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EFFECT OF LANTHANUM ON CALCIUM EXCHANGEABILITY IN MITOCHONDRIA

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Abstract—The present investigation was undertaken to investigate the involvement of lanthanum in processes other than the competition with membrane calcium receptors in mitochondria. The effect of lanthanum was evaluated on the calcium content of whole mitochondria and of mitochondrial membranes and matrix; the pool of exchangeable calcium was measured by radioassay. The results show that lanthanum can displace calcium from membrane receptors which are not specifically involved in the transport of this cation. Lanthanum, however, induces a significant increase in the pool of exchangeable calcium, while the total intramitochondrial calcium content is not modified. This suggests that lanthanum can release some of the intramitochondrial bound calcium, indicating that the intramitochondrial compartment may be partly accessible to lanthanum.

Lanthanum, having electropositive characteristics, affects calcium binding and has been used to outline Ca^{2+} movements within mitochondria. It was found to be a potent inhibitor of the energy linked accumulation of calcium by mitochondria [1–6] and consequently it was considered to affect only membrane calcium binding sites. More recently Reed and Bygrave [7] demonstrated that lanthanum binds to a large number of membrane sites, probably identical with the "low affinity calcium binding sites" described by Lehninger [8].

However, certain features of the interaction of lanthanum with mitochondria suggest that the action of La^{3+} might be more complicated. The inhibition of calcium uptake induced by lanthanum subsides with time suggesting that at a later time, the enhancement of calcium influx or of calcium exchange, also involving the matrix, might overlap with the action of lanthanum at membrane level. On the other hand, ^{45}Ca efflux from preloaded mitochondria is increased by lanthanum [9], suggesting that calcium release from the intramitochondrial compartment might be involved. Both these effects take place after several minutes of incubation in the presence of lanthanum supporting the view that these processes occur when lanthanum reaches a pattern of distribution different from that of early kinetics. The aim of this investigation was to study the mechanism of action of lanthanum under experimental conditions which grant a lanthanum distribution comparable with that of the above mentioned investigations. The possible involvement of lanthanum in processes other than the well known competition with the membrane calcium receptors has been evaluated by determining the amount of calcium bound to the mitochondrial membranes and matrix, and by measuring the extent of the exchangeable calcium.

EXPERIMENTAL

Heavy mitochondria were isolated from guinea-pig heart, using the mannitol–sucrose medium (0·225 M mannitol and 0·075 M sucrose) described by Mela [3]. Protein concentration was determined by the biuret method [10] with bovine serum albumin as standard. The incubation medium contained mannitol and sucrose as above, 30 mM Na succinate, 0·04 mM $CaCl_2$, and 0·5 mM Tris, pH 7·2; mitochondria were added to give 3·0 mg protein/ml.

$LaCl_3$ was added to give the final concentrations shown in the tables.

Mitochondria were incubated for 10 min, because preliminary experiments demonstrated that in this time the calcium turnover reached a steady-state both in the absence and in the presence of lanthanum, while the metabolic properties (oxygen consumption) were not significantly modified at the lanthanum concentrations tested (Table 1).

For the study of calcium turnover, ^{45}Ca (0·5 μCi/ml) was added to mitochondria incubated under the usual conditions; after 10 min, incubating vessels were chilled and mitochondria collected and washed three times by centrifugation at 4°. Loss of mitochondrial proteins and ^{45}Ca wash-out rate were checked and found to be equal in control and treated groups. Separation of the matrix from the membranes was achieved by osmotic shock and freezing–thawing, followed by centrifugation according to the method of Sottocasa *et al.* [11].

Intact mitochondria, membranes and matrix were mineralized at 210° with HNO_3–$HClO_4$ (1:1); each residue was assayed for total calcium by atomic absorption spectrometry and for ^{45}Ca by liquid scintillation counting.

Table 1. Effect of different concentrations of lanthanum on the oxygen consumption in mitochondria

Treatment	Concentration	O_2 Consumption (μl/min/mg proteins)	P
	—	0·86 ± 0·05 (12)	—
Lanthanum	10 μM	0·85 ± 0·03 (6)	n.s.
Lanthanum	100 μM	0·78 ± 0·04 (6)	n.s.

Number of determinations is given in brackets.

