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FUNICULOSIN: AN ANTIBIOTIC WITH ANTIMYCIN-LIKE INHIBITORY PROPERTIES

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SUMMARY

The antibiotic funiculosin mimics the action of antimycin in several ways. It inhibits the oxidation of NADH and succinate, but not TMPD+ascorbate. The titer for maximal inhibition in Mg^{2+}-ATP particles (0.4–0.6 nmol/mg protein) is close to the concentrations of cytochromes *b* and cc_1. Funiculosin also induces the oxidation of cytochromes cc_1 and an extra reduction of cytochrome *b* in the aerobic steady state, and it inhibits duroquinol-cytochrome *c* reductase activity in isolated Complex III. The location of the funiculosin binding site is clearly similar to that of antimycin. In addition, funiculosin, like antimycin, prevents electron transport from duroquinol to cytochrome *b* in isolated Complex III if the complex is pre-reduced with ascorbate. Funiculosin and antimycin differ, however, in the manner in which they modulate the reduction of cytochrome *b* by ascorbate+TMPD.

INTRODUCTION

Moser and Walter [1] recently introduced the antibiotic funiculosin [2] as a new site-specific inhibitor of the mitochondrial electron transport chain. These investigators showed that funiculosin inhibited oxidation of β-hydroxybutyrate and succinate, but not TMPD+ascorbate in rat liver mitochondria. An antimycin-like action was suggested for funiculosin [1] based upon its inhibition of succinate-ferricyanide reductase activity. In the present investigation the site of funiculosin inhibition has been more thoroughly analyzed. Results show that funiculosin inhibits electron transfer between cytochromes *b* and c_1, and, with minor differences, appears to act at, or near, the site(s) of antimycin and 2-heptyl-4-hydroxyquinoline *N*-oxide [3].

Abbreviations: TMPD, tetramethyl-*p*-phenylenediamine.

METHODS

Submitochondrial particles were prepared from beef heart mitochondria in the presence of Mg^{2+} and ATP as described [4]. Ubiquinol-cytochrome *c* reductase (Complex III) was prepared by the method of Rieske et al. [5]. Oxygen uptake was measured with a Clark-type oxygen electrode. Redox changes were measured with a dual wavelength spectrophotometer. Cytochrome *b* was measured at 562–575 nm, cytochrome cc_1 were measured at 550–540 nm in submitochondrial particles and cytochrome c_1 in Complex III was measured at 554–550 nm. Protein was measured with the biuret reagent. All solution of funiculosin (mol. wt. 428 [2]) were prepared in ethanol made slightly basic with NaOH. Funiculosin was a generous gift from Dr. P. Bollinger, Sandoz A. G., Basel.

RESULTS

In agreement with previous results [1], funiculosin inhibits electron transport in the cytochrome b-c_1 region of the respiratory chain. Mg^{2+}-ATP particles pre-incubated with 0.28 μM funiculosin do not oxidize NADH or succinate, but the oxidation of ascorbate+TMPD is not effected. Addition of FCCP did not overcome the inhibitory effect of funiculosin.

Fig. 1 shows the funiculosin titer for inhibition of NADH oxidase at two concentrations of particle protein. Maximal inhibition was achieved at approximately 0.4 nmol funiculosin/mg protein at both concentrations of protein. The concentrations of dithionite-reducible cytochromes cc_1 and cytochrome *b* in these particles were between 0.6 and 0.7 nmol/mg protein. NADH and succinate oxidation are inhibited with the same funiculosin titers (Fig. 2). The above results show that funiculosin inhibits nearly as effectively as antimycin, and is more effective than 2-heptyl-4-hydroxyquinoline *N*-oxide [3]. It should be pointed out that the maximal inhibition by funiculosin requires a short preincubation time of 1–2 min, in contrast to antimycin which acts immediately.

The site of funiculosin action is present in isolated Complex III, since both

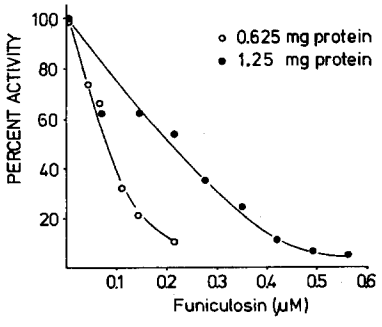

Fig. 1. Effect of protein concentration on the inhibition of NADH oxidase by funiculosin. Inhibition of NADH oxidase was measured using 0.625 mg (○) or 1.25 mg (●) Mg^{2+}-ATP particle protein per assay. The assay media contained 167 mM sucrose, 50 mM Tris · HCl, pH 7.5, and 1 mM NADH in a total of 1.6 ml. All samples were incubated for 1 min with funiculosin prior to addition of substrate. Control activity was 0.530 μg atoms O per min per mg protein.

