Ca^{2+} TRANSPORT ACROSS PLASMA AND MITOCHONDRIAL MEMBRANES OF ISOLATED HEPATOCYTES

W. P. DUBINSKY and R. S. COCKRELL
Department of Biochemistry, St. Louis University School of Medicine St. Louis, Missouri, 63104, USA

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1. Introduction

Calcium exerts many important metabolic effects upon isolated liver cells, e.g. it stimulates gluconeogenesis [1], elevates the mitochondrial NADH/NAD^+ ratio [2] etc. The influences of Ca^{2+} upon hepatocyte respiration are varied, e.g. they include cycles of stimulation [3] such as obtained with isolated mitochondria [4], sustained uncoupling [5] or a complex interplay of stimulation and inhibition [6]. The subcellular loci and mechanism of action of this ion have yet to be determined. The present studies indicate the relative plasma membrane permeability to Ca^{2+} is a major determinant of the influence of this ion upon respiration.

Ca^{2+} uptake by intact hepatocytes occurs at about 5% the rate obtained with cells treated with digitonin to render the plasma membrane freely permeable. Ca^{2+} accumulation by digitonin treated cells is a consequence of mitochondrial transport and occurs at rates and Ca^{2+}/O stoichiometries typical of mitochondria in vitro [4,7]. The influence of Ca^{2+} upon respiration and proton translocation of intact cells is apparently a combination of sluggish uptake by intact cells and rapid Ca^{2+} accumulation by a small fraction of damaged cells.

2. Materials and methods

Cells were isolated from livers of 24 h fasted 200–250 g Wistar rats by the enzymatic perfusion technique of Berry and Friend [8] as modified by Cornell et al. [9]. Cells were washed and resuspended in 136 mM NaCl, 10 mM Na-morpholinopropane sulfonate (MOPS) buffer, 1.0 mM $MgSO_4$, 1 mM K_2HPO_4 and 6 mM KCl at pH 7.4. Protein was determined by the biuret method [10]. Respiration and pH changes were measured simultaneously with a Clarke electrode and a Beckman combination pH electrode. Calcium transport was measured by the murexide indicator technique [7,11] with an Aminco DW-2 spectrophotometer.

3. Results

Digitonin was first employed by Zuurendonk and Tager to selectively disrupt the plasma membrane of isolated liver cells [12] and was adopted in the present studies to assess both the plasma membrane Ca^{2+} permeability of hepatocytes and the functional integrity of mitochondria in situ. Net Ca^{2+} fluxes for normal and digitonin treated cells are shown in fig.1.

Intact cells accumulated Ca^{2+} very slowly whereas rapid Ca^{2+} transport was observed with digitonin treated cells (fig.1). Ca^{2+} uptake by the latter could be attributed to mitochondrial accumulation since it was respiration-dependent (i.e., inhibited by Antimycin A) or driven by exogenous ATP which was abolished by oligomycin (not shown). Assuming mitochondria constitute approximately 25% of the total cell protein, the rate of calcium accumulation by mitochondria in situ compared favorably with values for isolated mitochondria [7]. Whether the slow uptake of Ca^{2+} by untreated cells involves mitochondrial transport or some other process, e.g. Ca^{2+} for Na^+ exchange across the plasma membrane [13,14] is not known. The influence of Ca^{2+} upon respiration and proton transport in control and digitonin treated cells is illustrated by the results of fig.2. Addition

