DEPENDENCY OF THE 2,4-DINITROPHENOL STIMULATED ATPase ACTIVITY ON K^+ AND RESPIRATION

A. Gómez-Puyou, F. Sandoval, M. Tuena de Gómez-Puyou, A. Peña and E. Chávez

Departamento de Bioquímica, Facultad de Medicina, Ciudad Universitaria, Apartado Postal 70159, México, D. F., México

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Summary

The 2,4-dinitrophenol (DNP) stimulated ATPase activity has been studied in rat liver mitochondria which have a K^+ content of 15 mM or less. It has been found that in these mitochondria the ATPase activity becomes strictly dependent on K^+ and on electron transport. The activity reaches a maximum with 20 mM KCl and is inhibited by rotenone and antimycin. Succinate, in some experiments, enhances the DNP stimulated ATPase activity in a malonate sensitive process.

Lardy and Wellman (1953) reported that KCl and NaCl increased the DNP stimulated ATPase activity, a finding that was later confirmed by Myers and Slater (1957). In agreement with these results Amons, Van Den Bergh and Slater (1968) showed that there is a strong dependency of the DNP stimulated ATPase activity for added cations and explained their results by assuming that cations facilitated the entrance of ATP into the mitochondria. More recently, Brierley et al., (1971) reported that the p-chloromercuriphenylsulfonate (CMS) activated ATPase of heart mitochondria had a marked requirement for K^+.

Although both the CMS and the DNP activated ATPases seem to depend on K^+, there is some controversy as to the role of electron transport on the CMS and DNP activated ATPases. Veldsema-Currie and Slater (1968) reported, in extension of previous studies (Myers and Slater, 1957), that cyanide which caused a strong reduction of the respiratory chain did not affect the DNP-activated ATPase, but on the other hand, Brierley et al., (1971) showed that the

CMS activated ATPase was enhanced by respiration. In this paper, it will be shown that in conditions in which the DNP activated ATPase becomes strictly dependent on K^+, the activity also becomes dependent on electron transport.

Methods

Rat liver mitochondria with a K^+ content of 15 mM or less prepared as described elsewhere (Gómez-Puyou et al., 1970) were used in all experiments. The ATPase activity was assayed by adding the mitochondria to 1.5 ml of an incubation media whose composition is detailed under Results. The mitochondrial mixture was incubated in 50-ml Erlenmeyer flasks for 10 minutes at 25° under vigorous shaking. The reaction was stopped with trichloroacetic acid at 5 percent final concentration. Inorganic phosphate was determined in the supernatant according to Sumner (1944).

Results

Figure 1 shows that the DNP stimulated ATPase activity of K^+ depleted mitochondria is progressively inhibited by increasing concentrations of sucrose. On the other hand, if the tonicity of the media is increased with KCl instead of sucrose, the activity diminishes, but at 100 mM KCl, the DNP stimulated ATPase activity is significantly higher than at 200 mM sucrose. These results suggest that at relatively high tonicities, the DNP stimulated ATPase activity may have a cation requirement.

Valinomycin has been shown to increase the permeability of the mitochondria to K^+ (Moore and Pressman, 1964). Thus, the effect of various concentrations of valinomycin was tested on the DNP activated ATPase of K^+ depleted mitochondria incubated at 200 mM sucrose and 20 mM KCl. Valinomycin at the concentrations indicated in Table I did not affect the ATPase activity, but induced a significant increase of the DNP stimulated ATPase activity. These results substantiate the above mentioned suggestion that the DNP activated

