# MIKAMYCIN, AN INHIBITOR OF BOTH MITOCHONDRIAL PROTEIN SYNTHESIS AND RESPIRATION.

Honor Dixon, G.M. Kellerman, Carolyn H. Mitchell, N.H. Towers and A.W. Linnane.

Department of Biochemistry, Monash University, Clayton, Victoria, 3168. Australia.

Received March 29, 1971

SUMMARY - Mikamycin, an antibiotic which inhibits bacterial protein synthesis, strongly inhibits mitochondrial protein synthesis at concentrations of the order of 1 μg/ml or less. At concentrations of the order of 25-50 μg/ml it also inhibits mitochondrial respiration at a site which appears to be localized between cytochromes *b* and $c_1$. A unitary concept for the two observed effects is discussed.

INTRODUCTION. A number of antibacterial antibiotics, including chloramphenicol, several macrolides, tetracycline, lincomycin, paromomycin and mikamycin, inhibit mitochondrial protein synthesis both *in vivo* and *in vitro* (for review 1). Recently we have suggested the possibility that the ribosome of yeast mitochondria is an integral part of the mitochondrial membrane and consequently that direct effects of the antibiotics on the mitochondrial ribosome may have secondary effects on other functions of the mitochondrial membrane, and vice versa (1,2,3). Indeed chloramphenicol at high concentrations has been recognized for some time to be an inhibitor of oxidative phosphorylation and electron transport (4-7). We have therefore extended our studies to include an examination of the effect of the other antibiotics on mitochondrial respiration and associated phenomena. The present communication details investigations on the mode of action of mikamycin on mitochondrial electron transport, and we discuss the results in relation to our hypothesis regarding ribosome-membrane interactions.

METHODS

Rat liver mitochondria were isolated by standard procedures in 0.34M sucrose - 1.0mM EDTA - 2mM Tris - 0.2% bovine serum albumin (BSA), pH 7.4. Yeast mitochondria were prepared from ethanol grown cells by methods previously detailed (2). Mitochondrial protein synthesis was assayed as described previously (5). NADH: oxygen oxidoreductase (EC 1.6.99.1), succinate: oxygen oxidoreductase (EC 1.3.99.1) and cytochrome c: oxygen oxidoreductase were assayed polarographically at 30° in isolation medium containing 10mM phosphate pH 7.4. Succinate (5mM), pyruvate (5mM) + malate (5mM) or cytochrome c (0.1mM) plus ascorbate (16mM) (8) were used as substrates for the relevant enzyme activities examined, and ADP (0.5mM) or 2,4-dinitrophenol (DNP) (0.1mM) were added when measurement of state 3 (9) or uncoupled respiration was required. Succinate:dichlorophenol indophenol oxidoreductase (EC 1.3.99.1) was assayed by the method of Arrigoni and Singer (10).

Cytochrome spectra were measured in a Cary Model 14 recording spectrophotometer at ambient temperature. Mitochondrial suspensions containing 10mM phosphate pH 7.4 and 5mM ADP were fully oxidised by aeration; ammonium persulphate (1.5mM) was then added to one cuvette, and succinate (16mM) or mikamycin (50 μg/mg protein) + succinate (16mM) to the other cuvette. Difference spectra were recorded 5 and 15 minutes later.

Mikamycin (a mixture of 89% mikamycin A and 11% mikamycin B) was obtained from Calbiochem, Los Angeles, Calif. U.S.A. Dilutions were prepared from a solution of 20 mg/ml in ethanol (methanol for the yeast experiments) so that the desired quantity of the antibiotic could be added in 10μl of solvent, a quantity shown to have no effect on the respiratory process.

## RESULTS AND DISCUSSION

The antibiotic mikamycin has at least two effects on mammalian mitochondria (Table 1). It acts as an inhibitor of mitochondrial protein synthesis at low concentrations, and as an inhibitor of respiration at higher concentrations. Maximal inhibition of protein synthesis viz. 80%, is observed at a concentration of about 1 μg mikamycin/mg mitochondrial protein.

TABLE 1.

