INHIBITED RESPIRATION AND ATPase ACTIVITY OF RAT LIVER MITOCHONDRIA UNDER CONDITIONS OF MATRIX CONDENSATION

D.G. NICHOLLS and O. LINDBERG

Wenner-Gren Institute, Norrtullsgatan 16, S-113 45 Stockholm, Sweden

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1. Introduction

It has long been recognised that a rather non-specific inhibition of mitochondrial respiration is obtained in the presence of high concentrations of sucrose [1–3] or uncouplers of oxidative phosphorylation [4,5]. Similar inhibitions may be obtained by the addition of ionophores under conditions resulting in ion efflux from the mitochondrial matrix [6–9]. The uncoupler stimulated ATPase activity of liver mitochondria also appears to be sensitive to inhibition under related conditions [10,11].

A common feature of these conditions is that they lead to an osmotically related decrease in the volume of the matrix compartment, either by an increase in external tonicity or by a loss of osmotic support within the matrix. Recent results from our laboratory [12] have shown that the respiration of hamster brown adipose mitochondria, in the presence of a variety of substrates, may be correlated with the size of the matrix compartment, suggesting a causal relationship between matrix condensation and respiratory inhibition. In the light of these results it was of interest to determine whether some of the inhibitions described above could be similarly ascribed.

2. Methods

Rat liver mitochondria were prepared and stored at 0° in 0.25 M sucrose. Oxygen uptake was determined amperometrically at 23° in a chamber having a volume of 1.4 ml. All media were potassium free and adjusted to pH 7.2 with Tris. The sucrose impermeable space was determined by a $[^3H]H_2O$, $[^{14}C]$sucrose dual label modification of the technique described by Malamed and Recknagel [13]. Mitochondrial protein was determined by the biuret method. ATPase activity was determined by the method of Lindberg and Ernste [14].

Abbreviations:

Tris : tris (hydroxymethyl) aminomethane;
DNP : 2,4-dinitrophenol.

3. Results and discussion

When rat liver mitochondria are incubated in a medium low in potassium with the addition of nigericin, or DNP and valinomycin, there is a loss of potassium, an inhibition of respiration and an increase in light scattering indicative of mitochondrial contraction [6, 8,9]. Fig. 1, however, shows that this respiratory in-

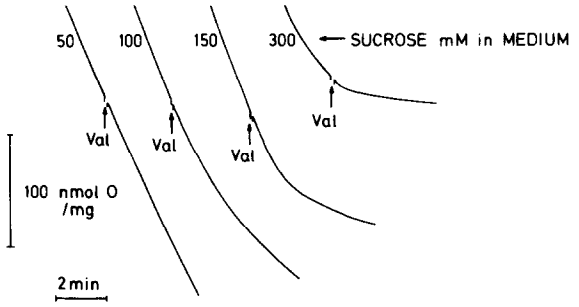

Fig. 1. The effect of valinomycin on the oxidation of malate and glutamate by mitochondria in the presence of DNP and varying sucrose concentrations. The incubation contained DNP 50 μM, Tris-phosphate 4 mM, $MgCl_2$ 2 mM, Tris-EDTA 1 mM, Tris-malate 3 mM, Tris-glutamate 3 mM and sucrose as shown. Where indicated valinomycin, 0.5 μM, was added. pH 7.2, 23°.

