BBA 41119

The reversibility of antimycin inhibition

Very low concentrations of antimycin inhibit respiratory chain electron flow between cytochromes *b* and c_1 (refs. 1, 2). Other compounds acting in the same region, including alkylhydroxynaphthoquinones and alkylhydroxyquinoline-*N*-oxides, operate in such a manner that their inhibition of electron transport is readily reversed upon the addition of uncouplers of oxidative phosphorylation[3,4]. Indeed, release of inhibition is produced, not only by classical uncouplers, such as 2,4-dinitrophenol, but also by other conditions leading to discharge of the high-energy state of mitochondria, including ion translocation and disruption of mitochondrial structure[5]. These results suggest that such inhibition in some way involves energy-conserving reactions as distinct from pure electron transfer[4].

On the other hand, antimycin gives rise to inhibition which has not, until now, been shown to be reversible under uncoupling conditions[6], a fact cited as evidence that its inhibitory action does not occur at the level of energy-transfer reactions[2]. This communication reports that antimycin inhibition of succinate oxidation may, under certain conditions, be released by various uncouplers and it discusses implications which those conditions present.

TABLE I

RELEASE OF ANTIMYCIN INHIBITION BY UNCOUPLERS

Mitochondria were isolated in 0.25 M sucrose. Oxygen consumption was measured using a vibrating platinum electrode and a reaction mixture containing 15 mM KCl, 2 mM EDTA, 50 mM Tris–HCl, 5 mM $MgCl_2$, 60 mM succinate, 30 mM glucose and 50 units of hexokinase (Sigma Type IV) in a volume of 2 ml. The reaction was followed at pH 7.4 and at 25°. Prior to the addition of succinate, the mitochondria were incubated with the reaction mixture for 2 min in order to reduce the concentration of intramitochondrial phosphate. Mitochondrial protein concentrations were 4.5, 4.5, and 6.7 in Expts. I, II, and III, respectively.

Expt. No.	*Additions*	*Oxygen consumption (μgatom/min)*
I	None	0.154
	+ antimycin (18 ng/mg protein)	0.064
	+ antimycin + CCCP (10 μM)	0.141
	+ antimycin + CCCP (20 μM)	0.096
II	None	0.144
	+ antimycin (13 ng/mg protein)	0.053
	+ antimycin + phosphate (2.5 mM)	0.039
	+ antimycin + phosphate + CCCP (10 μM)	0.139
III	None	0.176
	+ antimycin (9 ng/mg protein)	0.363

Table I illustrates release of antimycin inhibition upon addition of the uncoupler, carbonylcyanide-*m*-chlorophenylhydrazone (CCCP). It was found that inorganic phosphate facilitated inhibition by low concentrations of antimycin and, in the 3

Abbreviation: CCCP, carbonylcyanide-*m*-chlorophenylhydrazone.

experiments shown here, mitochondria were preincubated with a glucose and hexokinase "trap" in order to lower the intramitochondrial phosphate concentration. In the first experiment, the addition of a small amount of antimycin resulted in partial inhibition of succinate oxidation, after which substantial release was observed upon addition of CCCP. Addition of excess CCCP produced inhibition. In the second experiment, addition of inorganic phosphate, following that of antimycin, led to a further level of inhibition, but did not prevent subsequent stimulation of respiration by CCCP. On the other hand, attempts to repeat these release experiments without the glucose–hexokinase preincubation were unsuccessful. In the third experiment of Table I, addition of a very low concentration of antimycin to mitochondria led to a marked stimulation of respiration which should probably be regarded as uncoupling.

Fig. 1 shows a similar experiment in which addition of ADP *plus* phosphate does not lead to a release of antimycin inhibition while that of calcium does. In other experiments (not shown here) a similar release of inhibition was obtained with gramicidin, while attempts to obtain it with dicumarol have been unsuccessful. The influence of a doubling of the antimycin concentration following addition of calcium is shown on a separate trace. In this connection, it should be added that inhibition is reversible only within extremely narrow limits of antimycin concentration, inhibition by even a small excess being quite irreversible.

