THE EFFECT OF SOME ANIONS ON RESPIRATION INHIBITED BY PROGESTERONE

J. ŚWIERCZYNSKI and Z. ALEKSANDROWICZ
Department of Biochemistry, Medical School, Gdańsk, Poland

Received 6 October 1969

1. Introduction

It is known that progesterone can inhibit mitochondrial respiration with succinate as substrate but the mechanism of this inhibition does not seem to be clearly established [1, 2, 3]. The data obtained in our laboratory suggest that progesterone inhibits respiration by affecting proton reentry into the mitochondrion [4]. Popinigis et al. [5] suggested that some anions such as citrate and isocitrate penetrate the mitochondrion only in the non-ionised form (A^-/H^+ symport of Mitchell [6]), thereby conducting protons into the mitochondrion.

In this paper we studied the effect of some anions on progesterone-inhibited respiration. Using intact rat liver mitochondria we found that inhibition of succinate (+ rotenone) oxidation by progesterone is reversed by addition of citrate, isocitrate, malonate; partially reversed by additions of malate and oxoglutarate; but not reversed by fumarate.

2. Materials and methods

Rat liver mitochondria were prepared according to Weinbach [7] in 0.25 M sucrose + 3 mM tris chloride pH 7.3. Protein was estimated by the biuret method [8]. Respiration was measured with a Clark electrode.

Progesterone (Sigma Chem. Co.) was dissolved in ethanol and added in 10 μl aliquots. All anions examined were used as solutions (pH 7.3) of the K salt except isocitrate which was used as the Na salt.

3. Results

Inhibition by progesterone of succinate (+rotenone) oxidation by intact rat liver mitochondria was reversed by additions of citrate or isocitrate (fig. 1a,b). Citrate and isocitrate did not affect progesterone-inhibited respiration in the absence of phosphate (fig. 1c). Mersalyl and oligomycin prevented restoration of progesterone-inhibited respiration by citrate or isocitrate (fig. 2).

Fig. 3 shows the effect of malonate on progesterone-inhibited respiration. This anion like citrate or isocitrate also reversed inhibition of succinate (+ rotenone) oxidation by progesterone. If mersalyl and oligomycin were added to the medium after progesterone, malonate was without effect.

Malate and oxoglutarate were also studied. These anions partially reversed inhibition of succinate (+ rotenone) oxidation by progesterone in intact rat liver mitochondria (fig. 4). Mersalyl and oligomycin prevented restoration of the progesterone-inhibited respiration by these anions (not shown in fig. 4).

Fumarate on the other hand – which is known as a non-penetrant anion [9] – was without effect on inhibition of succinate (+ rotenone) oxidation by progesterone (fig. 5).

4. Discussion

We suggested recently that inhibition by progesterone of succinate (+ rotenone) oxidation by intact rat liver mitochondria is caused by affecting proton reentry into the mitochondrion [4]. It is possible that progesterone reacts with the mitochondrial membrane at the locus where back diffusion of protons take place, thereby preventing back diffusion of protons.

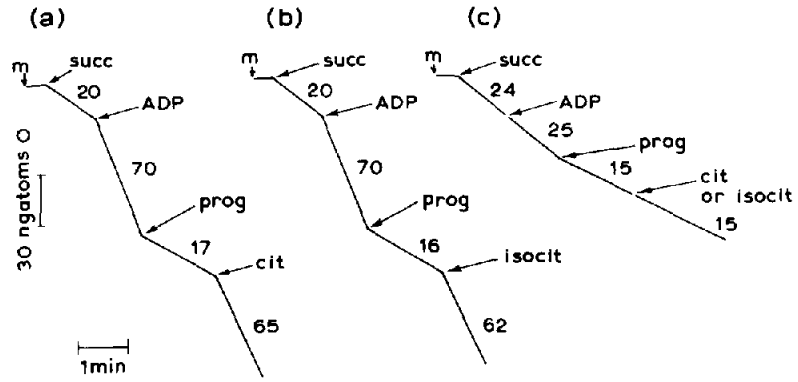

Fig. 1. The effect of citrate (a), isocitrate (b), and citrate or isocitrate without Pi (c) on respiration inhibited by progesterone. The respiration was measured with a Clark electrode in 3.5 ml medium pH 7.3 containing: 15 mM KCl, 50 mM tris chloride, 5 mM $MgSO_4$, 5 mM potassium phosphate (exeept c) and 4 μg rotenone. Other additions as indicated in figure: 3 mM succinate (succ), 1 mM ADP, 10^{-4} M progesterone (prog), 3 mM citrate (cit), 3 mM isocitrate (isocit). In experiment c 5 mM glucose + 1 mg hexokinase was added at zero time. Differences in oxygen consumption are expressed as ngatoms O/min/mg protein. The reaction was started by addition 8 mg protein of intact mitochondria (m).