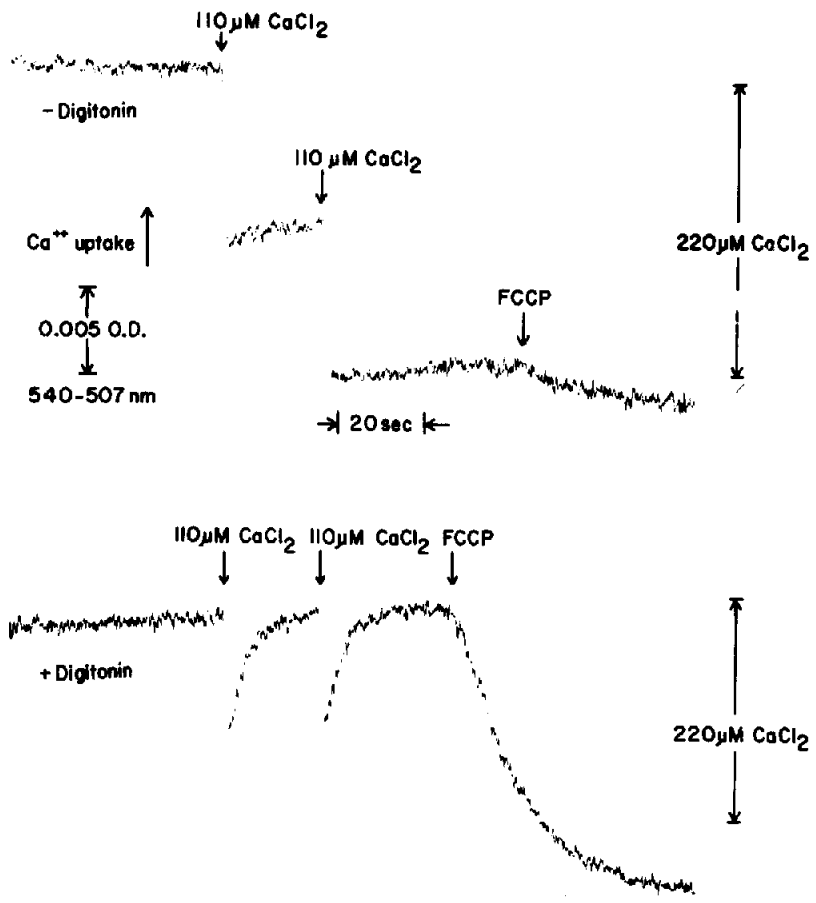

Fig.1. Ca^{2+} Uptake by intact and digitonin-treated hepatocytes. Control cells were suspended in NaCl medium (see Methods). For treatment with digitonin, cells were added to medium containing 0.01% digitonin in 142 mM KCl, 1 mM $MgSO_4$, 1 mM K_2HPO_4 and 10 mM Na-MOPS and assayed immediately. The reaction mixtures consisted of the above media plus rotenone (2 μg/ml), 2 mM K_2-succinate, oligomycin (1 μg/ml), bovine serum albumin (1 mg/ml) and 35 μM murexide at pH 7.4 and room temperature. FCCP (trifluoromethoxycarbonylcyanide phenylhydrazone) was added as indicated (4 μM). The cell concentration was 11.8 mg wet wt/ml.

of Ca^{2+} to an intact cell suspension increased the basal respiration rate by 40% and elicited a transient stimulation of proton ejection (fig.2A). The sustained stimulation of respiration by Ca^{2+} was not the result of accelerated ATP turnover since it was not affected by oligomycin. These results contrasted with those obtained with digitonin treated cells (fig.2B).

Cyclic stimulation of respiration and proton ejection occurred in response to successive Ca^{2+} additions to digitonin treated cells. These effects were prevented by uncoupling agent or the calcium ionophore A23187 [15] whilst oligomycin exerted no effect upon either the rate of Ca^{2+} uptake or the Ca^{2+}/O stoichiometry. These results indicated that Ca^{2+} uptake occurred via mitochondrial transport. Selected general properties of Ca^{2+} transport by hepatocytes with intact versus digitonin disrupted plasma membranes are summarized in table 1.

The rate of Ca^{2+} stimulated respiration and also the rate of Ca^{2+} uptake by intact cells were each about 10% and 5% respectively of comparable values for cells treated with digitonin (table 1). The Ca^{2+}/O and H^+/Ca^{2+} quotients for mitochondria in situ are typical by comparison to representative data for mitochondria in vitro [4]. The Ca^{2+}/O ratios are also in line with P/O quotients determined for digitonin treated cells.

