COMPETITIVE INHIBITION OF VALINOMYCIN-INDUCED K^+-TRANSPORT BY Mg^{2+}-IONS IN LIVER MITOCHONDRIA

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Received 28 April 1977

1. Introduction

Mitochondria are almost impermeable for K^+-ions. This restriction of K^+-ion permeability is apparently essential for mitochondrial function. As has been shown earlier the limitation of alkali-cation permeability depends on the presence of Mg^{2+}-ions. Simple chelation of Mg^{2+} by EDTA increased the K^+-ion permeability of isolated liver mitochondria [1–3]. EDTA increased also Na^+-ion permeability [4]. Addition of Mg^{2+}-ions restored the permeability for alkali cations to the original, low level. Very recently the divalent-cation ionophore, A 23187 was used in combination with EDTA to deplete the mitochondria of their endogenous Mg^{2+}-ion content: as a result the permeability for K^+-ions was greatly increased [5]. All these effects were taken as evidence that a postulated endogenous K^+–H^+ exchange system is operative in the absence of Mg^{2+}-ions and is inactivated in its presence [5]. It was supposed that either the same or a similar exchanger is responsible for the transport of Na^{2+}-ions [4]. The mechanism by which Mg^{2+}-ions would change the membrane permeability was left open. One of the possibilities is that Mg^{2+}-ions change the physical structure of the membrane, its inner fluidity, in a way that the intra-membrane mobility of the exchanger decreases. This hypothesis could be tested by investigating the effect of Mg^{2+}-ions on transport catalyzed by exogenous carriers.

The K^+-ion permeability of membranes is radically increased by the ionophore, valinomycin (see [6] for review). Valinomycin acts as a mobile carrier for K^+-ions and its characteristics as a mobile carrier are well established [7]. If Mg^{2+}-ions would change the endogenous K^+ permeability of mitochondria by decreasing the mobility of an endogenous carrier then the transporting activity of the exogenous mobile carrier should also be decreased by Mg^{2+}-ions. We found indeed that added Mg^{2+}-ions inhibited the effect of valinomycin in liver mitochondria: this inhibition was competitive with valinomycin. The results are compatible with the hypothesis outlined above.

Abbreviations: Cl-CCP, carbonyl cyanide-*m*-chlorophenylhydrazone; TMPD, 5,5,5′,5′-tetramethylparaphenylene-diamine

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2. Materials and methods

Valinomycin A grade, Cl-CCP (Calbiochem) and rotenone (K and K Laboratories Inc., Plainview, NY) were dissolved in ethanol and added as a volume of a few microliters.

Rat liver mitochondria were isolated according to Johnson and Lardy [8]. The action of valinomycin was assayed in two different types of experiments, either in respiring or in respiration-inhibited mitochondria.

2.1. *Effects of valinomycin in respiring mitochondria*

In respiring mitochondria three events which occur on addition of valinomycin parallel to K^+-ion uptake were followed [9,10].

(a) H^+-Ion extrusion in the absence of any 'permeant' anion added in a weakly buffered medium at room temperature was recorded by the glass electrode and a Radiometer pH meter 26 connected to a Goerz Servogor 2 recorder. The medium contained 248 mM sucrose, 10 mM KCl, 0.5 mM Tris–Cl, 1.3 mM Na-succinate and 1 μM rotenone: the initial pH was 7.0. In

this type of experiment valinomycin was added during the recording to the already respiring mitochondria to start H^+-ion extrusion. The addition of standard amount of HCl to the suspension served to calibrate the deflection of the recorder.

(b) The increase of the mitochondrial volume (swelling) which occurred when K^+-ions were accumulated in the presence of 'permeating' anions, either phosphate or acetate at ambient temperature was recorded optically by the decrease of absorbance using the Beckman DK2A spectrophotometer set at 546 nm with a high-absorbance standard in the reference path. In this case the ion uptake was initiated by the addition of the respiratory substrate to the suspension when valinomycin was already present. The medium contained 243 mM sucrose, 10 mM KCl, 1 mM Tris–phosphate and 1 μM rotenone, the final pH being 7.0. The substrate for respiration was either 1.3 mM Na-succinate, or in some experiments 6.7 mM Tris–ascorbate plus 100 μM TMPD. In some experiments the Tris–phosphate was replaced by 5 mM Tris–acetate.

(c) Respiration, which was increased by valinomycin in the presence of K^+ and of 'permeant' anions, was recorded in the same medium as was absorbance at 25°C by using the Clark-type electrode of the Gilson Oxygraph. The substrate was succinate and valinomycin was added as last during the course of respiration.