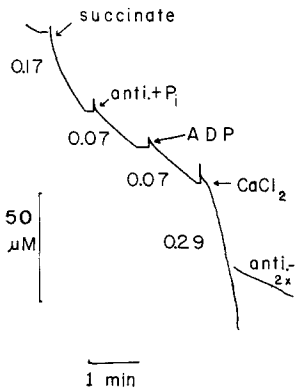

Fig. 1. Release of antimycin inhibition by calcium. Conditions as described in Table I including preincubation. Additions are indicated as follows: anti., antimycin (13 ng/mg protein); P_i (2.5 mM); ADP (1 mM); $CaCl_2$ (1.5 mM) and anti.-2×, antimycin (final concn. 26 ng/mg protein). Rates are given to the left of the trace in μgatoms of oxygen per min.

The observed release of antimycin inhibition by uncouplers suggests that the high-energy state of mitochondria influences the action of antimycin at the low concentrations used. These results can not be accounted for on the basis of a stimulation by uncouplers of the non-inhibited portion of electron flow, as neither ADP nor dicumarol, both of which stimulate State 4 respiration, have been observed to produce an effect. Indeed, it was found previously that neither compound was able to reverse inhibition of succinate oxidation by the alkylhydroxynaphthoquinone, hydrolapachol[4].

While it is not possible to offer a detailed mechanism on the basis of these observations alone, a useful point of view might be that the reversal phenomenon indicates a requirement for the active translocation of antimycin to its intramito-

chondrial site of action. Thus, uncouplers, by discharging the high-energy state of mitochondria might prevent them from maintaining a high internal inhibitor concentration and give rise to the observed release of inhibition. The requirement for active translocation would be expected to disappear with a higher inhibitor concentration, a condition under which uncouplers do not, in fact, give rise to release. A similar mechanism has been recently advanced by PALMIERI AND KLINGENBERG[7] to account for reversibility of inhibition by the respiratory chain inhibitor, azide.

Consideration of possible mechanisms should also be in light of the recent suggestion by VAN DAM AND SLATER[8] that anionic uncouplers act by virtue of their energy-linked translocation across the mitochondrial membrane. According to this idea, uncouplers may interfere with respiratory inhibition by competing directly with anionic inhibitors for entry, thus resulting in a lower intramitochondrial inhibitor concentration and the diminished inhibition which is observed. Anionic inhibitors include antimycin, alkylhydroxynaphthoquinones and alkylhydroxyquinoline-*N*-oxide, all bearing a phenolic hydroxyl group[9], and all having been shown to exhibit reversibility of inhibition on addition of uncoupling agents.

This investigation was supported by National Science Foundation Grant GB5460X. The excellent technical assistance of Mrs. M. WHITE and Mrs. A. SEYMOUR is gratefully acknowledged.

Department of Biology, Bowdoin College, Brunswick, Me. (U.S.A.) J. L. HOWLAND

1 V. R. POTTER AND A. E. REIF, *J. Biol. Chem.*, 194 (1952) 287.
2 R. W. ESTABROOK, *Biochim. Biophys. Acta*, 60 (1962) 236.
3 J. L. HOWLAND, *Biochim. Biophys. Acta*, 73 (1963) 665.
4 J. L. HOWLAND, *Biochim. Biophys. Acta*, 105 (1965) 205.
5 J. L. HOWLAND, *Abstr. 4th Meeting Federation European Biochem. Soc., Oslo, 1967*, p. 44.
6 F. HUIJING AND E. C. SLATER, *J. Biochem. Tokyo*, 49 (1961) 493.
7 F. PALMIERI AND M. KLINGENBERG, *European J. Biochem.*, 1 (1967) 439.
8 K. VAN DAM AND E. C. SLATER, *Proc. Natl. Acad. Sci. U.S.*, in the press.
9 A. L. TAPPEL, *Biochem. Pharmacol.*, 3 (1960) 289.

Received August 21st, 1967
Revised manuscript received October 13th, 1967