CHARGE TRANSFER DURING VALINOMYCIN-INDUCED Ca^{2+} UPTAKE IN RAT LIVER MITOCHONDRIA

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1. Introduction

Several lines of evidence indicate that the driving force of Ca^{2+} uptake in mitochondria is a membrane potential with negative intramitochondrial polarity [1–8]. Disputes exist, however, concerning the charge stoichiometry during Ca^{2+} flux. Measurements of K^+ and Ca^{2+} distribution ratios during respiration in the presence of valinomycin suggest that Ca^{2+} is distributed with 2 net positive charges according to Nernst equation [3]. However, other workers have failed to see a simple quantitative correlation between the cation ratios when experimental conditions were varied [7,9]. Measurements of H^+/Ca^{2+} flux ratios have also been used as indicators of the charge stoichiometry of Ca^{2+} transport (reviewed [10]). The variability of the results in the past have mainly been due to inability to exclude simultaneous H^+ movements unrelated to Ca^{2+} transport [11]. Moyle and Mitchell [12,13] have reported that a H^+/Ca^{2+} ratio of 1 is obtained at steady state after Ca^{2+} uptake [12] and they suggested that Ca^{2+} is translocated single-charged by a Ca^{2+}/phosphate symporter [13]. However, the results of Moyle and Mitchell [12,13] have been questioned by Reynafarje and Lehninger [14] who measured the initial rates of H^+ extrusion and Ca^{2+} uptake. They arrived at a ratio of 2 both from measurements of initial rates and steady state distributions of the ions [14]. They concluded that the difference could be due to the fact that Moyle and Mitchell [12,13] did not measure Ca^{2+} transport but approximated that all the Ca^{2+} added was taken up in their conditions. A H^+/Ca^{2+} ratio suggesting a transfer of 2 charges/Ca^{2+} translocated has also been determined in conditions where only the terminal part of the respiratory chain was active using ferrocyanide as electron donor [15]. This conclusion depends on the finding that 4 electrical charge equivalents are translocated per pair of electrons by cytochrome *c* oxidase [15,16]. As Ca^{2+} uptake can be driven by an artificial potassium ion diffusion potential induced by valinomycin [17], the aim of present work is to determine the K^+ efflux/Ca^{2+} influx stoichiometry in such conditions and hence the charge stoichiometry of Ca^{2+} uptake. The results indicate that 2 K^+ are extruded/Ca^{2+} taken up and hence that Ca^{2+} is taken up into mitochondria as a bivalent cation.

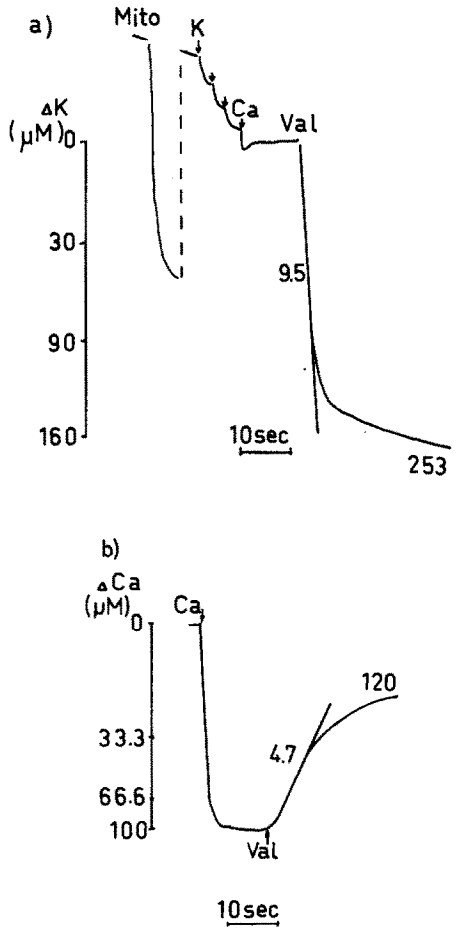
2. Materials and methods

Liver mitochondria were prepared from young male Sprague Dowley rats by a conventional method [18]. Ca^{2+} uptake was measured either with a Radiometer F 2112 Ca selectrode connected through a Datex IM-555 pH meter to a recorder or with the murexide method [19] using the wavelength pair 540–507 nm in an Aminco DW 2 spectrophotometer. The electrode was calibrated as in [20]. K^+ transport was measured with a Beckman no. 5826 K^+-sensitive electrode and pH changes with an Ingold T 7209 combination electrode. The measured response times for the Ca^{2+} electrode were in the order of 1–2 s, K^+ and pH electrodes below 1 s. The standard reaction medium contained 0.25 M sucrose, 10 mM Hepes, pH 7.2 Tris, 10 μM rotenone, 10 ng/ml oligomycin, 5 mM NaCN and 0.5 μg/ml BSA (bovine serum albumin). Reaction temp. 25°C.

All reagents used in this work were commercial products of the highest grade.