Mitochondria were added to give a final concentration of 1.2 mg protein/ml in (a) and (b) and 1.8 mg/ml in (c). When added, $MgCl_2$ was always present before valinomycin.

2.2. *Osmotic swelling of mitochondria with inhibited respiration*

Osmotic swelling was recorded optically at 546 nm in a medium containing 150 mM K-acetate, 5 mM Tris–Cl and 1 μM rotenone at pH 7.4. The swelling was initiated by addition of 1.7 μM Cl-CCP and valinomycin. Mitochondria were added to give a final concentration of 0.8 mg protein/ml.

3. Results

3.1. *Respiring mitochondria*

Freshly prepared respiring rat-liver mitochondria take up K^+-ions very slowly, with very slow H^+-ion extrusion, no swelling occurs and the respiration is at a low level, because the K^+-ion permeability of the mitochondrial membrane is low. Valinomycin complexes with K^+-ions and the complex is able to cross the mitochondrial inner membrane. With valinomycin present, respiring mitochondria take up rapidly and extensively K^+-ions with H^+-ion extrusion and with swelling and acceleration of respiration, these latter two depending on the presence of 'permeant' anions. All these processes are a function of valinomycin added and show saturation kinetics (figs 1–3). The amount of valinomycin which gives in the different systems one-half of the maximal rate of change was calculated from double reciprocal plots: in the different mitochondrial preparations it was found to be between 1.05 ng and 2.4 ng valinomycin/mg protein which corresponds to a concentration of about $1–2 \times 10^{-9}$ M.

Any inhibition by Mg^{2+} of the valinomycin-induced transport would either decrease the maximum rate of transport (V_{max}) or would change the affinity of the system for valinomycin.

Figure 1 shows that 6 mM $MgCl_2$ inhibited considerably the rate of H^+-ion extrusion caused by valinomycin. The inhibition was counteracted by increasing the amount of the ionophore.

The rate of swelling induced by valinomycin in the presence of phosphate was inhibited by 1 mM and by 5 mM $MgCl_2$, as is shown in the double reciprocal plot on fig.2. The inhibition was competitive with valinomycin: the amount of the ionophore which gave half-maximal rate of swelling was 1.05 ng/mg protein

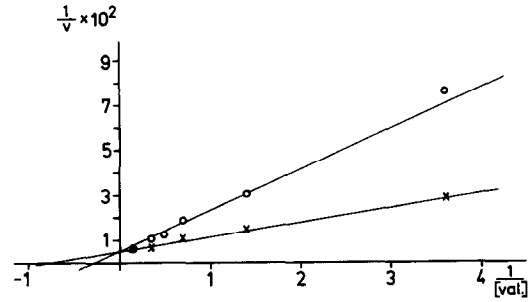

Fig.1. Effect of $MgCl_2$ on the proton extrusion induced by valinomycin: double reciprocal plot. The values for valinomycin are given as ng/mg protein; those for H^+-ion extrusion as ng ion/min/mg protein. (o——o) 6 mM $MgCl_2$; (X——X) control.

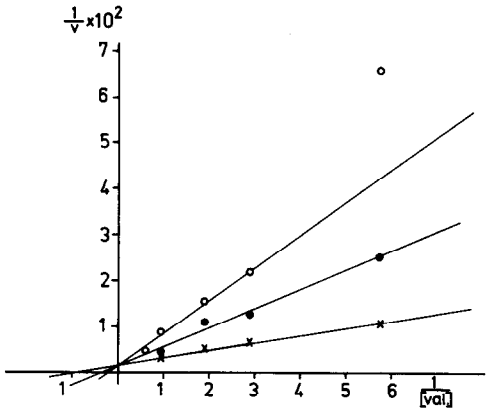

Fig.2. Effect of $MgCl_2$ on the rate of swelling induced by valinomycin: double reciprocal plot. The ion uptake was linked to respiration (see Materials and methods, 2.1. (b)). The values for valinomycin are given as ng/mg protein; for the rate of swelling as the change in absorbance/min. (o——o) 5 mM $MgCl_2$; (•——•) 1 mM $MgCl_2$; (X——X) control.

