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Inhibition of K^+ transport in liver mitochondria

The local anesthetic nupercaine has been shown to diminish the loss of K^+ and the uptake of protons induced by 2,4-dinitrophenol in isolated mitochondria[1]. However, it remained an open question whether nupercaine inhibited K^+ or H^+ transport, and to what extent the inhibition was specific.

Moreover, as the transport of K^+ through the mitochondrial membrane is accelerated several times by the antibiotic valinomycin[2-4], the question arises of whether the effect of this antibiotic is counteracted by nupercaine.

Rat liver mitochondria, prepared as described previously[5], were used in all experiments. K^+ and H^+ movements were recorded by using specific glass electrodes. Oxygen was measured polarographically, and ATP formation by following the proton uptake that accompanies phosphorylation[6]. Mitochondrial swelling was followed at 546 mμ with an Eppendorf photometer, equipped with a recorder. Mitochondria suspended in buffered isoosmotic media with a low K^+ content, in the presence of an inhibitor of electron transport such as rotenone or antimycin, released their endogenous K^+ very slowly. Valinomycin or FCCP accelerated the rate of release. Valinomycin and FCCP together induced the maximum rate of K^+ efflux from mitochondria. Gramicidin alone gave a rate of release of the endogenous mitochondrial K^+ similar to that induced by valinomycin and FCCP together.

Low concentrations of nupercaine were found to inhibit the rate of spontaneous release of K^+ and the rate of the parallel uptake of protons. When valinomycin and/or FCCP were added, and the K^+ efflux rate accelerated several times, nupercaine was as effective as on the spontaneous release of K^+. The concentration of nupercaine for 50% inhibition of the rate of K^+ release was 100 μM. Increased concentrations of valinomycin did not remove the effect of nupercaine.

In order to understand whether nupercaine inhibited K^+ or H^+ transport, or both, the following experiments were carried out. After having loaded the mitochondria with Ca^{2+}, antimycin was added, and the uptake of protons, that is parallel to Ca^{2+} release, was followed. The presence of fairly large concentrations of nupercaine did not influence the rate and the extent of proton uptake, which occurred parallel to the release of Ca^{2+}. Thus the inhibition of the transport of K^+ is not due to inhibition of the coupled transport of protons. On the other hand, the swelling that occurs at pH 8.8 and in the presence of valinomycin, due to the transport of K^+ together with Cl^- through the mitochondrial membrane[7], was also inhibited by low concentrations of nupercaine. Thus K^+ transport is inhibited also when it does not occur in exchange with protons.

The lack of effect of nupercaine on electron transport or energy conservation is indicated by the findings that there was no effect of nupercaine up to the concentration of 500 μM on the rate of FCCP-stimulated respiration or ADP-stimulated respiration. The rate of phosphorylation was also unaffected within a wide range of concentrations of nupercaine. The energy-dependent Ca^{2+} transport was not influenced at the concentration of nupercaine that maximally inhibited the rate of release of K^+ from mitochondria.

Abbreviation: FCCP, carbonylcyanide *p*-trifluoromethoxyphenylhydrazone.

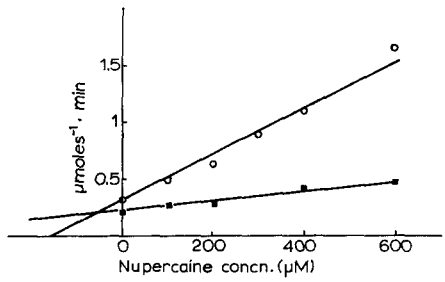

Fig. 1. Competitive inhibition of K^+ uptake by nupercaine. Rat liver mitochondria (14 mg protein) were incubated in a medium containing 250 mM sucrose, 5 mM Tris, pH 7, 4 mM sodium succinate, 3 μM rotenone, 5 mM P_i, 6 mM KCl (○—○) or 12 mM KCl (■—■), 0.1 μg valinomycin. K^+ uptake was measured by a specific glass electrode and a Beckman pH-meter.

The energy-linked transport of K^+ was strongly inhibited by nupercaine. The presence or the absence of a permeant anion such as P_i, did not influence the degree of inhibition. The effect of nupercaine was counteracted by increasing the concentration of K^+. As shown in Fig. 1 nupercaine acts as a competitive inhibitor of K^+ transport. The K_i of nupercaine for K^+ transport was close to 50 μM.

At higher concentrations (0.5–1 mM) nupercaine also inhibited to a certain extent the energy-linked Ca^{2+} transport. The K_i of this inhibition was of the order of 700 μM. No inhibitory effect of Ca^{2+} on passive K^+ movements has been found.

From the above-reported findings it is concluded that nupercaine at low concentrations is a specific inhibitor of K^+ transport in mitochondria. Nupercaine acts by competing with K^+ at some step of the transport process, but it does not influence the availability of energy for the transport. As to the mechanism of cation transport in mitochondria it is possible to conclude that K^+, during the transport, is bound at a fixed site, and that nupercaine acts by competing for the binding at the same site.

The reported results also suggest that, due to the lack of inhibition by Ca^{2+} of K^+ transport and the different K_i of nupercaine for the active K^+ and Ca^{2+} uptake, the mechanisms for K^+ and Ca^{2+} transport in liver mitochondria are, at least in part, different.

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