THE REVERSIBLE INHIBITION OF SUCCINOXIDASE BY NAPHTHOQUINONES

David Hendlin and Thomas M. Cook

Merck Sharp & Dohme Research Laboratories
Division of Merck & Co., Inc., Rahway, New Jersey

Received December 14, 1959

Ball, Anfinsen, and Cooper (1947) demonstrated that 2-hydroxy-3-alkylnaphthoquinones, which possess antimalarial activity, exert their inhibitory effect on respiration by inhibiting succinoxidase. They pinpointed the locus of action of these compounds to a site between cytochromes b and c in the electron transport system. The recent discovery of a benzoquinone, coenzyme Q_{10} (CoQ_{10}), as a cofactor essential for electron transport (Crane, Hatefi, Lester, and Widmer, 1957) prompted us to investigate the possibility that the naphthoquinones were inhibiting at the CoQ_{10} site.

The present communication describes studies which demonstrate that the inhibitory effect of the antimalarial naphthoquinones on the succinoxidase system of beef heart electron transport particles (ETP) can be reversed by CoQ_{10} and related compounds. Antimycin A, also known to inhibit between cytochromes b and c in the electron transport chain (Potter and Reif, 1952), is not so antagonized.

METHODS

The preparation of ETP from bovine heart has been described previously (Crane, Glenn, and Green, 1956). Succinoxidase activity of these particles was determined manometrically according to Crane, Widmer, Lester, and Hatefi (1959). In the present studies unextracted enzyme was used and no phospholipid was added to the

reaction vessel. The tests were conducted in a Warburg vessel containing 1 mg of enzyme protein in a total volume of 1.5 ml.

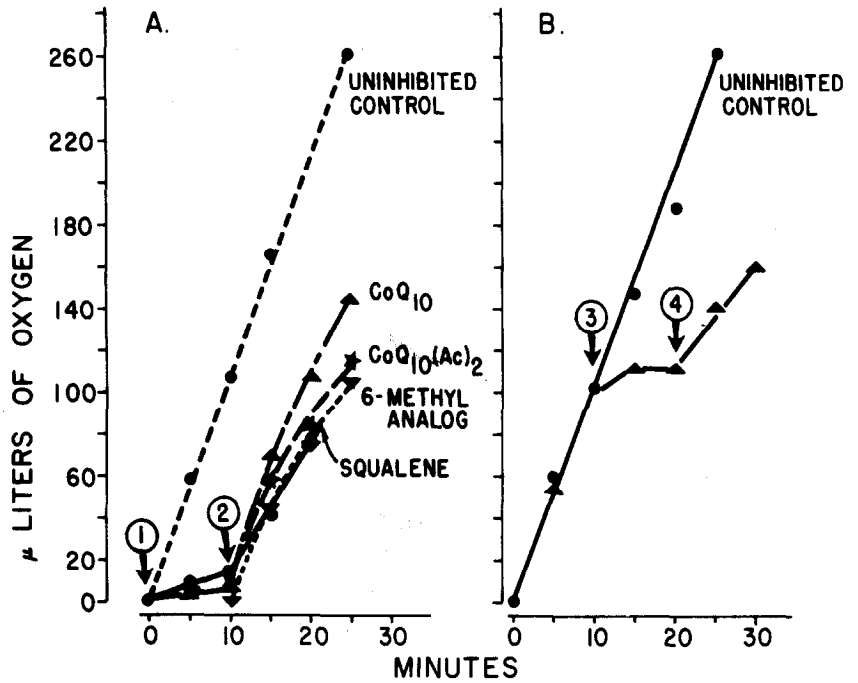

Figure 1. Reversal of Naphthoquinone Inhibition

A. Inhibition by 2-hydroxy-3-(3',7'-dimethyl) octyl-1,4-naphthoquinone. (1) 1 μg naphthoquinone tipped into vessel. (2) 300 μg of indicated reversing agents tipped into vessel. B. Inhibition by 2-hydroxy-3-cyclohexylpropyl-1,4-naphthoquinone. (3) 10 μg naphthoquinone tipped into vessel. (4) 300 μg CoQ_{10} tipped into vessel.