INHIBITION OF RAT LIVER MITOCHONDRIAL PROTEIN SYNTHESIS AND RESPIRATION BY MIKAMYCIN.

| Mikamycin concentration μg/ml | % inhibition of protein synthesis | % inhibition of state 4 respiration | | |
|---|---|---|---|---|
| | | Pyruvate + malate | Succinate | Cytochrome *c* + ascorbate |
| 0.03 | 23 | - | - | - |
| 0.1 | 50 | - | - | - |
| 0.3 | 66 | - | - | - |
| 1 | 75 | - | - | - |
| 10 | 83 | 17 | 13 | - |
| 25 | 84 | 58 | 76 | 0 |
| 50 | - | 63 | 92 | 8 |

The results represent values from typical experiments. Samples for protein synthesis and respiration contained 0.8-1.6 and 0.4-0.7 mg mitochondrial protein/ml medium respectively.

State 4 respiratory rates (μmoles oxygen/mg protein/min) and respiratory control ratios were respectively: succinate 1.7, 3.4; cytochrome *c* + ascorbate 1.3,2.5, pyruvate + malate 0.6, 4.2.

At an antibiotic to protein ratio of 50 μg/mg protein, mikamycin almost completely inhibited oxygen uptake in the presence of succinate; it had only a slight inhibitory action on oxygen uptake in the presence of cytochrome c + ascorbate. Strong inhibition of respiration was also observed in the presence of a number of NADH-linked substrates such as pyruvate + malate. The values given in Table 1 refer to respiratory inhibition in state 4 (minus ADP) but similar levels of inhibition were observed in state 3 (plus ADP) and also when mitochondria were uncoupled by DNP. These observations suggested that mikamycin was inhibiting the electron transport system directly, and also that the site of action was located between flavoproteins and cytochrome c. The possibility that mikamycin was acting on succinate dehydrogenase itself was excluded by direct assay of the enzyme in sonicated preparations of mitochondria, using phenazine methosulphate and 2,4-dichlorophenol indophenol as electron acceptors. No inhibition of the enzyme was observed in the presence of up to 50μg mikamycin/mg protein and at higher concentrations a small stimulation occurred.

Difference spectra for succinate reduced versus oxidised rat liver mitochondria in the absence and presence of mikamycin are shown in Fig.1. Curve (a) shows the steady state spectrum obtained within 5 minutes after addition of substrate to mitochondria in the absence of mikamycin. When mikamycin (50 μg/mg protein) was added before the succinate, the spectrum shown in curve (b) was obtained, and persisted for at least 15 minutes. The antibiotic prevented the reduction of cytochromes a, $a_3$, c and $c_1$ so that only the absorption peaks at 563-564 nm and 432 nm due to reduced cytochrome b were seen. At lower concentrations of mikamycin (10-20 μg/mg protein)

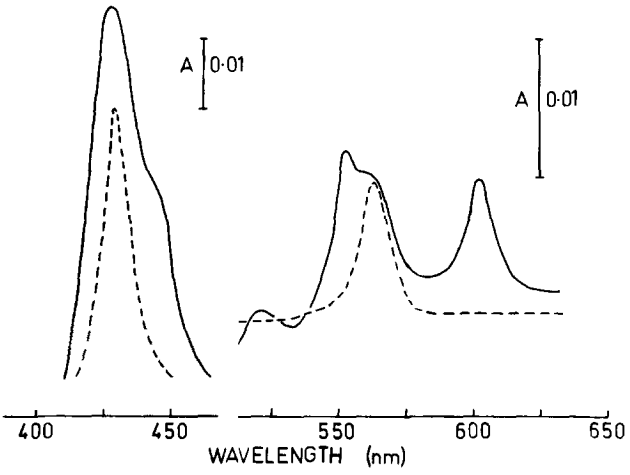


Fig. 1. Difference absorption spectra of rat liver mitochondria reduced with succinate versus oxidised with ammonium persulphate. (a)——————— Mitochondrial suspension (3.5 mg protein/ml) plus succinate. (b) ---------- mikamycin (50 μg/ml protein) added to aerated suspension before succinate. In sample (a), absorption maxima of reduced cytochrome a (603 nm), $a_3$ (444 nm), b (563-564 nm) c + $c_1$ (553 nm) and a broad Soret band at 428-430 nm are observed. In sample (b) only cytochrome b peaks (563-564 nm, 432 nm) are present.

