GUANIDINE SENSITIVE TRANSPORT OF Na^+ AND K^+ IN MITOCHONDRIA

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SUMMARY

Na^+ induce the release of K^+ from mitochondria through a process that is accompanied by a high respiratory rate. With succinate as substrate, octylguanidine, an inhibitor of oxidative phosphorylation at Site I, blocks the Na stimulated oxygen uptake and the Na^+ induced release of K^+. These findings indicate that mitochondria have a guanidine sensitive system that is involved in the translocation of Na^+ and K^+.

During the last years evidence that indicates that guanidine derivatives interfere with a wide variety of biological processes involving metal ions has been accumulating (1-4), but with respect to the site specific action of guanidines in mitochondria, the mechanism through which they inhibit respiration (5-8) is still a matter of discussion (9,10). However, it is possible that in mitochondria guanidines affect also a system involving metal ions mainly because octylguanidine inhibits the action of Na^+ in an apparently competitive form (11), and also because guanidines have been shown to inhibit the uptake of Mg^{2+} (12). Along the same line Davidoff (9) reported that cations inhibited the accumulation of guanidines by mitochondria.

In this work, the possibility that guanidines affected a cation transport system in mitochondria was investigated by measuring the effect of octylguanidine on the Na^+ stimulated oxygen uptake and on the Na^+ induced release of K^+ that occurs when mitochondria are incubated with Na^+, EDTA (ethylene diamine tetra acetate), phosphate and succinate (13). The results obtained indicate that in the presence of succinate, octylguanidine, an inhibitor of

oxidative phosphorylation at Site I (5), diminished the Na^+ induced loss of K^+ and the Na^+ stimulated oxygen uptake. These findings strongly suggest that mitochondria possess a guanidine sensitive system involved in the movement of Na^+ and K^+ across the membrane.

Material and Methods

The technique for the preparation of liver mitochondria in 0.25 M sucrose and 1 mM EDTA, as wells as the methods for measuring oxygen uptake and K^+ release have been described (13).

Results

The results of Table I show that octylguanidine inhibits the Na^+ induced release of mitochondrial K^+ when either glutamate or succinate (the latter in the presence of rotenone) support the process. Nevertheless, in the presence of glutamate the system, on a concentration basis, is more sensitive to octylguanidine than when succinate is the oxidizable substrate.

As coupled respiration is more sensitive to octylguanidine at Site I (5), these findings could suggest that the inhibition of the Na^+ induced K^+ release was a consequence of the inhibition of electron transport. However, when the effect of several concentrations of octylguanidine was assayed on the ADP and on the Na^+ stimulated oxygen uptake, it was found that the ADP stimulated oxygen uptake was less sensitive to octylguanidine than the stimulation of respiration induced by Na (Fig. 1). These results would indicate that the primary action of octylguanidine is on a cation transport system .

Nevertheless, the polarographic traces of Figure 1 show that in an octylguanidine poisoned system, Na^+ induces a gradual increase in the respiratory rate. This could be due to a gradual displacement of octylguanidine from its

TABLE I

EFFECT OF OCTYLGUANIDINE ON THE Na^+ INDUCED LOSS OF K^+

	nmoles K^+ lost per mg of protein	
Octylguanidine (uM)	Glutamate	Succinate
-	106	110
78	77	89
156	57	72
312	43	77

Mitochondria from approximately one half of rat liver were incubated in 12.5 ml of a reaction mixture that contained 10 mM phosphate, 10 mM Tris-HCl (pH 7.3), 10 mM substrate, 1 mM EDTA, 80 mM sucrose, and 50 mM NaCl. In addition the mixture contained the indicated concentrations of octylguanidine and 20 uM rotenone when the mixture contained succinate.

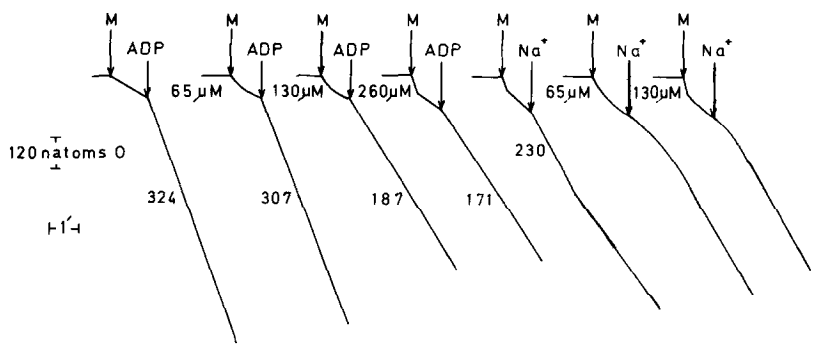

Figure 1. Effect of Octylguanidine on the ADP and Na^+ Stimulated Oxygen Uptake. Mitochondria (5 mg of protein) were added to an incubation mixture that contained 10 mM phosphate, 10 mM succinate, 20 mM Tris-HCl (pH 7.3), 1 mM EDTA, 80 mM sucrose and 20 uM rotenone in a final volume of 3.0 ml. To the vessels in which ADP (1 mM) was added, the mixture contained 100 mM KCl. The mixture also contained the indicated concentrations of octylguanidine. 100 mM NaCl was added where indicated. The numbers at the side of the trace indicate the respiratory rate (natoms O min^{-1}). The respiratory rate of the last two traces is not indicated since the respiration increased gradually with time.

locus of action; further experiments are in progress to clarify this point.

Discussion

The experiments described in this communication are strongly suggestive

that mitochondria possess a guanidine sensitive system involved in the transport of Na^+ and K^+ across the membrane. Thus in the light of these findings it is of interest to point out that many transport system are affected by guanidines i.e. the transport of Na^+ in the toad bladder (1) and axon membranes (2), the K^+ fluxes of perfused liver (3), the nigericin induced K^+ influx in mitochondria (4), and the uptake of Mg^{2+} in mitochondria (12). Furthermore, tetrodotoxin, the potent inhibitor of Na^+ transport (14), is also a guanidine derivative . Therefore, it is possible that guanidine sensitivity is a common characteristic of a wide variety of biological systems involved in ion transport.

At the present it is not possible to ascertain the relation of this cation transport system to oxidative phosphorylation, but it should be recognized that the sensitivity of the Na^+ induced K^+ efflux to guanidines depends on the segment of the respiratory chain that supports the process. This may suggest that each site of phosphorylation possesses a particular system for the translocation of cations.

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