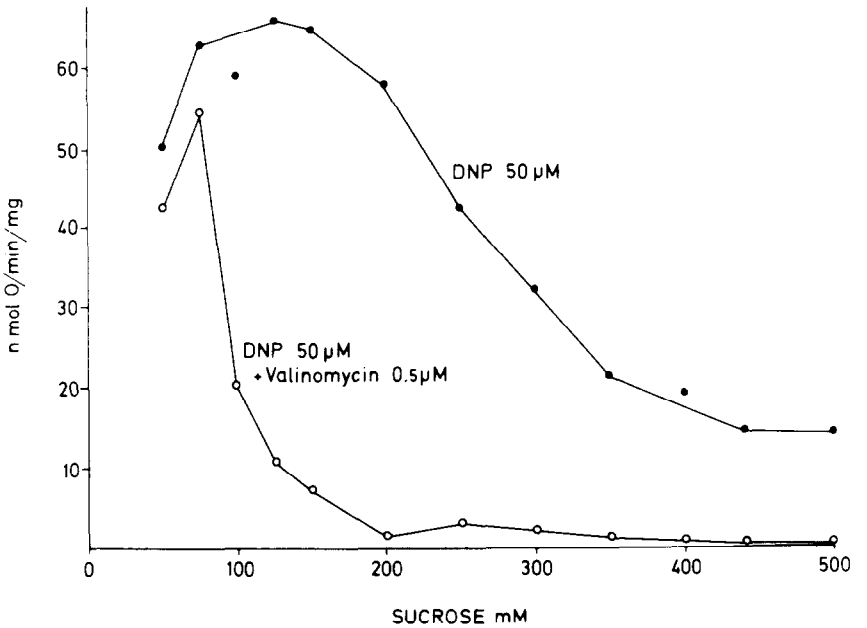

Fig. 2. The inhibition by sucrose of malate and glutamate oxidation in the presence of DNP and DNP plus valinomycin. Incubation conditions were as described for fig. 1. Oxygen uptake in the presence of DNP alone was measured immediately after addition of mitochondria, while when valinomycin was present, rates were measured after 5 min.

hibition is dependent on the osmolarity of the incubation medium. At a low tonicity no effect of added valinomycin can be observed within the time scale of the trace, but on increasing the sucrose concentration the delay before inhibition is apparent decreases, while the extent of inhibition increases. Fig. 4 contains controls in the presence of 50 μM DNP alone. We interpret these results to imply that under hypotonic conditions, the medium does not exert sufficient osmotic pressure on the matrix to collapse it to an inhibitory level even after the valinomycin induced loss of osmotically active ions.

Support for this contention is provided by fig. 2, which compares the inhibitory effect of sucrose on malate and glutamate oxidation by mitochondria in the presence of DNP, and after the further addition of valinomycin. It is clear that this latter condition greatly increases the sensitivity of the sucrose-induced inhibition. Hamster brown adipose mitochondria show a similar high sensitivity when freshly prepared [12], and are believed to be depleted of potassium [15] and phosphate [16] on preparation. These results are consistent with respiratory inhibition resulting from osmotically induced collapse of the matrix compartment.

More direct confirmation of a valinomycin induced decrease in matrix volume is given in fig. 3, where the sucrose impermeable space has been determined under the same conditions as fig. 2. Both in the presence and absence of valinomycin, inhibition of malate and glutamate oxidation therefore appears to set in when the sucrose impermeable space decreases below 0.5 μl/mg protein (determined by biuret).

The inhibition of malate and glutamate oxidation by DNP alone bears qualitative resemblance to that induced by DNP and valinomycin. Thus fig. 4 shows that in hypotonic sucrose even 180 μM DNP causes only a moderate respiratory inhibition, whereas in hypertonic medium 54 μM DNP is sufficient for almost complete inhibition within the course of the experiment. With both 300 mM sucrose and high uncoupler concentration the inhibition is most pronounced but is still evidently time dependent. In this latter case dilution of the incubation with water is sufficient to reactivate respiration. It is thus likely that at least some of the uncoupler-induced respiratory inhibitions which have been reported may be related to the osmotic collapse of the matrix compartment.

The sensitivity of the uncoupler stimulated ATPase

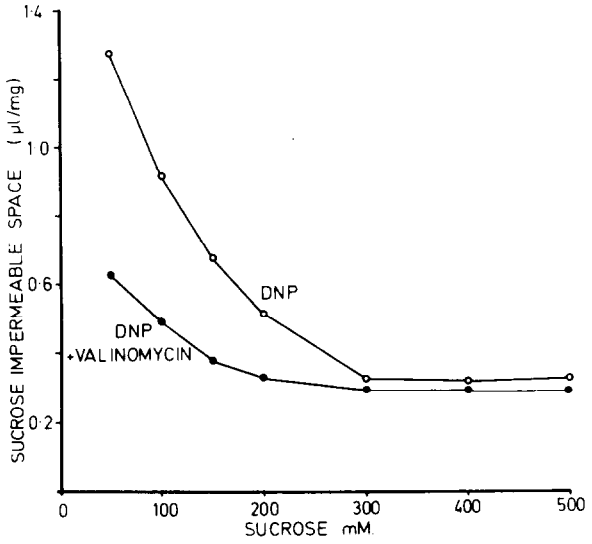

Fig. 3. The sucrose impermeable space of mitochondria. The incubation media were as described for fig. 2 with the inclusion of [^{3}H]H_2O (1 μCi) and [^{14}C]sucrose (0.1 μCi). The spaces in the presence of DNP alone were determined 3 min after addition of mitochondria, while 10 min was allowed for the incubation with the inclusion of valinomycin.

