Å, [19]). In 3 experiments the amounts of Dy^{3+} needed for 50% inhibition were 40–55% of the quantities of Ho^{3+} and Gd^{3+}. An equivalent selectivity for Dy^{3+} is not shown by heart mitochondria. The essential point, nevertheless, is that the pattern of lanthanide potency is similar in the two cases. This pattern disagrees with the data of Tew [20], who reported that Sm^{3+} and Nd^{3+} are the strongest inhibitors of Ca^{2+} uptake by liver mitochondria.

Figure 2b reports the sensitivity of the Na^+–Ca^{2+} antiporter of heart mitochondria to lanthanides (C). This carrier is much less sensitive to lanthanides than the carrier that catalyses Ca^{2+} uptake. Moreover, the pattern of inhibitory potency is opposite to that observed for uptake, i.e., La^{3+} and Ce^{3+} are the best inhibitors (0.4 nmol · mg protein^{-1} for 50% inhibition) and the sensitivity decreases markedly with the smaller lanthanides to Tm^{3+}.

These observations are difficult to explain as effects of the lanthanides on uptake and release of

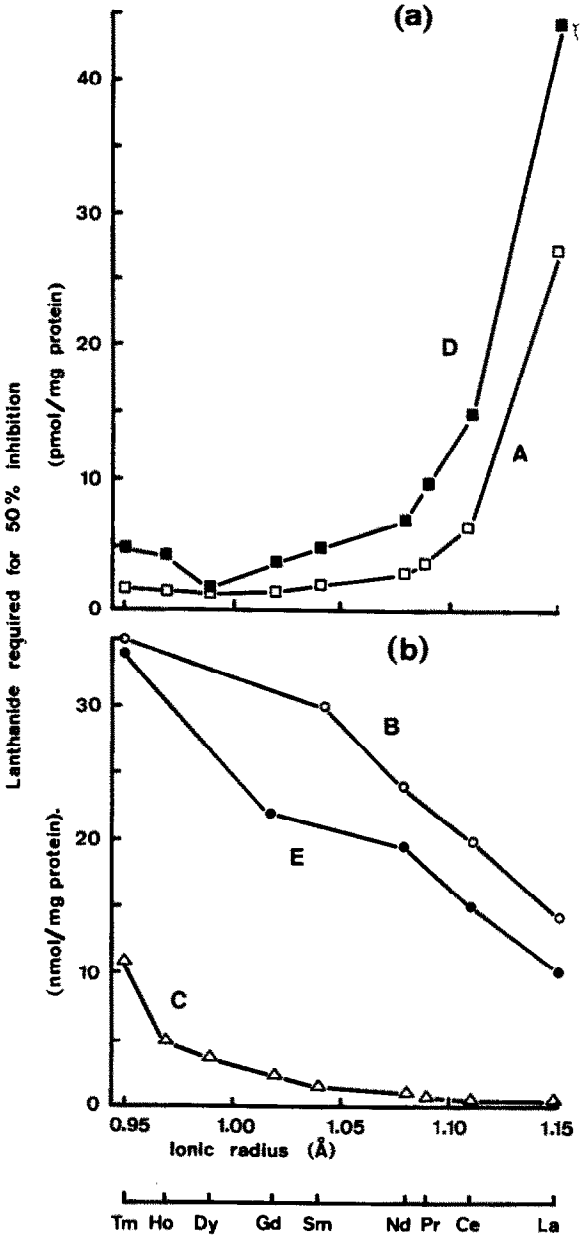

Fig.2. The sensitivity to different trivalent lanthanides of the uptake and release of Ca^{2+} by heart and liver mitochondria. The amounts of lanthanide needed for 50% inhibition of the initial rate of phases A to E (fig.1) were determined as described in the methods section. Symbols: ■, Ca^{2+} influx into liver mitochondria (D); □, Ca^{2+} influx into heart mitochondria (A); ○, Ca^{2+} efflux from heart mitochondria in the absence of Na^+ (B); ●, Ca^{2+} efflux from liver mitochondria in the absence of Na^+ (E); △, Ca^{2+} efflux from heart mitochondria in the presence of 10 mM NaCl (C).

Ca^{2+} via a common system, but they may be readily interpreted in terms of widely differing affinities for lanthanides of two distinct systems, i.e., the Ca^{2+} uniporter catalysing influx and the Na^+–Ca^{2+} antiporter catalysing efflux of Ca^{2+} [2–6].

The remaining question concerns the identity of the process responsible for net efflux of Ca^{2+} in the absence of Na^+ (phases B and E, fig.1). In cardiac mitochondria, and in other mitochondria that possess the Na^+–Ca^{2+} antiporter [5], this basal efflux of Ca^{2+} is very slow below 30°C (< 1 nmol $Ca^{2+} \cdot$ mg protein$^{-1} \cdot$ min^{-1} at 25°C). Nevertheless, it is important to establish whether the basal efflux is due to an ability of the Na^+–Ca^{2+} antiporter to function, albeit very slowly, in the absence of Na^+.

Figure 2b shows that the basal efflux (B) is much less sensitive to lanthanides than the Na^+-induced efflux (C). This disparity is particulary pronounced with the larger lanthanides, e.g., whereas the difference with Tm^{3+} is 3 fold, it is 35-fold with La^{3+}. On this basis, it appears that the basal efflux of Ca^{2+} from heart mitochondria is probably not catalyzed by the Na^+–Ca^{2+} antiporter.

Similar conclusions may be drawn for the basal efflux from liver mitochondria (E, fig.2b), which is also quite insensitive to lanthanides. This sensitivity was not significantly changed in the presence of Na^+ (phase F, fig.1b; data not shown). It may be remarked that the effects of lanthanides on the two basal effluxes of Ca^{2+} may well be due to non-specific effects on mitochondrial function. Nevertheless, such a possibility does not detract from the fundamental utility of this approach, which permits the basal effluxes in liver and heart mitochondria to be attributed to a process distinct from the Na^+–Ca^{2+} antiporter. It is possible that the basal effluxes in the two mitochondrial types, though differing in activity, are catalysed in the same way; they both show a similar, poor sensitivity to lanthanides.

In conclusion, the present data support the proposal [2,5] that mitochondria exhibit two, fundamentally different, modes of Ca^{2+} extrusion in vitro. In mitochondria exemplified by those from heart, the Na^+–Ca^{2+} antiporter is very active, whereas the Na^+-insensitive system has a very low activity. On the other hand, the Na^+–Ca^{2+} antiporter is not detectable in liver mitochondria, but, in these, the Na^+-insensitive system is more active. Finally, at variance with some proposals [12,13], the data agree with the concept that both efflux systems are quite distinct from the carrier that catalyses Ca^{2+}-uptake.

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