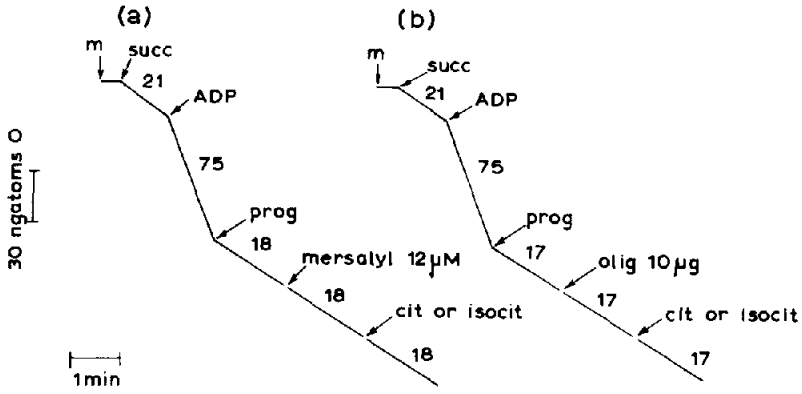

Fig. 2(a). The effect of mersalyl and (b) oligomycin (olig) on restoration of the progesterone-inhibited respiration by citrate and isocitrate. Experimental conditions as in fig. 1. Mitochondrial protein 8 mg.

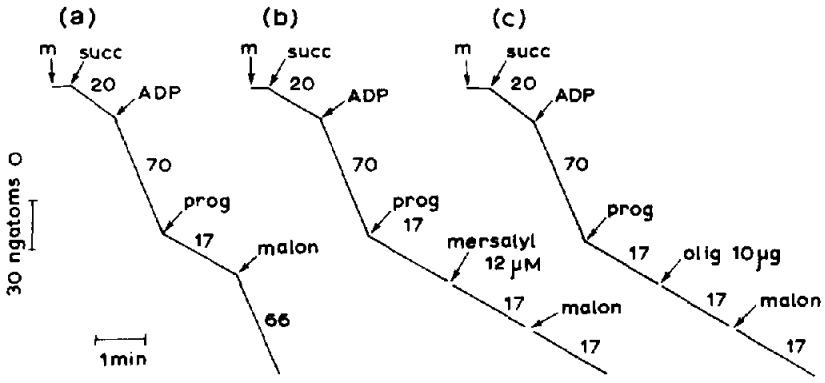

Fig. 3. The effect of malonate (malon) on respiration inhibited by progesterone (a); mersalyl was added as indicated (b); oligomycin was added as indicated (c). Succinate 10 mM, malonate 0.6 mM. Experimental conditions as in fig. 1. Mitochondrial protein 7 mg.

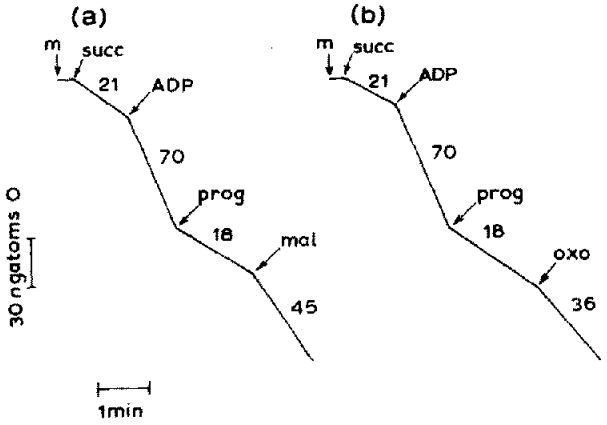

Fig. 4. The effect of malate (a) and oxoglutarate (b) on respiration inhibited by progesterone. Malate 5 mM (mal), oxoglutarate 5 mM (oxo). Mitochondrial protein 7 mg. Experimental conditions as in fig. 1.