activity to conditions resulting in a decrease in the matrix volume was also investigated. High concentrations of sucrose [10], or the addition of ionophores under conditions leading to ion depletion of the matrix, [11], both lead to a decrease in ATPase activity. Fig. 5 shows that the inhibition resulting from the inclusion of valinomycin is sensitive to the osmolarity of the sucrose medium in much the same way as was the mitochondrial respiration shown in fig. 2.

Almost certainly it is an oversimplification to ascribe all inhibitory effects of uncouplers, ionophores or hyperosmolarity simply to the osmotic collapse of the matrix. It is, however, a phenomenon which must be carefully eliminated before alternative explanations are accepted.

It is possible only to speculate on the mechanism of the observed inhibitions. The loss of water from the matrix is of course parallelled by an increase in the concentration of the material enclosed by the inner membrane, and a level of dehydration could easily be reached when the matrix components precipitate or the matrix becomes so viscous as to limit diffusion. In addition, electron micrographs of highly condensed

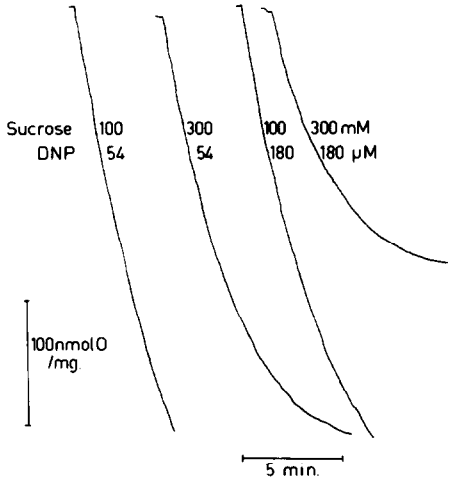

Fig. 4. The effect of DNP alone on malate and glutamate oxidation. The medium was as described for fig. 1.

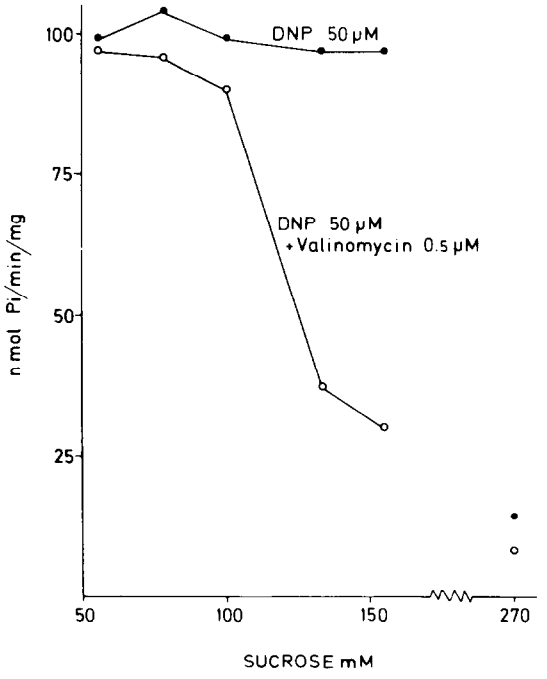

Fig. 5. DNP-ATPase activity of mitochondria. Mitochondria were preincubated for 5 min at 23° in a medium containing 20 mM Tris-Cl, pH 7.2, DNP 50 μM and sucrose and valinomycin as indicated. 3 mM ATP was then added and the incubation continued for 10 min.

brown adipose mitochondria [17], show that large areas of inner membrane may be deprived of contact with matrix, thus rendering a large proportion of permease or translocase activity non-functional.

It is unlikely that such extensive matrix condensation could occur *in vivo*, but it should be considered as a factor complicating the interpretation of *in vitro* experimentation.

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