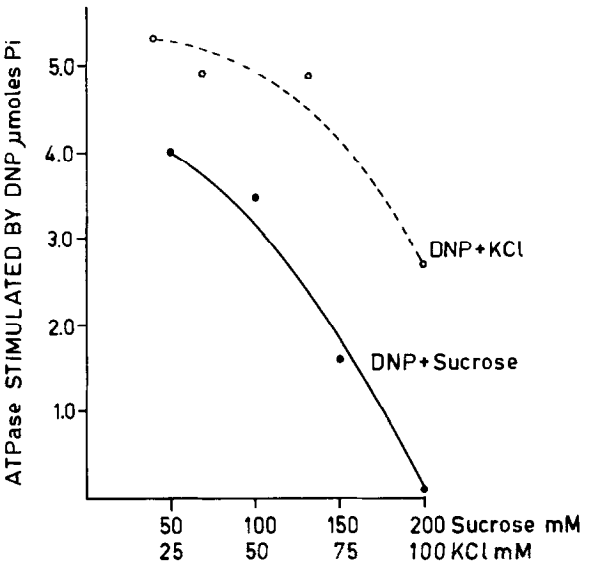

Figure 1. Effect of Sucrose and KCl on the DNP Stimulated ATPase Activity. The incubation conditions were: 11 micromoles of ATP, 2 mM Tris-HCl (pH 7.3) 1 mM EDTA. The indicated concentration of sucrose or KCl and 3.7 mg of mitochondrial protein. The increment of inorganic phosphate (Pi) produced by the addition of 0.1 mM DNP in 10 minutes of incubation is expressed.

TABLE I

EFFECT OF VALINOMYCIN ON THE DNP STIMULATED ATPase ACTIVITY

Valinomycin ng per mg	µmoles of Pi formed	
	-DNP	+DNP
-	0.6	1.3
0.3	0.4	1.8
1.5	0.7	2.2
3.0	0.9	2.5

3.7 mg of mitochondrial protein were added to 1.5 ml of a mixture which contained 11 micromoles of ATP, 20 mM KCl, 200 mM sucrose, 2 mM Tris-HCl (pH 7.3), 1 mM EDTA, 0.1 mM DNP and the indicated concentration of valinomycin. The micromoles of inorganic phosphate (Pi) formed in 10 minutes are expressed.

ATPase indeed has a K^+ requirement since valinomycin, by augmenting the permeability of the mitochondria to K^+, enhances the DNP stimulated ATPase activity.

The dependency of the DNP activated ATPase on K^+ is shown in Figure 2. Up to a concentration of 20 mM KCl, the DNP activated ATPase increases as the concentration of K^+ is raised. Above this concentration, the DNP plus valinomycin stimulated ATPase activity starts to diminish.

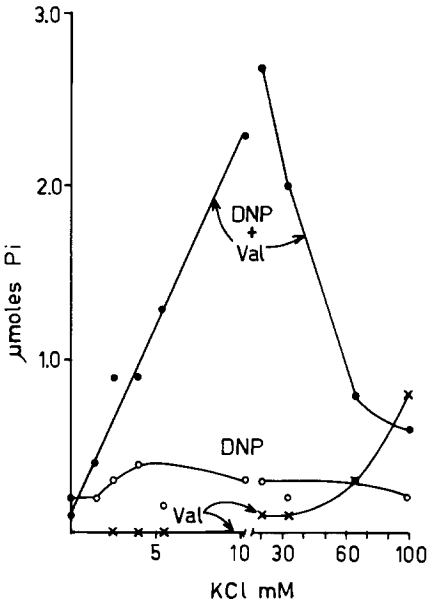

Figure 2. Effect of KCl on the DNP Stimulated ATPase Activity. The incubating conditions were as in Table I except that the mixture contained the indicated concentrations of KCl and 12 ng of valinomycin (Val). The increment of inorganic phosphate (Pi) produced by 0.1 mM DNP and/or valinomycin in 10 minutes of incubation is plotted.

The effect of respiratory inhibitors and succinate on the K^+ dependent ATPase is shown in Table II. Rotenone and antimycin inhibit the DNP plus valinomycin stimulated ATPase activity, the latter being somewhat more effective. The inclusion of succinate in the incubation media produced two types of response. In some experiments, succinate induced a significant in-

TABLE II

EFFECT OF ROTENONE, ANTIMYCIN, MALONATE AND SUCCINATE ON THE DNP STIMULATED ATPase ACTIVITY

		μ moles of Pi formed			
Exp.	Additions	-	DNP	Valinomycin	Valinomycin + DNP
1	-	0.5	0.9	1.0	4.0
	Rotenone	0.7	1.0	1.2	2.9
	Antimycin	0.9	0.9	1.3	2.2
2	-	0.7	1.4	1.2	2.1
	Succinate	0.3	1.5	0.7	4.2
3	-	0.7	1.1	0.7	3.8
	Succinate	0.5	1.1	1.3	3.8
	Succinate + Rotenone	0.5	1.1	1.4	3.3
	Succinate + Antimycin	1.0	1.0	1.2	1.5
	Succinate + Malonate	0.6	1.0	1.4	1.5