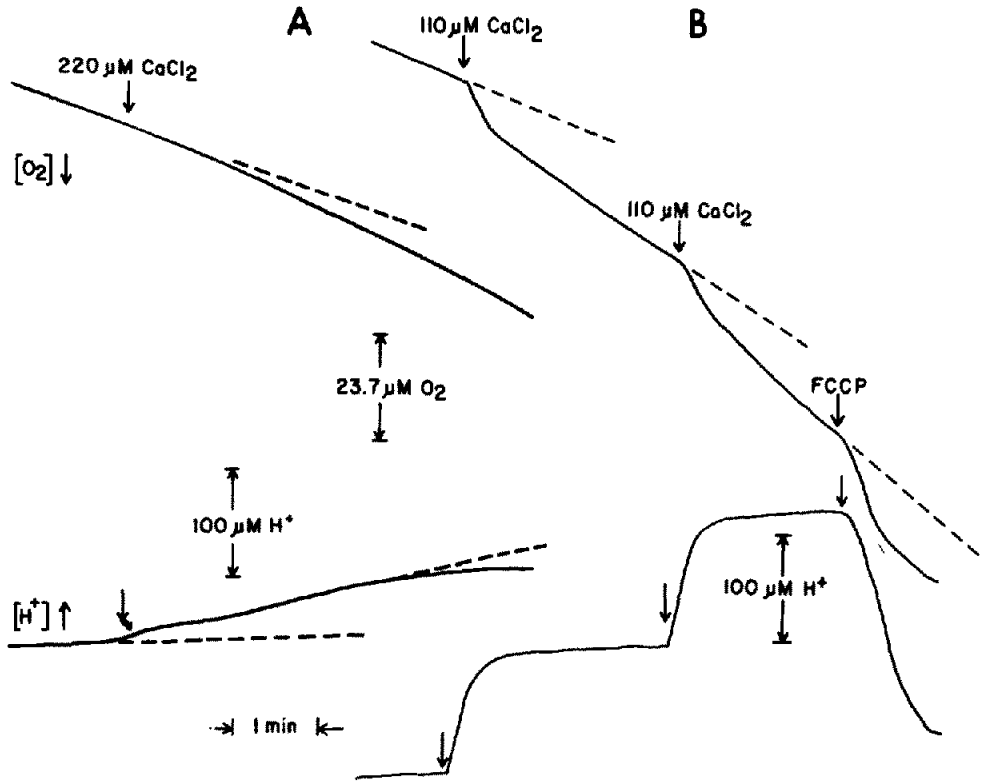

Fig.2. The influence of Ca^{2+} upon oxygen consumption and proton transport. The media and reaction conditions were identical to those described in the legend to fig.1 for control (A) and digitonin-treated cells (B). The final cell concentrations were, 16.4 and 11.8 mg wet wt/ml, respectively.

Table 1
Characteristics of Ca^{2+} transport by hepatocytes

Parameter	Control Cells	Digitonin treated cells
Ca^{2+} stimulated respiration (μmol O_2/min g wet wt)	0.49 ± 0.19 (8)	5.17 ± 1.93 (6)
Ca^{2+} uptake rate (μmol Ca^{2+}/min g wet wt)	0.81 (2)	29 ± 16 (3)
Ca^{2+}/O ratio	0.8[a]	3.8 ± 0.1 (6)
H^+/Ca^{2+} ratio	–	0.8 ± 0.2 (7)
P/O ratio	2.0 ± 0.1 (lactate)[b] 0.1 ± 0.02 (succinate)[b] 2.04 (pyruvate)[c]	1.4 ± 0.2 (5)

[a] Estimated from separate measurements of Ca^{2+} uptake rate and the steady state rate of Ca^{2+}-dependent respiration.
[b] Taken from ref. [17].
[c] Taken from ref. [18].
The data summarized were obtained from experiments conducted as described in the previous legends. The P/O ratios for succinate oxidation were determined polarographically [19]. The values in parentheses refer to the number of separate cell preparations tested.

4. Discussion

Maximal respiratory rates of 5.2 μmol O_2/min g wet wt have been obtained with digitonin treated cells oxidizing succinate in the presence of uncoupler. Calcium stimulates respiration 0.5 μmol O_2/min g wet wt. This extra oxygen utilization could be accounted for by a 10% damaged cell population which is completely uncoupled. This is supported by the low observed rate of Ca^{2+} uptake (5% that of digitonin treated cells) and correlates with trypan blue staining which demonstrates 90–95% cell viability for cell preparations used in the present studies. The low Ca^{2+}/O ratio (0.8; cf. table 1) for untreated cells would therefore reflect massive Ca^{2+} uptake by the mitochondria of a small number of damaged cells. Alternatively, it is possible that very slow Ca^{2+} permeation of the plasma membrane of intact cells yields a low Ca^{2+}/O quotient which has been demonstrated in vitro by slow infusion of Ca^{2+} into suspensions of isolated mitochondria [4].

The low plasma membrane Ca^{2+} permeability demonstrated here is at variance with the results of Kleineke and Stratman [3]. Extensive washing with Ca^{2+} free medium involved in their cell preparation could have caused cell damage or depletion of Ca^{2+} which may itself result in an increased plasma membrane permeability. Along these lines, the Ca^{2+} dependent rapid oxidation of pyridine nucleotides observed by Ontko, et al. [16] may occur in a small population of damaged hepatocytes as mentioned by these authors. Correspondingly, Ca^{2+} would be expected to decrease membrane ionic permeability.

Table 2
The effect of Ca^{2+} upon substrate permeation of the plasma membrane

Condition	Basal	Oxygen uptake rate (μmol O_2/g wet wt min) + FCCP	Δ
–Digitonin	0.7	2.0	1.3
–Digitonin, + Ca^{2+} (220 μM)	1.4	1.4	0.0
+ Digitonin, + Ca^{2+} (330 μM)	1.3	4.5	3.2

The experimental conditions were the same as indicated in the legend to fig.2.

Indeed it was indicated previously that Ca^{2+} appeared to decrease the plasma membrane permeability to oxidizable substrates [6]. This effect is aptly demonstrated by the results of table 2. Uncoupler-stimulated substrate oxidation by intact cells is abolished by exogenous Ca^{2+}. If the plasma membrane permeability barrier to substrate is eliminated by digitonin, uncoupling agent causes the usual stimulation of oxygen utilization. Thus, the observed effects of calcium upon hepatocyte metabolism may result from its action upon a damaged population of cells and/or an influence upon plasma membrane permeability.

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