INTERACTION OF CALCIUM ION WITH THE MITOCHONDRIA OF RABBIT SPERMATOZOA

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1. Introduction

Mitochondria isolated from many mammalian tissues accumulate calcium ions in a rapid reaction which is energy linked competes successfully for the high-energy intermediates that drive oxidative phosphorylation [1, 2], and is inhibited by lanthanide ions and Ruthenium red [3, 4]. Lehninger [5] has proposed that the biological function of this reaction is to produce a high local concentration of calcium and phosphate ions which then react to form amorphous tricalcium phosphate in 'micropackets'. These are then excreted by the mitochondrion and eventually by the cell, from whence they are transported to a target collagen structure and deposited. A plausible mechanism for the formation of calcified tissue is provided by this proposal. The liver, heart, kidney, brain, and spleen of the rabbit all yield mitochondria that show the calcium accumulation reaction [6], which raises the question: to what extent is this reaction genetically programmed into the mitochondria of all differentiated tissues of the mammal? The recent demonstration that the mitochondria of rabbit epididymal spermatozoa become accessible to reagents in the suspending medium when the spermatozoa are treated with hypotonic buffer, but themselves remain undamaged by this treatment [7], offers the chance to examine the calcium accumulation reaction by the mitochondria of a class of highly differentiated mammalian cells which can scarcely be involved in the formation of hard tissue. In this paper we show that there is an uptake of calcium, but by a mechanism which appears to be fundamentally different from that observed with mitochondria from the other tissues of the rabbit.

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2. Methods

Spermatozoa were flushed from the excised epididymides of mature male white New Zealand rabbits and washed twice with a medium composed of 113 mM KCl, 15 mM KP_i, 3 mM $MgCl_2$, 0.4 mM EDTA, 20 mM Tris, pH 7.4 [7, 8]. They were then treated with 10 mM KP_i solution, pH 7.4, as described previously [7], washed once with the KCl medium with magnesium omitted and EDTA at 1.6 mM, then washed twice with a medium composed of 113 mM KCl and 35 mM Tris, pH 7.4. The washed, treated cells were then resuspended in a minimal quantity of this latter medium.

The respiratory activity of sperm cell suspensions was assayed at room temperature using the miniaturized oxygen electrode assembly described previously [7]. Assays were carried out in the KCl–Tris medium described above. Uptake of added Ca^{2+} was measured by the murexide absorbance change technique [9] described by Scarpa [10], using the dual