difference spectra of type (b) developed soon after succinate addition but the cytochromes a, $a_3$, c and $c_1$ gradually became reduced, giving a steady state spectrum similar to curve (a) after 15 minutes, presumably due to incomplete inhibition at this concentration. Thus at high levels, mikamycin causes an inhibition of the respiratory chain close to the site at which antimycin A inhibits respiration.

The effects of mikamycin on yeast mitochondria appear similar to those on rat liver mitochondria in that both protein synthesis (2) and respiration are inhibited by concentrations strictly comparable to those affecting the mammalian system. Progessive inhibition of oxygen uptake occurs with concentrations of the order of 20-100 μg mikamycin/mg protein, reaching about 90% at the higher antibiotic concentration with succinate as substrate. The spectral changes observed in the presence of

succinate and mikamycin (50-100 μg/mg protein) are similar to those seen with liver mitochondria (reduction of cytochrome b and oxidation of the other cytochromes). For the reliable demonstration of the specific spectral effect with yeast mitochondria, it is essential for them to be prepared in the presence of 0.2% BSA. When yeast mitochondria are prepared in the absence of BSA, mikamycin still inhibits respiration, but the localized inhibition between cytochromes b and $c_1$ is not often seen in the spectrum. That this is not simply due to the preservation of respiratory control by BSA is shown by the fact that mitochondria made in the presence of BSA and uncoupled by DNP still show the specific spectra effect. Thus it appears that some subtlety in the organizational integrity of the mitochondrial membranes is necessary for the expression of the mikamycin specificity, and further work is in progress to characterize its nature.

At this stage of the investigations it is not clear whether the inhibitions of mitochondrial protein synthesis and respiration by mikamycin represent two related phenomena or whether they are independent. However we have also recently established that the antibiotics carbomycin, oleandomycin and paromomycin, like mikamycin and chloramphenicol, interfere with respiration at high concentrations (1). The probability that such a diverse group of chemical compounds would all have the same two unrelated effects appears unlikely. We have therefore suggested as a working hypothesis (1,2,3), a unitary concept in which antibiotics which inhibit mitochondrial protein synthesis are considered to interact with an integrated ribosome-membrane system, with consequent secondary effects on other membrane functions e.g. electron transport as reported herein for mikamycin.

## REFERENCES


1. Linnane, A.W. and Haslam, J.M. in Current Topics in Cellular Regulation. Volume 2. Ed. by Horecker, B.L. and Stadtman, E.R. Academic Press. (New York) 101 (1970).
2. Bunn, C.L., Mitchell, C.H., Lukins, H.B. and Linnane, A.W. Proc. Natl. Acad. Sci. U.S., 67, 1233 (1970).
3. Linnane, A.W. and Kellerman, G.M. Abstracts 8th Internatl. Con. of Biochem. 263 (1970).
4. Hanson, J.B. and Hodges, T.K. Nature Lond., 200 1009 (1963).
5. Firkin, F. and Linnane, A.W. Biochem. Biophys. Res. Commun. 32, 398 (1968).
6. Firkin, F. and Linnane, A.W. FEBS Letters 2, 330 (1969).
7. Freeman, K.B. Can. J. Biochem. 48, 479 (1970).
8. Sottocasa, G.L., Kylenstierna, B., Ernster, L. and Bergstrand, A. J. Cell Biol. 32, 415 (1967).
9. Chance, B. and Williams, G.R. in "Advances in Enzymology" Vol. 17. Ed. by Nord, F.F. Interscience Publishers Inc., N.Y. 65 (1956).
10. Arrigoni, O. and Singer, T.P. Nature Lond., 193, 1256 (1962).
11. Dixon, H., Towers, N., Kellerman, G.M. and Linnane, A.W. In Preparation (1971).