

## FUNICULOSIN; A NEW SPECIFIC INHIBITOR OF THE RESPIRATORY CHAIN IN RAT LIVER MITOCHONDRIA

Ulrich K. MOSER and Paul WALTER

Medizinisch-chemisches Institut der Universität Bern, Bülhstrasse 28, 3000 Bern 9, Switzerland

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### 1. Introduction

The antibiotic funiculosin ( $C_{27}H_{41}NO_7$ ) has been isolated in crystalline form from *Penicillium funiculosum* Thom [1]. The antibiotic has a broad antifungal spectrum and it also shows some antiviral activity, but is ineffective against both gram positive and negative bacteria [1]. The results of this communication show that in rat liver mitochondria, funiculosin blocks the electron transfer from 3-hydroxybutyrate and succinate, however, not from cytochrome *c* to oxygen. The compound also inhibits the electron transfer from succinate to ferricyanide but shows no effect on the reversed electron transport from succinate to NAD and on the dinitrophenol stimulated ATPase. Its inhibitory properties are similar to those of antimycin and it is concluded that funiculosin is a specific inhibitor of the respiratory chain blocking the electron transport between coenzyme Q and cytochrome *c*.

### 2. Materials and methods

Male rats (CFN, SIV 50) with an average weight of 200–240 g from the Tierzucht Institut of the University of Zurich were used. Liver mitochondria were prepared according to Johnson and Lardy [2] in 0.25 M mannitol–0.07 sucrose [3]. The mitochondria were suspended in the sucrose medium at 1 g of original liver weight per ml. Because mannitol interferes [4] with the Lowry-Lopez assay for phosphate determination [5], mitochondria were isolated in 0.25 M sucrose for the experiments of tables 1 and 3. The reaction media are given in the legends.

Table 1  
Effect of funiculosin on mitochondrial ATPase

Funiculosin $\mu M$	DNP	Pi liberated $\mu mol$
None	–	0.1
0.5	–	0.2
2.0	–	0.3
None	+	3.9
0.5	+	4.0
2.0	+	3.8

The reactions were carried out according to Walter et al. [8]. When indicated, 0.05 mM dinitrophenol (DNP) was added. Final volume was 1.0 ml containing 1.7 mg of mitochondrial protein.

Table 2  
Effect of funiculosin on the reversed electron transport from succinate to NAD

Funiculosin $\mu M$	3-Hydroxybutyrate formed $\mu mol$
None	1.72
0.1	1.99
0.25	1.91
0.60	1.80
1.0	1.85

The assay system is similar to that of Ernster and Lee [9] containing 1.8 mM acetoacetate, 7.5 mM magnesium chloride, 45 mM potassium chloride, 20 mM glycyl-glycine buffer pH 7.5, 20 mM ascorbate, 1 mM *N,N,N',N'*-tetramethyl-*p*-phenyldiamine (TMPD), 1  $\mu g$  antimycin, 20 mM succinate, 6 mg mitochondrial protein and mannitol–sucrose solution to make a final isotonic solution of 2.0 ml. Reactions were carried out in 25 ml shaking erlenmeyer flasks for 25 min at 30°C.

Table 3  
Effect of funiculosin on oxidative phosphorylation from ascorbate as substrate

Additions	Oxygen uptake	Phosphate uptake	P : O
	ngatoms	$\mu\text{mol}$	
Control	$16.11 \pm 1.95$	$13.42 \pm 1.17$	$0.84 \pm 0.04$
Funiculosin ( $0.67 \mu\text{M}$ )	$15.24 \pm 1.52$	$12.64 \pm 0.88$	$0.83 \pm 0.03$

The measurements were carried out in Warburg vessels in principle according to Sanadi and Jacobs [10]. The medium contained 2 mM ATP, 6.7 mM potassium phosphate buffer pH 7.4, 16.7 mM triethanolamine buffer pH 7.4, 10 mM magnesium sulfate, 13.3 mM ascorbate, 0.27 mM TMPD, 0.02  $\mu\text{M}$  rotenone, 6.7 mg mitochondrial protein and sucrose solution to make a final isotonic solution of 3.0 ml. 0.5 mg of hexokinase type III (Sigma) in 0.2 ml 0.25 M glucose were tipped in from the sidearm after the equilibration period. Incubation time was 15 min at 37°C. Results are averages of 5 incubations  $\pm$  SD with  $n = 5$  with the same mitochondrial preparation.

The reactions were stopped with trichloroacetic acid (tables 1, 3, 4) or perchloric acid (table 2). Unless otherwise indicated, the results represent average values of duplicate incubations with the same mitochondrial preparation.

Funiculosin was a gift of Sandoz AG in Basel. Antimycin A (B grade) was obtained from Calbiochem (Luzern, Switzerland). Both antibiotics were dissolved in maximally 20  $\mu\text{l}$  ethanol. This amount of ethanol was also added to the controls.