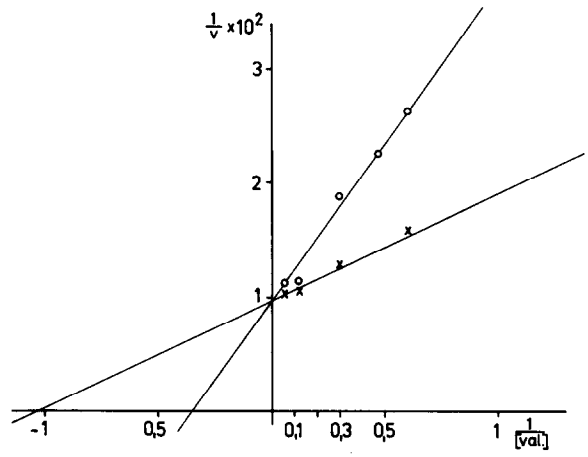

Fig.3. Effect of $MgCl_2$ on the rate of respiration stimulated by valinomycin: double reciprocal plot. The values for valinomycin ar given as ng/mg protein; for the rate of respiration as ng atom oxygen/min/mg protein. (o——o) 5 mM $MgCl_2$; (X——X) control.

without added Mg^{2+} and this value was increased to 4.0 ng/mg in the presence of 5 mM Mg^{2+}. Identical results were obtained when phosphate was replaced by 5 mM acetate. The substrate for respiration was succinate in fig.2: similar competitive inhibition by Mg^{2+} was also seen when ascorbate together with TMPD served as substrate. From these experiments it follows that the site of inhibition by Mg^{2+}-ions is neither the transport of permeant anions, phosphate or acetate, as both enter the mitochondria by entirely different mechanisms, nor the accessibility of succinate as electron donor.

In the presence of Mg^{2+} the activation of respiration by valinomycin was much less than in its absence and the inhibition was in this case also competitive (fig.3). Mg^{2+} did not affect the respiration stimulated by ADP (State 3) nor the uncoupled respiration with succinate as substrate.

3.2. *Non-respiring mitochondria: osmotic swelling*

The swelling of respiration-inhibited mitochondria in iso-osmotic K-acetate requires the presence of both an uncoupler (Cl-CCP was used in our experiments) and of valinomycin [11,12]. At a fixed Cl-CCP concentration (1.7 μM) the initial rate of swelling depended on the amount of valinomycin added, but much higher levels of valinomycin were required to give swelling in this type of experiment. Without added Mg^{2+}-ions half-maximal rate of swelling was obtained with 50 ng valinomycin/mg protein and this value was increased to 100 ng/mg in the presence of 5 mM $MgCl_2$.

4. Discussion

The alkali-cation permeability of the mitochondrial inner membrane resembles that of simple phospholipid bilayers, the liposomes. It is extremely unfavourable energetically for these cations with a highly concentrated charge to penetrate the apolar phase of the bilayer resp. inner membrane. In the valinomycin–K^+-ion complex the charge is distributed within the molecule and the apolar character of the outer part of valinomycin makes the complex lipid-soluble. According to current views [6,7] valinomycin resides mainly within the membrane phase because of its partition coefficient between lipids and water. It reacts with the K^+-ions of the water phase at the boundary of the membrane, removes the solvation shell of the ions and the whole complex thus formed gains mobility within the apolar membrane phase. The complex dissociates again at the opposing surface of the membrane. Both the amount of valinomycin available and the speed with which it diffuses in the membrane determine the

rate of transport. We postulated that Mg^{2+}-ions bound to the membrane surface change the fluidity of the interior of the membrane and thus decrease the mobility of the valinomycin–K^+-complex in it. Changes in the lipid microviscosity of sarcoplasmic reticulum membranes caused by Mg^{2+}-ions were reported very recently [13].

If Mg^{2+} acts by changing the membrane fluidity then it is expected that an ionophore which does not require free 'shuttling' movement within the membrane would be insensitive against Mg^{2+}-ions. Gramicidin, a channel-forming peptide which induces alkalication permeability in different membranes [6] is an obvious candidate for performing this type of experiment: the action of Mg^{2+}-ions on gramicidin-induced transport is presently under investigation.

At the present state of information it is possible that Mg^{2+}-ions act in a different way: bound to the outer surface of the membrane they charge it positively and this charge could interfere with the formation of the valinomycin–K^+-ion complex. Transport kinetics of ions will turn sigmoidal if the surface of the membrane will be charged in the same sense as the transported ion [14,15]. If Mg^{2+}-ions would act by simply charging the membrane then the plot of transport rate versus concentration (either valinomycin or K^+) would be changed from hyperbolic into sigmoidal. No such change was found as yet in preliminary experiments.

It is reasonable to assume that Mg^{2+}-ions inhibit induced K^+ permeability and endogenous K^+ permeability by the same mechanism.

Acknowledgements

This work is part of the research project 'Carrier mechanisms of biological membranes' and was supported by the grant No. 1-01-0306-01-1/F of the Hungarian Ministry of Health. The authors thank Mrs Julianna Szilassay for expert and devoted te technical assistance and Dr J. Harmath (ORMI, Budapest) for the use of the Beckman DK2A spectrophotometer.

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