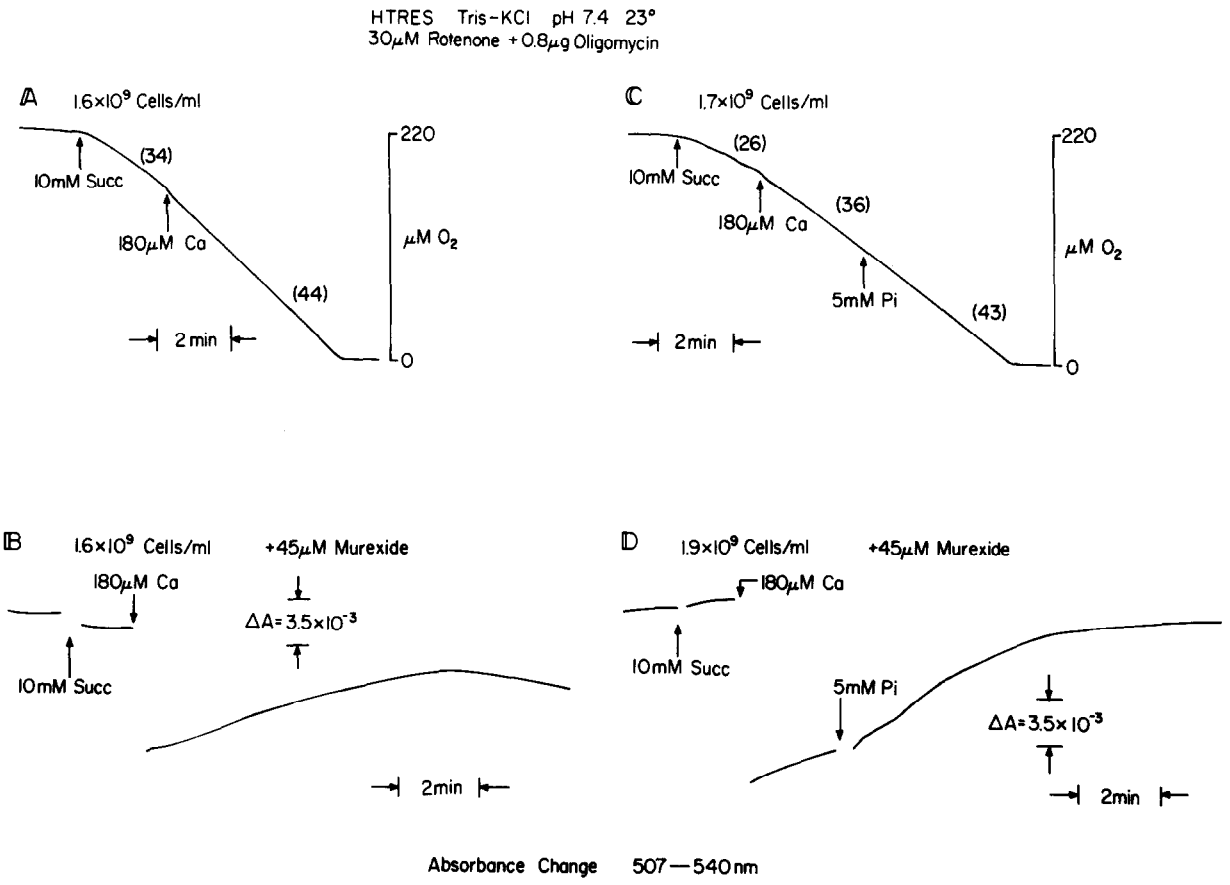

Fig. 1. Oxygen uptake (A) and Ca^{2+} uptake (B) by the mitochondria of hypotonically treated rabbit epididymal sperm (HTRES) suspended in KCl–Tris medium; no permeant anion is present. The effect of adding the permeant anion P_i is shown for oxygen uptake in (C) and for Ca^{2+} uptake in (D).

wavelength spectrophotometer with the wavelength pair 507 and 540 mm. The optical path length was 0.5 cm.

All reagents were of the best grade available commercially and were used without further purification. The uncoupler designated '1799' (hexafluoroacetonyl acetone) was generously provided by Dr. Peter G. Heytler of the Central Research Department of E.I. DuPont de Nemours Co.

3. Results

The effect of adding 180 μM Ca^{2+} to hypotonically treated rabbit epididymal sperm respiring with succinate in the presence of rotenone and oligomycin in KCl–Tris medium is shown in fig. 1A. The rate of oxygen consumption is increased by about 30%, but there is no cycle of rapid respiration followed by a slower state 4 rate [1]. Further, no inhibited state 6 [1, 11] is observed in this system in the absence of a permeant anion such as P_i. The slow uptake of Ca^{2+} under these conditions is shown by fig. 1B, in which the rapid absorbance change, shown by murexide on addition of Ca^{2+} to the suspending medium, is reversed [9, 10]. On anaerobiosis, a slow leakage of Ca^{2+} into the medium is observed (fig. 1B). If the permeant anion P_i is added to the sperm cell suspension after addition of Ca^{2+}, there is a further stimulation of respiration by 20% (fig. 1C) and a corresponding increase in the rate of Ca^{2+} uptake from the medium (fig. 1D). This rate slows and becomes zero

