DEPLETION OF Mg^{2+} AND PERMEABILITY INCREASE OF THE MITOCHONDRIAL INNER MEMBRANE BY PRIMYCIN

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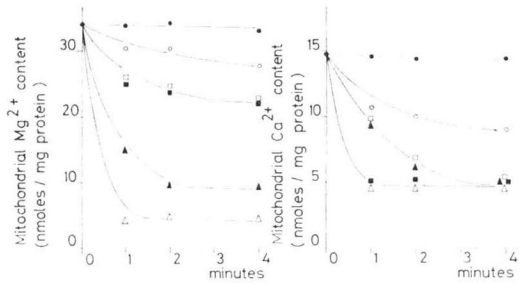
Recently we have shown[1] that primycin below 2~3 nmoles/mg protein concentration ("low dose") renders their inner membrane permeable to K^+, Na^+ and $Tris^+$ but not to Tea^+. This effect was seen only under energized conditions. It has been concluded that this effect of the antibiotic is due to its ionophore-like action. This conclusion has been supported by the experiments of BLASKÓ *et al.*[2] made on erythrocytes and artificial lipid membranes. On the other hand, we have also reported[1] that primycin at a concentration higher than 2~3 nmoles/mg protein ("high dose") can also interact with non-respiring (*i.e.* deenergized) mitochondria increasing their inner membrane permeability to protons and chloride, too. Since the ATPase activity induced by a "high dose" of primycin was significantly higher in the presence of added Mg^{2+} than in its absence, the depletion of Mg^{2+} from the mitochondria by the antibiotic has been proposed to be responsible for the non-selective permeability increase. In this note we present direct evidence in favour of this proposal.

As it can be seen in Fig. 1 primycin in a "high dose" (6.6 nmoles/mg protein) induces a rapid—and in the presence of EDTA practically complete—depletion of Mg^{2+} from mitochondria, while adding it in "low dose" (1 nmole/mg protein) the Mg^{2+} loss is significantly less and slower.

Our previous proposal that primycin in a "high dose" depletes Mg^{2+} from mitochondria is now experimentally verified. Thus the non-selective permeability changes can be well explained by the depletion of Mg^{2+} from mitochondria as suggested by others[3~7].

Fig. 1. Mg^{2+} and Ca^{2+} efflux from mitochondria induced by primycin.

The media contained 140 mM sucrose, 120 mM mannitol, 2 mM HEPES, 2.5 mM succinate and 5 μM rotenone. The pH of reagents and the media was adjusted to 7.4 with KOH. Further additions were: ●—● none, ○—○ 0.5 mM EDTA, ■—■ 1 nmole of primycin/mg protein alone, □—□ plus EDTA, ▲—▲ 6.6 nmoles of primycin/mg protein alone, △—△ plus EDTA. The reaction was started at 25°C by addition of mitochondria prepared by the method of JOHNSON and LARDY[10], and was stopped by cooling the samples to 0°C and by centrifugation (2 min, 16,000×g). The amount of bivalent cations was measured by atomic absorption spectrometry following the method of REED and LARDY[11]. The mitochondrial protein content of all tubes was 5.4 mg determined according to SCHACTERLE and POLLACK[12].



To see whether primycin in a "high dose" also induces a Mg^{2+} influx, swelling experiments were made in isoosmotic $Mg(NO_3)_2$. Figs. 2 a, b, c show that in the presence of "low dose" of the antibiotic neither energized nor deenergized mitochondria swell in magnesium nitrate, though they do swell in isoosmotic KNO_3. On the contrary, primycin in a "high dose" induces a high amplitude swelling of non-respiring mitochondria in magnesium nitrate (Fig. 2a). Thus it can be concluded that primycin only in a "high dose" renders the membrane permeable to Mg^{2+}.

The question arises whether primycin in "high dose" acts as a bivalent cationophore.

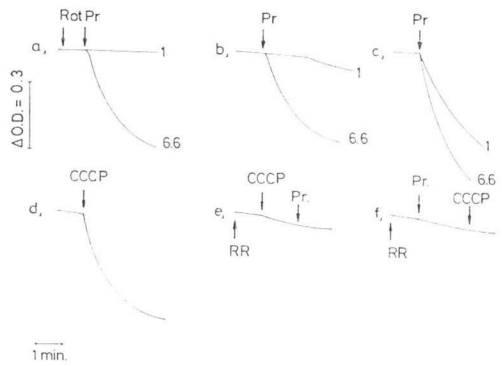
As it is shown in Fig. 1 primycin in "low" and "high dose" equally induces a Ca^{2+} efflux from mitochondria. The rate of this efflux is increased in the presence of EDTA presumably preventing the mitochondrial reuptake of Ca^{2+}.

Non-respiring mitochondria swell in isoos-

Abbreviations: Tris, Tris (hydroxymethyl) aminomethane; Tea, triethanolamine; EDTA, ethylenediaminetetraacetic acid; HEPES, N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone.

Fig. 2. Osmotic swelling of mitochondria induced by primycin in isoosmotic solutions.

The swelling was followed monitoring the changes of optical density at 610 nm. The medium contained 100 mM $Mg(NO_3)_2$ in a, b; 140 mM KNO_3 in c and 100 mM $Ca(CH_3COO)_2$ in d, e, f. 5 mM glutamate and 1.7 mM malate were also present in a, b and c. The pH was adjusted to 7.4 with 5 mM Tea-NO_3 in a, b, c and with acetic acid in d, e and f. The amount of mitochondrial protein was 3.9 mg in each experiment. Other additions at the arrows were: rotenone (3 μM Rot) in d, e, f; CCCP (3 μM); ruthenium red (RR 6 nmoles) and primycin. The numbers by the curves of a, b, c represent the concentration of antibiotic expressed in nmoles/mg protein. The concentration of primycin in d, e and f was 6.6 nmoles/mg protein.



motic calcium acetate in the presence of protonophore, and this swelling can be prevented by ruthenium red which inhibits the natural Ca-translocator of the inner membrane (Figs. 2d and e). The synthetic Ca-ionophore, as found by CARONI *et al.*[8], induces a swelling of mitochondria also in the presence of ruthenium red, by facilitating the Ca^{2+} influx through the inner membrane. On the contrary, primycin does not induce a swelling of ruthenium red treated mitochondria indicating that it does not facilitate the transport of Ca^{2+} (Figs. 2e and f).

These experimental findings make very unlikely that primycin in "high dose" acts as a bivalent cationophore. Thus, the mechanism by which the antibiotic increases the Mg^{2+} permeability of the inner membrane of mitochondria requires further experiments. In this respect it should be mentioned, that oleficin, an antibiotic of quite different structure, increases the Mg^{2+} permeability of the mitochondrial inner membrane in a similar manner[9].

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