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Variability of the K^+–Na^+ Discrimination of Beauvericin in Mitochondrial Membranes

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Abstract

In intact mitochondria supplemented with succinate or β-hydroxybutyrate, the rates of oxygen consumption induced by beauvericin followed the ionic selectivity pattern: $Na^+ > Rb^+, Cs^+, K^+, Li^+$.

When the respiratory substrate is glutamate plus malate in the absence of phosphate, the selectivity pattern is: $K^+ > Rb^+ > Cs^+ > Li^+ > Na^+$.

When the media are supplemented with phosphate, the Na^+/K^+ discrimination of beauvericin is considerably modified with all the respiratory substrates, being $K^+ > Na^+$ with succinate and $Na^+ > K^+$ with glutamate plus malate, whereas no significant ionic selectivity differences were obtained with β-hydroxybutyrate.

The respiratory control induced by oligomycin in submitochondrial particles is released by beauvericin only in the presence of a nigericin-like carboxylic antibiotic and an alkali metal cation, being far more effective in K^+ than in Na^+.

This selectivity is maintained regardless of whether NADH or succinate is used as a respiratory substrate.

Release of respiratory control can also be obtained with a combination of beauvericin and NH_4Cl.

This information indicates that the ionic selectivity pattern obtained with beauvericin in mitochondrial membranes is an intrinsic property of the antibiotic which, however, can be significantly modified by factors such as the nature of the translocatable substrate anion or other anionic species, as well as the possible operation of a Na^+/H^+ antiporter existent in the membrane.

Introduction

One of the outstanding unsolved problems in biology is that of the mechanism of the ionic selectivity in membranes. This problem has been

experimentally approached with the advent of the macrocyclic antibiotics and model membrane systems. The selectivity and transport-induced properties of the antibiotics follow a close parallelism when studied in the natural [1-3] or the artificial membrane [4, 5].

Beauvericin is a cyclic hexadepsipeptide antibiotic containing in alternating sequence three D-α-hydroxyisovaleryl and three *n*-methyl-L-phenyalanyl residues [6]; The antibiotic was originally found by Dorschner and Lardy [7] to induce the transport of Li^+ more than of Na^+ in intact mitochondria. It was independently synthesized by the groups of Roeske [8] and Ovchinikov [9]. The conductometrically determined [9] ionic selectivity of beauvericin is the following: Rb^+, $Cs^+ > K^+ > Na^+ > Li^+$, while in bulk phase partition measurements, the antibiotic extracted Na^+, K^+ and Rb^+ with similar effectiveness while Li^+ about one-third as effectively as Na^+ [8].

The present contribution describes the variable ionic selectivity properties of beauvericin in mitochondrial membranes as obtained from its effects on ion transport and energy coupling both in intact mitochondria and submitochondrial sonic particles.

Materials and Methods

Mitochondria were prepared from livers of male rats weighing 150 g as described by Johnson and Lardy [10]. Submitochondrial particles derived by sonic disruption of beef-heart mitochondria were prepared by the method of Fessenden and Racker [11].

A continuous recording of oxygen-consumption and its derivative was carried out by means of an apparatus designed, developed and constructed by Chance, Mayer, Pressman and Graham [12-14].

Protein was determined by the biuret method [15]. The antibiotic beauvericin was kindly supplied by Dr. Robert W. Roeske, Indiana University School of Medicine. Nigericin and monensin A were a kind gift from Dr. Henry A. Lardy, The Institute for Enzyme Research, The University of Wisconsin and Dr. Marvin Gorman, The Eli Lilly Laboratories. All chemicals used were of the highest purity commercially available. Glass-redistilled water was used throughout.

Results

Ionic Selectivity of Beauvericin in Intact Mitochondria

(a) Substrate dependence of the ionic selectivity of beauvericin. The influence of beauvericin on mitochondrial respiration is stringently

dependent on the nature of the alkali metal cation and the oxidizable substrate added.

The addition of saturating concentrations of beauvericin (13×10^{-6} M) to mitochondria oxidizing succinate, in the absence of added phosphate and phosphoryl acceptor, results in a stimulation of the rate of oxygen consumption, which is about three-fold faster in Na^+ than in K^+ supplemented media. On the other hand, when the oxidizable substrates are glutamate plus malate (Fig. 1B) an opposite ionic dependence of the respiration rate stimulated by the antibiotic is obtained, that is, $K^+/Na^+ \cong 6$.

A detailed study of the ionic selectivity of beauvericin on mitochondrial respiration, under experimental conditions analogous to those described in Fig. 1 is presented in Table I. A remarkable contrast between the compared substrates is evident; for the case of succinate, the ionic selectivity is $Na^+ > K^+$, Rb^+, Cs^+, Li^+, whereas the selectivity pattern: $K^+ > Rb^+ > Cs^+ > Li^+ > Na^+$ is found for glutamate plus malate. It clearly emerges that the main discrimination conferred by beauvericin is that between K^+ and Na^+. Hence, the experiments to be described will be restricted to this particular pair.