3. Results

Addition of valinomycin to non-respiring mitochondria causes a fast release of K^+ into the medium and concomitant uptake of Ca^{2+} (fig.1). No significant K^+ extrusion occurs in the absence of Ca^{2+} (in the presence of EGTA) in the present conditions. Note that there is a small lag period in the Ca^{2+} electrode response upon addition of valinomycin. A similar lag is often also seen upon starting Ca^{2+} uptake by the addition of succinate (not shown). The rate of Ca^{2+} uptake was always determined from the end of the lag period. From the data a K^+ efflux versus Ca^{2+}



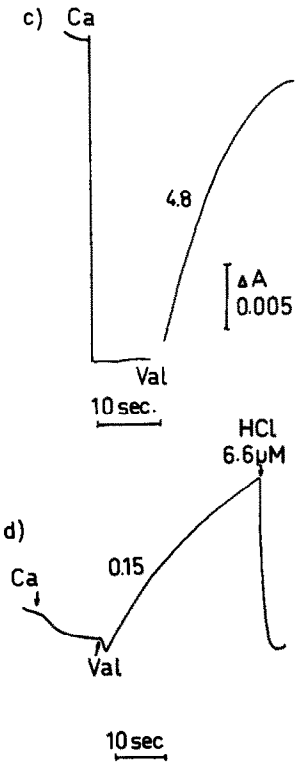


Fig.1. Initial rates of K^+ efflux and Ca^{2+} influx upon addition of valinomycin to non-respiring mitochondria. Mitochondria (2 mg protein/ml) were incubated in the standard reaction medium. In (a) a trace obtained with the K^+ electrode. Addition of mitochondria (Mito), change in O position (– – –), additions of 12 nmol KCl (K), 100 μM Ca^{2+} and 100 ng/l valinomycin (Val) as indicated. Calibration of the electrode response in similar conditions (scale on the left) except that the valinomycin addition was omitted and 12 nmol pulses of KCl were added to the medium. Volume 1.5 ml. In (b) a Ca^{2+} electrode trace. Mitochondria and 36 nmol KCl added before experiment. Addition of 100 μM $CaCl_2$ (Ca) and 100 ng/l valinomycin (Val) as indicated. Electrode response (scale on the left) calibrated by adding 10 nmol Ca^{2+} pulses in identical conditions and was controlled by the addition of 2 μM ruthenium red after uptake experiment and Ca^{2+} pulses subsequently. Volume 1.5 ml. In (c) a trace with the murexide technique. Mitochondria and 60 nmol KCl added before experiment. Additions of 100 μM $CaCl_2$ (Ca) and 100 ng/l valinomycin (Val) as indicated. Volume 2.5 ml. In (d) a trace with the pH electrode. Mitochondria and 36 nmol KCl added before experiment. Addition of 100 μM Ca^{2+} (Ca), 100 ng/ml valinomycin (Val) and 8 μM HCl as indicated. Medium same as (a–c) except that 1 mM Tris–Cl was used as buffer. Numbers on slopes indicate calculated initial rates expressed as nmol/mg protein/s and at the completion of experiment extent of change expressed as nmol.

Table 1
Initial rates of K^+ and Ca^{2+} fluxes induced by valinomycin

	K^+ efflux	Ca^{2+} influx (electrode)	Ca^{2+} influx (murexide)	K^+/Ca^{2+} electrode	Murexide
Initial rate (nmol/mg protein/s)	10.7 ± 0.75	4.7 ± 0.2	4.7 ± 0.1	2.2	2.2
Steady state (nmol/mg protein)	88.4 ± 3	43 ± 2	46 ± 2	2.05	1.92

Conditions as in fig.1

influx ratio of near 2 is obtained both for the initial rate values and at steady state.

Measurements of H^+ movements in similar conditions (except with 1 mM Tris–Cl as buffer) show that no or only insignificant proton uptake occurs during the initial Ca^{2+} uptake phase (fig.1). Table 1 summarizes the mean stoichiometry from 5 experiments in the present conditions.

4. Discussion

The results of the present work show that 2 K^+ are extruded/Ca^{2+} taken up when valinomycin is added to non-respiring mitochondria suspended in a sucrose medium suggesting that 2 positive charges are transferred across the membrane/Ca^{2+} translocated. The results thus also indicate that the measured H^+/Ca^{2+} ratios [14,15] appear to represent ratios of charge transfer across the membrane. Since much controversy exists for the time being concerning the stoichiometry of H^+ translocation versus e^- transfer in the respiratory chain [16,21,22] it is of importance to determine the charge transfer of Ca^{2+} with other methods than only using H^+/Ca^{2+} or Ca^{2+}/O ratios.

It has been argued [23,24] that if Ca^{2+} is distributed with 2 net charges with a membrane potential of 180 mV [25–27] or more [28] very high gradients of Ca^{2+} would be created across the mitochondrial membrane [23,24].

Furthermore K^+ and Ca^{2+} distribution ratios do not show a simple relationship according to Nernst equation when experimental conditions are varied [3,7,9]. Thus the factors determining the steady state retention of Ca^{2+} are more complex than the uptake mechanism. A charge transfer of 1 charge/Ca^{2+} translocated has been measured during nitriloacetate-induced Ca^{2+} efflux from de-energized mitochondria [8]. This would suggest that Ca^{2+} efflux might be mediated by a different mechanism [6,29], which possibly involves an H^+/Ca^{2+} exchange [29], because Ca^{2+} is released from mitochondria upon addition of small acid pulses [30]. The uptake mechanism could simply involve a conduction of Ca^{2+} through a channel [31,32].

Clarification of these points is of importance concerning the ability of mitochondria to translocate Ca^{2+} (i.e., the ability of these particles to create Ca^{2+} gradients) and hence their role in regulation of cytosolic Ca^{2+}.

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