RESULTS

The addition of several 3-substituted 2-hydroxynaphthoquinones* to the ETP system strongly inhibited succinoxidase activity. Of the analogs tested, the 3-(3',7'-dimethyloctyl) derivative was the most potent. The inhibition was proportional to the dose over a narrow range. Fifty per cent inhibition occurred with 0.5 μg

* We wish to thank Prof. L. Fieser for making these compounds available to us.

($1.6X10^{-9}$ moles) and 1.0 μg caused complete inhibition. A considerable degree of protection was obtained by including CoQ_{10} in the reaction vessel.

More detailed studies demonstrated that the protective effect was reproducible and that protection and reversal could be obtained with a variety of CoQ_{10} analogs as well as with α-tocopherol, vitamin K_1, and squalene. Complete reversal of the naphthoquinone inhibition was obtained with the diacetate of CoQ_{10} hydroquinone and several CoQ_{10} analogs (6-phytyl, 6-farnesyl, 6-heptyl, 6-(3'-methyl-2' butenyl), 6-methyl, and the monoethoxy homolog of CoQ_{10}). Figure 1A shows the results obtained with some of these compounds. The oxygen uptake so restored could be completely stopped by tipping in antimycin A from the side arm. Antimycin A toxicity could not be reversed by CoQ_{10} or its analogs. Higher levels of the naphthoquinone could not be reversed by further CoQ_{10} supplementation.

Similar results were obtained with another analog, 2-hydroxy-3-cyclohexylpropyl-1,4-naphthoquinone. When 10 μg of this compound were added to the system, complete inhibition of succinoxidase occurred. This inhibition was completely reversed by the subsequent addition of 300 μg CoQ_{10} (Fig. 1B).

DISCUSSION

Crane, Widmer, Lester, and Hatefi (1959) demonstrated that CoQ_{10} functions between cytochromes *b* and *c*. This is the same area which is sensitive to the naphthoquinones and antimycin A. If either of these inhibitors acts at the CoQ_{10} site, it would be expected that their toxicity could be reversed by CoQ_{10}. CoQ_{10} did indeed reverse the inhibitory effect of the naphthoquinones but not of antimycin A. This may indicate that: (a) the naphthoquinones and antimycin A act at different loci, or (b) that they inhibit at the same locus but that antimycin A is more

tenaciously bound to the enzyme.

The ability of several CoQ_{10} analogs (including those which show no CoQ_{10} activity), α-tocopherol, squalene and vitamin K_1 also to reverse naphthoquinone toxicity casts doubt on the hypothesis that naphthoquinones act at the CoQ_{10} locus.

It is apparent from our data the naphthoquinone toxicity can be relieved by either quinones or lipophilic substances. These findings are reminiscent of the results obtained by Weber, Gloor, and Wiss (1958). They observed a similar requirement for the restoration of cytochrome c reductase of isooctane-extracted ETP.

It is possible that the quinones act as an electron "by-pass" (Mahler, 1956; Weber, Gloor, and Wiss, 1958) transferring electrons around the blocked reaction. If such is the case, then the site of antimycin A and naphthoquinone must be different to account for the sensitivity of the "by-pass" to antimycin A.

The activity of squalene and the diacetates of CoQ_{10} and vitamin K_1 hydroquinones may reside in their lipophilic properties. They may act by displacing the naphthoquinone from the lipoprotein complex.

It is evident that the elucidation of the exact site of action of naphthoquinones will require further investigation. Such stuies should be directed at the individual steps in the reaction sequence between cytochromes b and c.

REFERENCES

Ball, E. G., Anfinsen, C. B., and Cooper, O., J. Biol. Chem., 168, 257 (1947).

Crane, F. L., Glenn, J. L., and Green, D. E., Biochim. et Biophys. Acta, 22, 475 (1956).

Crane, F. L., Hatefi, Y., Lester, R. L., and Widmer, C., Biochim. et Biophys. Acta, 25, 220 (1957).

Crane, F. L., Widmer, C., Lester, R. L., and Hatefi, Y., Biochim. et Biophys. Acta, 31, 476 (1959).

Mahler, H. R., Advances in Enzymol., 17, 223 (1956).

Potter, V. R. and Reif, A. E., J. Biol. Chem., 194, 287 (1952).

Weber, F., Gloor, U., and Wiss, O., Helv. Chim. Acta, 41, 1046 (1958).