THE EFFECT OF ARSENITE ON RAT-LIVER MITOCHONDRIA

András CSILLAG
Experimental Research Department, Semmelweis University Medical School, Budapest VIII, Hungary

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1. Introduction

It has been known for some time that arsenite inhibits the oxidation of 2-oxoglutarate by specifically blocking vicinal dithiols of the 2-oxoglutarate dehydrogenase complex [1]. In liver mitochondria the inhibition is maximal at about 1 mM arsenite [2].To get complete inhibition it was earlier found essential to preincubate mitochondria with arsenite for approx. 10 min [3]. In this work we have confirmed these data: without preincubation, arsenite only inhibited 2-oxoglutarate oxidation after 10 min. In addition we have shown that the effect of arsenite set in considerably sooner in the presence of either valinomycin or uncouplers. We attribute this phenomenon to alterations in the penetration of arsenite into the mitochondria.

2. Experimental

Rat-liver mitochondria isolated by the method of Johnson and Lardy [4] were used. Oxygen consumption was measured at 25° by the vibrating platinum electrode. The respiratory substrates were either 2-oxoglutarate or succinate (the latter in the presence of rotenone). Other experimental details are given in the legends of the figures.

3. Results

Using 2-oxoglutarate as substrate the inhibition of respiration by arsenite commences considerably earlier in the presence of valinomycin than in its absence (fig. 1). In the control experiment arsenite, without preincubation, takes 10 min to decrease the rate of respiration to a minimum. Adding valinomycin to mitochondria respiring in the presence of arsenite causes a short initial period of fast respiration, with an almost complete block of respiration occurring in approx. 1 min. Subsequent addition of inorganic phosphate does not enhance the respiratory

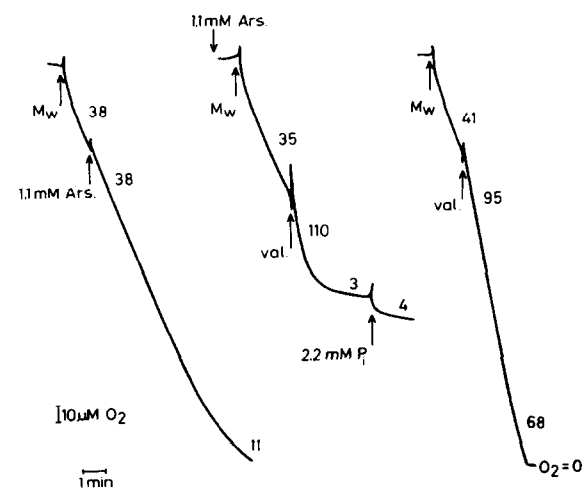

Fig. 1. Effect of valinomycin and arsenite on respiration. Substrate: Tris-2-oxoglutarate 5.1 mM. Reaction medium: 9 mM KCl; 5 mM $MgCl_2$; 92 mM sucrose; 26 mM Tris-HCl, pH 7.4, and where indicated 1.1 mM Na-arsenite in a final volume of 2.7 ml. Temperature 25°. Mitochondrial protein 2.1 mg/ml. Valinomycin 0.5 μg/2.7 ml. The figures above the tracings represent respiratory rates in μM O_2/min.

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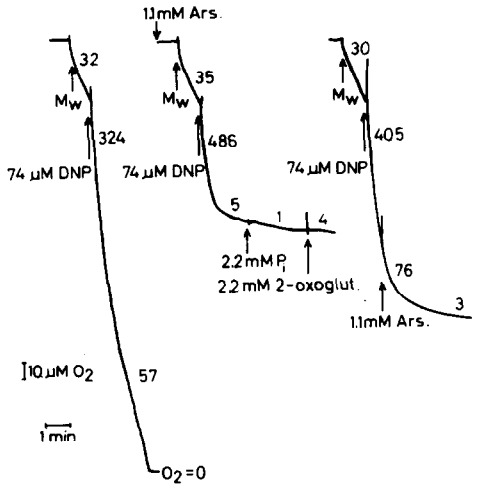

Fig. 2. Effect of DNP and arsenite on respiration. Conditions as in fig. 1.