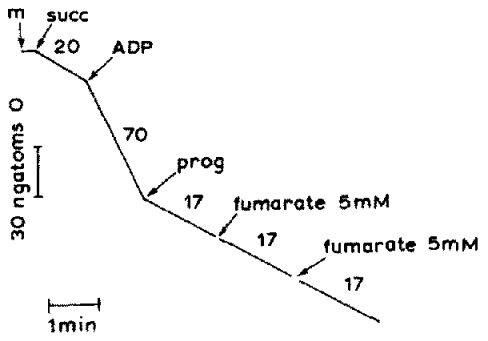

Fig. 5. The effect of fumarate on respiration inhibited by progesterone . Experimental conditions as in fig. 1. Mitochondrial protein 7 mg.

Popinigis et al. [5] suggested that citrate and isocitrate, in the presence of protamine – which probably prevents back diffusion of protons into mitochondrion – penetrate the mitochondrion only in the non-ionised form (A^-/H^+ symport of Mitchell [6]) thereby conducting the protons into mitochondrion. These protons react with OH^- ions generated by respiration or by ATP hydrolisis.

Probably in the presence of progesterone, penetrating anions such as citrate, isocitrate, malonate, malate and oxoglutarate can pass through the mitochondrial membrane in the non-ionised form. Protons enetering the mitochondrion in the form of undissociated acids react with OH^- ions generated by respiration and ATP hydrolysis and reverse progesterone oxidation.

As may be seen in fig. 1c the observed effect of anions is dependent on their ability to pass across the mitochondrial membrane; this indicates that citrate and isocitrate affect progesterone-inhibited respiration only when the conditions required for their passage through the mitochondrial membrane are fulfilled [10]. This view is also supported by the results of our experiments in which mersalyl – a blocking agent of inorganic phosphate entry into mitochondria [11] – was added to a medium containing progesterone.

According to Popinigis et al. [5] tricarboxylic anions require protons from ATP hydrolysis and respiration for their output from mitochondria. This view is also supported by our experiments with oligomycin. As oligomycin prevents restoration of the progesterone-inhibited respiration by dicarboxylic anions such as malonate, malate and oxoglutarate it is possible that these anions require protons both from ATP and respiration for their output from mitochondria.

The conslusion that the observed effect of anions are dependent on their ability to pass across the mitochondrial membrane in the non-ionised form is supported by the results of experiment (not shown here) at various pH values. In a medium at pH 7.1, the effects of anions on progesterone-inhibited respiration is largest, smaller at pH 7.5 and at pH 7.9 all anions examined are without effect.

The supposition that the observed effect of some tricarboxylic and dicarboxylic anions on progesterone-inhibited respiration are related to their ability to pass across the mitochondrial membrane is supported also by the experiments with fumarate. Fumarate, which is known as a non-penetrant anion [9] did not affect inhibition of succinate (+ rotenone) oxidation by progesterone in intact rat liver mitochondria.

Our suggestions are in agreement with results of Chance et al. [12] that citrate induces acidification inside mitochondria and with the results of Liberman and coworkers [13] that accumulation of penetrating anions is coupled to an alkalinization of the suspending medium.

References

[1] R. Wade and H.W. Jones, J. Biol. Chem. 220 (1965) 553.
[2] B. Chance and G. Hollunger, J. Biol. Chem. 238 (1963) 418.
[3] R.H. Vallejos and A.O.M. Stoppani, Biochim. Biophys. Acta 131 (1967) 295.
[4] Z. Aleksandrowicz and J. Swierczyński in press.
[5] J. Popinigis, W. Rzeczycki and J. Swierczyński, FEBS Letters 8 (1970) 149.
[6] P. Mitchell and J. Moyle, Nature 208 (1965) 1205.
[7] E.C. Weinbach, Anal. Biochem. 2 (1961) 335.
[8] E. Layne, Methods of Enzymology, Vol. 3 (Academic Press, New York, 1957) p. 447.
[9] J.B. Chappel and K.N. Haarhoff in: Biochemistry of Mitochondria, eds. E.C. Slater, Z. Kaniuga and L. Wojtczak (Academic Press and Polish Scientific Publishers, London and Warsaw, 1967) p. 75.
[10] J.B. Chappel, Brit. Med. Bull. 24 (1968) 150.
[11] D.D. Tyler, Biochem. J. 111 (1969) 665.
[12] A.K. Ghosh and B. Chance, Arch. Biochem. Biophys. 138 (1970) 483.
[13] E.A. Liberman and V.P. Skulachew, Biochim. Biophys. Acta 216 (1970) 30.