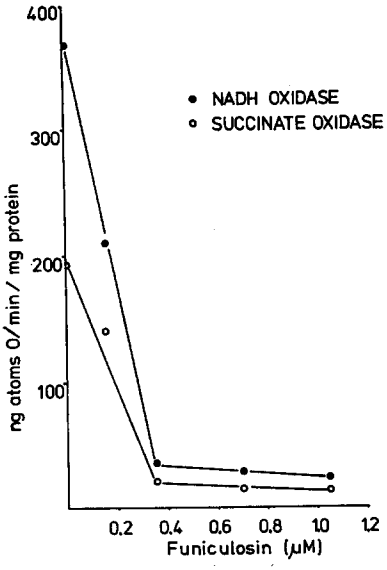

Fig. 2. Funiculosin titer for the inhibition of NADH- and succinate-oxidases. Assay conditions are as in Fig. 1. Each assay contained 1.25 mg of Mg^{2+}-ATP particle protein.

duroquinol- and succinate-cytochrome *c* reductases are strongly inhibited (Table I) (the latter enzyme is considered to be a contamination in Complex III). In contrast, 2-thenoyltrifluoroacetone (TTFA) has little or no effect on duroquinol-cytochrome *c* reductase while it strongly inhibits both succinate-cytochrome *c* and succinate-PMS reductase. These results indicate that duroquinol feeds electrons into Complex III beyond succinate dehydrogenase, and that funiculosin inhibits between the dehydrogenase and cytochrome *c*.

Fig. 3 shows that funiculosin, like antimycin, induces a cross over in the redox states of cytochromes *b* and cc_1, as well as an extra reduction of cytochrome *b* in Mg^{2+}-ATP particles oxidizing NADH. Thus, funiculosin acts at or near the antimycin site.

It has been reported that antimycin prevents reduction of cytochrome *b* if a

TABLE I

FUNICULOSIN INHIBITION OF DUROQUINOL- AND SUCCINATE-CYTOCHROME *c* REDUCTASE ACTIVITIES IN ISOLATED COMPLEX III

TTFA, 2-thenoyltrifluoroacetone.

	μmol cytochrome *c* reduced/min/mg	
	DQH_2-cytochrome *c* reductase	Succinate-cytochrome *c* reductase
Expt. 1 Control	4.60	0.73
Funiculosin (3 nmol/nmol c_1)	0.12	0.01
Expt. 2 Control	1.40	0.25
TTFA (300 μM)	1.10	0.00

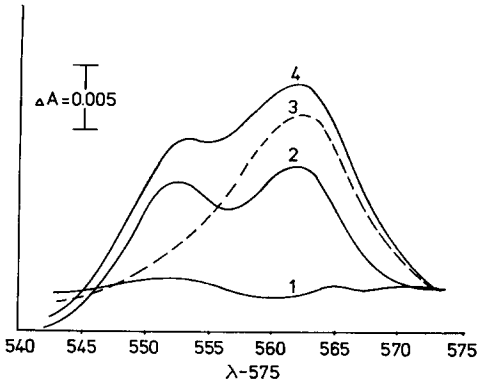

Fig. 3. Effects of funiculosin on the redox states of cytochromes *b* and c_1. Mg^{2+}-ATP particles (2.5 mg protein) were suspended in a volume of 1 ml containing 167 mM sucrose and 50 mM Tris · HCl, pH 7.5. Spectra were scanned with the reference wavelength fixed at 575 nm. Curve 1: baseline containing Mg^{2+}-ATP particles; Curve 2: NADH (1 mM), cuvette anaerobic; Curve 3 : 3 μM funiculosin plus oxygen added to curve 2; Curve 4: dithionite.

component in the cytochrome *b*-c_1 region is pre-reduced with ascorbate [6, 7], ascorbate+TMPD [8] or 2,3-dimercaptopropanol [9]. To test if funiculosin also shares this unusual effect with antimycin, experiments were carried out on isolated Complex III (Fig. 4). Reduction of cytochrome *b* by duroquinol is slowed or completely eliminated by pre-reduction of the complex with ascorbate in the presence of antimycin (Fig. 4D). Funiculosin produces the same effect upon cytochrome *b* reduction, but inhibition is not so complete as with antimycin (Fig. 4B and 4C). In addition, funiculosin produced a small extra reduction of cytochrome *b* in isolated Complex III (compare Fig. 4A and 4B), which we have never observed with antimycin.