Table 2. Effect of 100 μM lanthanum on the calcium content in whole mitochondria, mitochondrial membranes and matrix

	Controls	Lanthanum	P
Membranes	7·14 ± 0·64 (8)	5·00 ± 0·52 (8)	<0·05
Matrix	46·17 ± 7·36 (9)	47·96 ± 10·84 (10)	n.s.
Whole mitochondria	64·5 ± 8·2 (15)	62·1 ± 8·2 (14)	n.s.

Values are μ-equiv/g protein. Number of determinations is given in brackets.

Table 3. Effect of 100 μM lanthanum on the specific activity and exchangeable calcium in mitochondria

	Controls	Lanthanum	P
Specific radioactivity dis/min/n-equiv Ca	272·7 ± 54·2 (9)	1450·0 ± 274·8 (11)	<0·01
Exchangeable calcium per cent	2·1 ± 0·3 (13)	9·6 ± 1·2 (10)	<0·001

Number of determinations is given in brackets.

Counting efficiency (55% in all tested materials) was measured by internal standardization.

Calcium exchangeability in mitochondria was evaluated as the ratio between the specific radioactivity (dis/min/μ-equiv Ca) of mitochondria and that of the incubation medium.

RESULTS AND DISCUSSION

Table 2 shows that the calcium content of mitochondrial membranes is about 40% lower in lanthanum-treated preparations than in control mitochondria, while it is not modified in the matrix. The amount of total calcium in whole mitochondria does not change significantly, in spite of the diminution in the membranes. This probably depends on the limited amount of membrane calcium which has been released by lanthanum. These results agree with those of other workers suggesting that lanthanum displaces calcium principally from membrane located binding sites, and support the view of Reed and Bygrave [7] that lanthanum-insensitive calcium sites are present in the mitochondrial membranes. The matrix-bound calcium is not modified by lanthanum. This fact seems to rule out the possibility that lanthanum interferes with calcium movements at levels other than membranes. However, the determination of total calcium content of the intramitochondrial compartment gives a limited picture of the whole process, because it does not consider the dynamics.

Total calcium could remain unchanged in the matrix even if there is an increase of transmembranes exchange of calcium which occurs when the influx of the ion from the external medium is balanced by the efflux of an equal amount of intramitochondrial calcium. An increase of calcium exchangeability could account for the increase of release of ^{45}Ca from preloaded mitochondria [9] in the presence of lanthanum.

In order to check this hypothesis, the pool of exchangeable calcium was evaluated. Mitochondria take up radiocalcium to a specific activity which reaches a much larger value in the presence of lanthanum than in control mitochondria (see Table 3). This suggests that the calcium transporting properties of the mitochondrial membranes are not blocked by lanthanum. Also the pool of exchangeable calcium increases in lanthanum-treated mitochondria. Since the total calcium content of the matrix is not modified, it is possible that in the presence of lanthanum some bound calcium is set free in the intramitochondrial compartment. This suggests that under these experimental conditions lanthanum could cross the membranes and displace calcium from intramitochondrial storage sites.

This assumption could probably justify the apparent discrepancy between the reported increase of the wash-out rate of calcium and the inhibition of uptake. Since the last process was studied by short term experiments, it is possible that only the more accessible membrane receptors were reached by lanthanum, whereas lanthanum might achieve a different pattern of distribution during the time-course of wash-out experiments.

On the other hand, the data indicate that calcium can be transferred across the mitochondrial membranes in the presence of lanthanum, indicating that this element does not block the membrane receptors specifically involved in calcium translocation. Also the results obtained by Reed and Bygrave [7] suggest that lanthanum binds to the low-affinity calcium binding sites. As a consequence, it could be postulated that calcium transport in mitochondria utilizes specific receptors which do not react with lanthanum. These data are in agreement with the findings of Lehninger [8], Rejnafarie *et al.* [12] and Carafoli [9, 13], indicating that at least two calcium receptors are present in the mitochondrial membranes, one of them principally interested in calcium storage, the other one specifically involved in the processes leading to calcium translocation. Lanthanum at concentrations which do not affect mitochondrial respiration, mainly blocks the first kind of receptors.

This behaviour of lanthanum in mitochondria is in agreement with the results obtained in sarcoplasmic reticulum vesicles, demonstrating that calcium transport is not affected by lanthanum [14, 15].

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