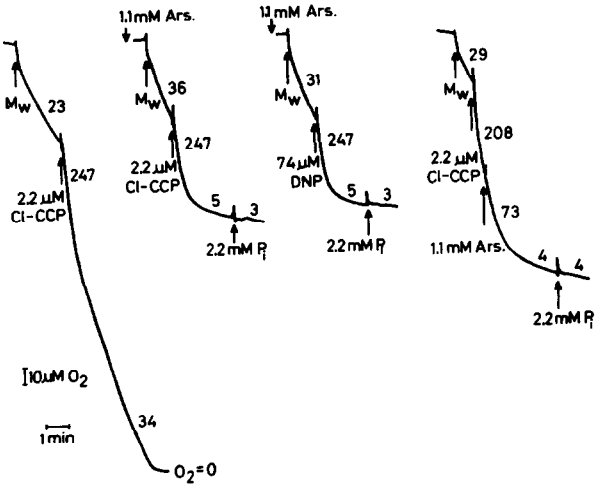

Fig. 3. Effect of Cl-CCP and arsenite on respiration. Conditions as in fig. 1.

rate. Valinomycine alone, without arsenite, has no inhibitory effect in these conditions.

Unexpectedly, uncouplers show an effect very similar to that of valinomycin in this system (figs. 2 and 3). Both 2,4-dinitrophenol (DNP) and carbonyl-cyanide *m*-chlorophenylhydrazone (Cl-CCP) increase the rate of respiration in the absence of arsenite. When arsenite is present, addition of either of the two uncouplers is followed after a brief period of rapid respiration by inhibition of respiration. Neither addition of phosphate, nor a further addition of substrate activated the inhibited respiration. The effects of DNP and Cl-CCP were similar when added prior to arsenite; in the presence of these uncouplers added arsenite inhibited respiration rapidly. The similar response to the two uncouplers is shown in fig. 3. With succinate as substrate, in the presence of rotenone, arsenite was ineffective in blocking respiration both alone and together with valinomycin or uncouplers.

In the experiments shown in figs. 1–3 the medium did not contain inorganic phosphate, though in its presence the results were the same. Mersalyl did not alter the response to arsenite either with or without valinomycin or uncouplers. Therefore arsenite penetration is independent of the mersalyl-sensitive phosphate carrier [5–6].

4. Discussion

The arsenite anion has to penetrate the mitochondrial membrane in order to produce its inhibitory effect. This penetrating process by itself is rather slow; in the presence of valinomycin or uncouplers it can be greatly accelerated. The problem is: by what means do valinomycin and uncoupling agents act?

The increased rate of respiration caused either by valinomycin or by uncoupling agents is accompanied by a rapid penetration of arsenite. With valinomycin we may assume that arsenite follows the accumulation of K^+ (fig. 4). According to Pressman [7], Chappell and Crofts [8] and Lardy and his coworkers [9] the K^+-influx caused by antibiotics is compensated in part by the influx of penetrating anions. Consistent with that, arsenite may play the role of a permeating anion. This explanation can be compared with the finding of Palmieri and Klingenberg [10]: in their experiments azide acted as the permeant anion and was accumulated maximally during energy-dependent cation transport. Nevertheless, on the basis of the effect of uncouplers, discussed below, it cannot be excluded that a common mechanism of some sort exists in the actions of the two kinds of agents, valinomycin and uncouplers. This mechanism would possibly involve their property of activating

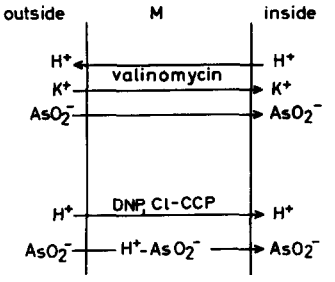

Fig. 4. Scheme of arsenite penetration into mitochondria. For explanation see text.

the respiration-dependent proton pump including the generation of certain local H^+-shifts within the membrane.

There is one important difference between our results and those of Palmieri and Klingenberg. These authors report inhibition of azide accumulation by uncoupling agents, while the present data suggest uncouplers increase penetration of the anion (arsenite). Uncouplers may inhibit the accumulation of anionic substrates [11, 12]. On the basis of these observations Meyer et al. postulate that uncouplers might also inhibit the penetration of arsenite [2]. Our results presented here seem to be contradictory to the latter. Apparently, the penetration of arsenite is not an energy-dependent process in our system, the uncouplers presumably acting as proton conductors, thus enabling H^+ ions to penetrate the mitochondrial membrane, postulated by Mitchell to be impermeable to H^+ and OH^-(fig. 4). Following H^+, arsenite may penetrate down its concentration gradient in the form of a dipole. As an analogy see Mitchell and Moyle [13] and Klingenberg [14]. DNP promotes swelling of liver and heart mitochondria in of NH_4Cl and NH_4NO_3 , respectively [15, 16]. Thus proton conductors may increase anion penetration.

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