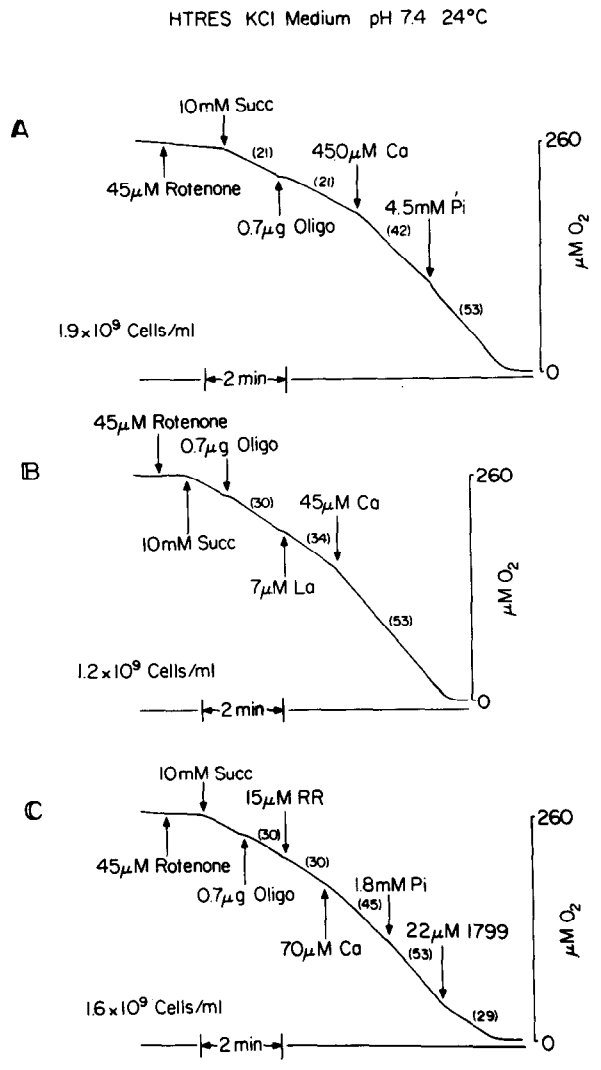

Fig. 2. Effect of the inhibitors La^{3+} and Ruthenium red (RR) on oxygen uptake stimulated by Ca^{2+} followed by P_i. The control experiment without inhibitor is shown in (A). The effect of La^{3+} is shown in (B), and the effect of Ruthenium red (RR) is shown in (C).

on anaerobiosis, but no subsequent leakage of Ca^{2+} into the medium is observed under these conditions. If the permeant anion P_i is present in the medium before addition of Ca^{2+}, a cycle of increased followed by decreased respiration is observed with a Ca^{2+}/O ratio of about 0.6.

Addition of La^{3+} at concentrations more than sufficient to inhibit Ca^{2+} uptake in other mitochondria [3, 12] has no effect on the Ca^{2+} uptake in these mitochondria (fig. 2B). Neither does Ruthenium red (fig. 2C). With the latter inhibitor, which does not form an insoluble complex with P_i as does La^{3+}, one can also demonstrate the further stimulation of the respiratory rate by addition of P_i (fig. 2C). Addition of the uncoupler 1799 not only stops accumulation of Ca^{2+} as monitored with murexide (not shown), it also inhibits the rate of respiration as shown in fig. 2C.

Comparison of the O_2 uptake experiments of fig. 1 and fig. 2 also indicate the variability in degree of stimulation observed on addition of Ca^{2+} to the cells respiring in KCl–Tris medium: The percent stimulation observed in a series of experiments to range between 30% and 60%, with a stimulation of 100% occasionally observed as shown in fig. 1. The concentrations of Ca^{2+} to give half maximal stimulation is estimated to be 15 μM. Subsequent addition of P_i consistently gives a rate increase of 25%–35%.

4. Discussion

These results show that Ca^{2+} does stimulate respiration of rabbit spermatozoa mitochondria utilising succinate as substrate and is taken up in a slow but energy-dependent reaction, which is not sensitive to inhibition by either La^{3+} or Ruthenium red. Further, there are no cyclical bursts of respiration caused by addition of limited amounts of Ca^{2+} in the absence of P_i, as observed with mitochondria from other rabbit tissues; nor does one observe an inhibited respiration corresponding to state 6. These observations make it most unlikely that these mitochondria take up Ca^{2+} by the same mechanism – most probably involving a specific carrier – as do the mitochondria from liver, kidney, brain, and spleen. During the maturation of the sperm cell, this reaction is lost, either through loss of the carrier, or its immobilization by mechanisms as yet unknown. This lends support to Lehninger's proposal concerning the biological function of Ca^{2+} uptake: In a group of cells where transport of micropackets of amorphous calcium phosphate cannot occur, the mitochondria do not take up Ca^{2+} in the usual manner. It seems possible that the stimulation of respiration by Ca^{2+}, with concomitant uptake of Ca^{2+} from the medium, reflects

increased transport of substrate anion accompanied by Ca^{2+}. This hypothesis is undergoing further investigation.

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