The above experiments indicate that antimycin and funiculosin act in a similar manner. A difference between the two antibiotics is observed, however, as demon-

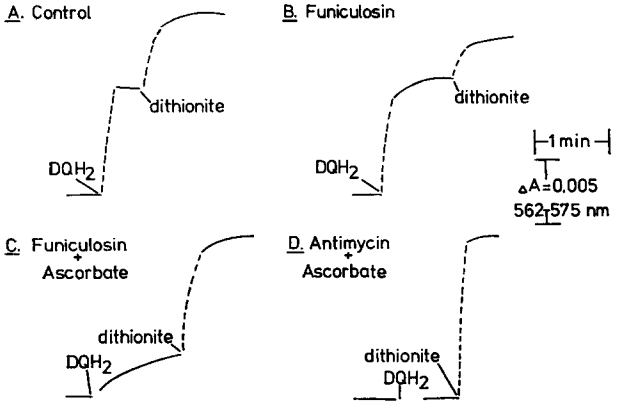

Fig. 4. Funiculosin control of cytochrome *b* reduction in isolated Complex III. Complex III (160 μg protein) was suspended in 1 ml of buffer containing 167 mM sucrose and 50 mM Tris · HCl, pH 7.5. Other additions were: 10 mM ascorbate, 1.5 μM funiculosin, and 0.3 μg antimycin. Cytochrome *b* was measured at 562–575 nm. DQH_2, duroquinol.

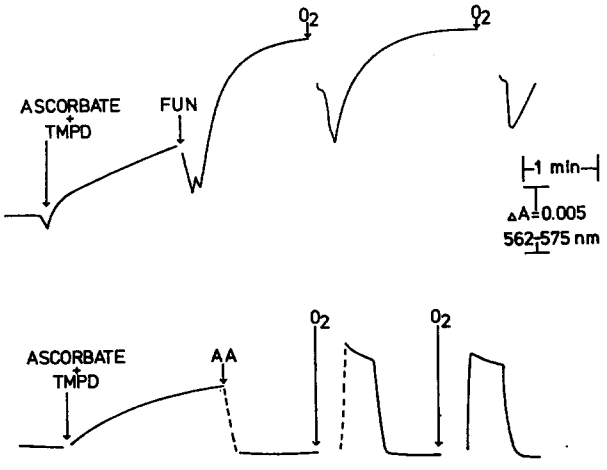

Fig. 5. Effects of funiculosin (FUN) and antimycin (AA) on the aerobic-reduction of cytochrome *b* in Mg^{2+}-ATP particles, Mg^{2+}-ATP particles (2.5 mg protein) were suspended in 1 ml of buffer containing 167 mM sucrose and 50 mM Tris · HCl, pH 7.5. Other additions were: 5 mM ascorbate, 240 μM TMPD, 3 μM funiculosin, and 0.5 μg antimycin.

strated in Fig. 5. In the absence of oxygen, antimycin induces the complete oxidation of cytochrome *b* reduced in Mg^{2+}-ATP particles by ascorbate+TMPD (lower curve). Addition of oxygen under these conditions leads to an extra reduction of cytochrome *b* followed by re-oxidation when oxygen is depleted. In contrast to antimycin, funiculosin induces a further reduction of cytochrome *b* in the absence of oxygen, and the subsequent addition of oxygen leads to oxidation of cytochrome *b* rather than an extra reduction, as with antimycin (Fig. 4B, upper curve). Similar results have been obtained in the presence of uncouplers and with lyophilized membranes, and are therefore not dependent upon the state of energization of the membrane.

DISCUSSION

The mechanism of action of the antibiotic funiculosin [2] is similar to that of antimycin. Like antimycin, funiculosin inhibits NADH and succinate oxidation at concentrations near to that of cytochrome cc_1 in the respiratory chain, and is thus a more efficient inhibitor than 2-heptyl-4-hydroxyquinoline 4-oxide [3]. Funiculosin also induces the oxidation of cytochrome cc_1 and the reduction of cytochrome *b* in the aerobic steady state, and it inhibits duroquinol-cytochrome *c* reductase activity in isolated Complex III. The latter result clearly locates the site of action of funiculosin. In addition to the above, funiculosin, like antimycin, induces an extra reduction of cytochrome *b*, and it places constraints on electron transport from duroquinol to cytochrome *b* in purified Complex III if the complex is pre-reduced with ascorbate [6–8].

The only difference observed between funiculosin and antimycin is the manner in which the two antibiotics modulate reduction of cytochrome *b* by ascorbate+TMPD in the aerobic steady state (Fig. 5). In view of our lack of understanding of the mechanism underlying the aerobic-reduction of cytochrome *b* (see ref. 11 for review),

and thus the role of antimycin, we cannot conclude from these experiments that the two antibiotics have different modes of action. Such a result might be explained on a kinetic basis due to less efficient control of funiculosin on cytochrome *b* reduction (Fig. 4). On the other hand, there is reason to believe that compounds such as 2-heptyl-4-hydroxyquinoline *N*-oxide and 2,3-dimercaptopropanol act differently from antimycin [8–10, 12], even though they exert an "antimycin-like" inhibition on the electron transport chain [8–10]. This raises the possibility that an "antimycin-like" inhibition can result from alterations at several different sites in Complex III. This possibility is strengthened by observations that the effects of antimycin on cytochrome *b* reduction and electron transport can be separated in antimycin-resistant yeast mutants [13], and may be related to different antimycin binding sites [14]. Whether funiculosin has a novel mechanism of action at the molecular level or if it acts similarly to antimycin remains to be determined.

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