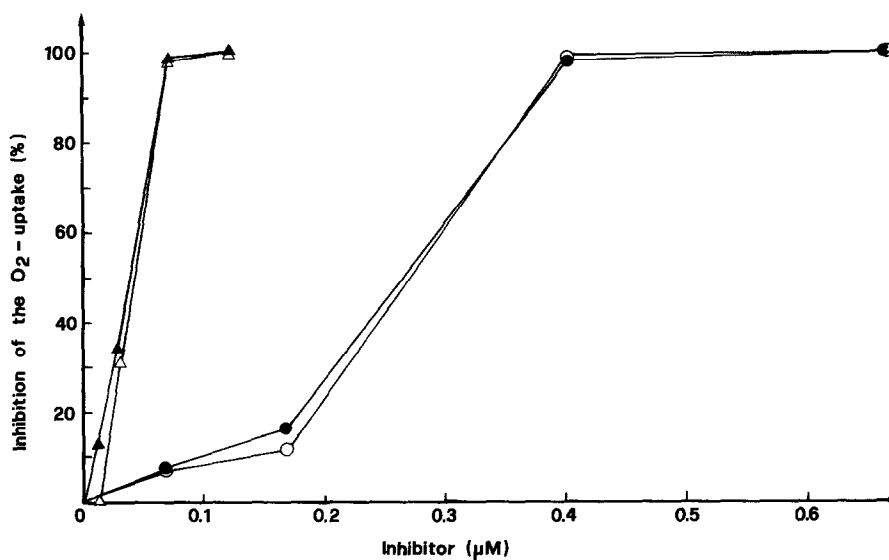


Fig. 1. Inhibitory effects of funiculosin and antimycin on coupled respiration from 3-hydroxybutyrate or succinate as substrates. Oxygen consumption was measured with a Clark-type oxygen electrode (Yellow Springs Instr. Ohio, US). The medium contained 6.7 mM potassium phosphate pH 7.4, 6.7 mM triethanolamine buffer pH 7.4, 10 mM magnesium sulfate, 3.3 mM of either succinate or 3-hydroxybutyrate, 0.67 mM ADP, 8 mg mitochondrial protein and mannitol-sucrose solution to make a final isotonic solution of 3.0 ml. Round symbols: Inhibition by funiculosin; triangles: inhibition by antimycin; filled symbols: 3-hydroxybutyrate as substrate; open symbols: succinate as substrate.

### 3. Results and discussion

The plots in fig.1 show a nearly complete inhibition by funiculosin of the coupled respiration from 3-hydroxybutyrate as well as from succinate. The sensitivity towards the inhibitor is the same in the presence of either substrate. For comparison, the results with antimycin are given showing the same inhibitory pattern as funiculosin, however, antimycin exerts its effect at an about 7 times lower concentration (on a molar basis) than the new antibiotic. Addition of  $3 \cdot 10^{-5}$  M dinitrophenol to the inhibited system did not result in an increased oxygen uptake (not shown) indicating that the antibiotic is acting on the respiratory chain and not on the energy coupling processes leading to the formation of ATP. This conclusion is supported by the measurements of the ATPase activity which was neither stimulated in the absence of dinitrophenol nor inhibited in the presence of the uncoupler (table 1).

In order to pinpoint the site of action of funiculosin the effects of the antibiotic were measured on various segments of the respiratory chain. As shown in tables 2 and 3, funiculosin did not inhibit the respiration linked to the first and third phosphorylation site, whereas the electron transport at site 2 from succinate to ferricyanide was blocked (table 4, exp. 1).

In the reactions described so far, funiculosin showed an antimycin like effect. Even though the structure of funiculosin has not yet been elucidated enough is known to be sure that the two compounds are not identical. Besides differences in the UV-spectra as well as in optical rotation and melting point [1, 6], it is especially noteworthy that funiculosin possesses neither phenolic nor enolic hydroxyl nor aldehyde groups [1] whereas antimycin contains an *o*-formylaminophenol group [6]. When antimycin was preincubated with ferricyanide in the absence of mitochondria, antimycin was shown to lose its inhibitory properties, probably because of a transformation of the *o*-formylaminophenol group [7]. When a similar experiment was carried out with funiculosin (table 4, exp. 2) only a small change in the inhibitory activity could be observed. This experiment indicates that funiculosin and antimycin may act by different mechanisms.

The results presented show that funiculosin is a very specific inhibitor acting near or at the same site as antimycin.

Table 4  
Effect of funiculosin and antimycin on electron transport from succinate to ferricyanide under different incubation conditions

Additions	Ferricyanide reduced	
	Exp. I	Exp. II
$\mu\text{M}$	$\mu\text{moles/20 min}$	
None	26.2	23.9
0.67 Antimycin	3.3	23.8
1.34 Antimycin	2.6	21.8
0.29 Funiculosin	26.2	22.2
0.83 Funiculosin	2.7	12.6
1.67 Funiculosin	3.6	7.0
3.33 Funiculosin	3.0	7.0

The incubations were carried out in shaking Erlenmeyer flasks at 30°C. They contained 2 mM ATP, 5 mM magnesium sulfate, 16.7 mM phosphate buffer pH 7.4, 33 mM triethanolamine buffer pH 7.4, 6.7 mM succinate and mannitol-sucrose solution to make an isotonic solution of 2.3 ml. Further additions were in exp. I: at zero time, 0.3 ml of mitochondrial suspension (7 mg protein); after 8 min of preincubation, 0.2 ml 0.25 M potassium ferricyanide and 0.2 ml 0.25 M glucose containing 0.5 mg hexokinase; in exp. II: potassium ferricyanide at zero time, mitochondria and glucose-hexokinase at 8 min. Final volume was 3 ml for all incubations. Ferricyanide utilization was measured spectrophotometrically [7].

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