The incubating conditions were as in Table I; where indicated 12 ng of valinomycin, 160 ng of rotenone, 1 μg of antimycin, 3.0 mM succinate, and 40 mM malonate were included. The μ moles of inorganic phosphate (Pi) formed in 10 minutes of incubation by 3.7 mg of mitochondrial protein are expressed.

crease of the DNP plus valinomycin activated ATPase, while in other experiments, no such effect was observed (Experiments 2 and 3 of Table II respectively). This variation is probably due to the different content of endogenous substrates of the mitochondrial preparations. Nevertheless, Experiment 3 of Table II shows that in the presence of succinate, the DNP plus valinomycin stimulated ATPase activity is inhibited by antimycin and malonate, but not by rotenone; the latter, in the absence of succinate, does inhibit the ATPase ac-

tivity. These results indicate that the valinomycin facilitated ATPase activity induced by DNP is respiration dependent.

Discussion

Although the specificity of the DNP stimulated ATPase activity for cations has not been explored in this work, the results summarized in this communication show that only in the presence of valinomycin and K^+ does DNP stimulate high rates of ATPase activity. As the concentration of valinomycin employed does not affect the activity, it may be concluded that valinomycin is merely facilitating the action of DNP by diminishing the mitochondrial permeability to K^+, a conclusion that is substantiated by the fact that the DNP activated ATPase is higher in 100 mM KCl than in 200 mM sucrose. In our experimental conditions, the DNP activated ATPase also becomes dependent on electron transport.

This K^+ and respiration dependency of the DNP activated ATPase is remarkably similar to the behavior of the CMS activated ATPase described by Brierley et al., (1971). Thus, the mechanism of the DNP activated ATPase of K^+ depleted mitochondria can be fully explained according to Brierley's model (1971), based on the Mitchell's chemiosmotic hypothesis (1968), in which the action of K^+ would be to facilitate the entrance of ATP into the mitochondria. The role of respiration, with the subsequent generation of OH^- in the matrix as the chemiosmotic hypothesis implies, would be visualized, also in Brierley's model, as the driving force for a $H^+ - K^+$ antiport which would set up high ATPase rates. The $H^+ - K^+$ antiport in our experimental conditions would be facilitated by valinomycin and mediated by DNP. In this respect, Karlish et al., (1968) explained the synergistic uncoupling action of valinomycin and DNP in chloroplasts, on the grounds that DNP and valinomycin permitted the equilibrium across the membrane of H^+ and K^+ respectively.

However, there are several questions that cannot be satisfactorily answered by Brierley's model (1971). One of these is that the K^+ dependency of the DNP activated ATPase in our experimental conditions is much more marked than in the system of Lardy and Wellman (1953) and Amons et al (1968). Furthermore, in Brierley's paper (1971) no effect of added cations was detected on the DNP activated ATPase. In view of the fact that the main difference between the experimental conditions employed by other authors and ours is the K^+ content of the mitochondria, it is possible that at some stage of the oxidative phosphorylation sequence, K^+ exerts a direct action. In the preparations of Lardy and Wellman (1953) and Amons et al., (1968), the relatively higher K^+ content of their mitochondria might have masked this possible direct action of K^+ and only the cation facilitated entrance of ATP would have been detected. In this respect, there are several lines of evidence which indicate that K^+ (or other monovalent cations) may have a direct influence on oxidative phosphorylation: first, a preference for K^+ relative to Na^+ has been detected in mitochondrial ATPase either in whole mitochondria (Lardy and Wellman, 1953) or in a partially purified ATPase (Peña-Díaz et al., 1964); second, the State 3/State 4 ratios depend on the K^+ content of the mitochondria (Gómez-Puyou et al., 1970); and third, K^+ and other monovalent cations increase the P:O ratios of K^+ depleted mitochondria (Gómez- Puyou et al., to be published). Thus, it is possible that K^+ may have a direct influence on one of the steps involved in oxidative phosphorylation.

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