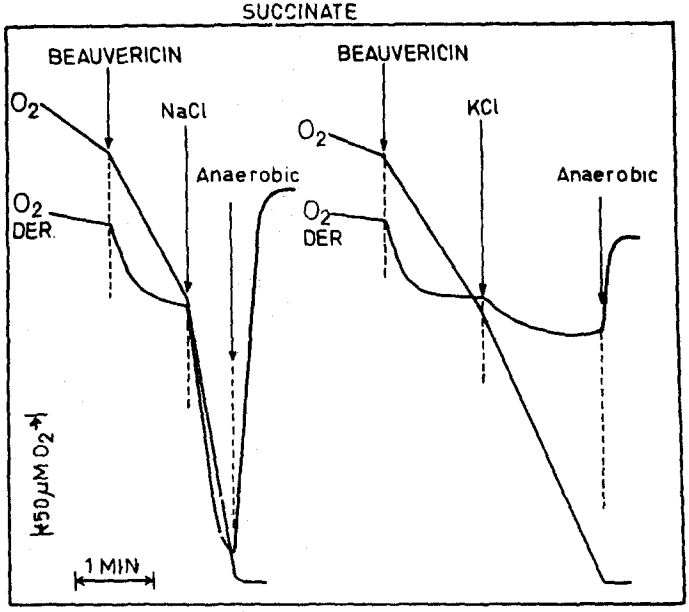

Figure 1A. Effect of beauvericin on succinate oxidation by rat liver mitochondria in the presence of K^+ or Na^+. Reaction mixture: 1.2 mg/ml of mitochondrial protein, 0.15 M sucrose, 0.003 m triethanolamine-HCl, pH 7.4, 0.002 M succinate (triethanolamine-salt), 2 μg/ml rotenone, and where indicated: 0.008 M KCl or NaCl, and 13×10^{-6} M beauvericin. Final volume: 5.0 ml. Temperature: 25°C.

TABLE I. The ionic selectivity of beauvericin to enhance the rate of succinate or glutamate plus malate oxidation by rat liver mitochondria

Alkali metal cation added	Succinate Oxygen uptake in natoms O_2/min	ΔCation*	Glutamate + Malate Oxygen uptake in natoms O_2/min	ΔCation*
None	281	–	28	–
Na^+	1139	4.1	73	2.6
K^+	538	1.9	192	6.7
Rb^+	530	1.9	144	5.1
Cs^+	523	1.9	120	4.0
Li^+	498	1.8	96	3.4

Reaction mixture: The mitochondrial protein concentration is: 0.4 mg/ml and 1.7 mg/ml for succinate and glutamate + malate (triethanolamine-salts) oxidation, respectively; 0.15 M sucrose, 0.003 M triethanolamine-HCl, pH 7.4, 0.016 M KCl or NaCl, 13.6×10^{-6} M beauvericin and where indicated: 0.002 M succinate with 2 μg/ml of rotenone and 0.01 M glutamate + 0.01 M malate. Temperature: 25° C.

* These values represent the ratio of oxygen consumption induced by addition of beauvericin in the presence and absence of alkali metal cations.

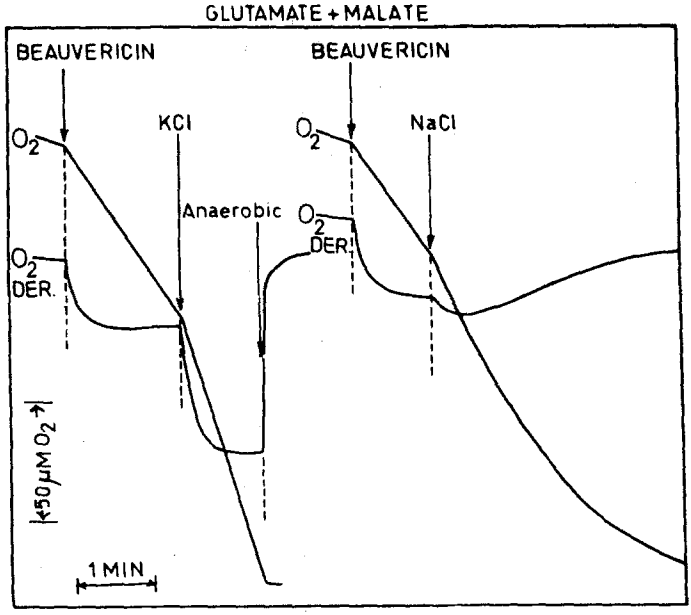

Figure 1B. Effect of beauvericin on glutamate + malate oxidation by rat liver mitochondria in the presence of K^+ or Na^+. Reaction mixture: 1.2 mg/ml of mitochondrial protein, 0.15 M sucrose, 0.003 M triethanolamine-HCl, pH 7.4, 0.01 M glutamate, 0.01 M malate (triethanolamine-salts), and where indicated: 0.008 M KCl or NaCl and 13×10^{-6} M beauvericin. Final volume: 4.0 ml. Temperature: 25° C.

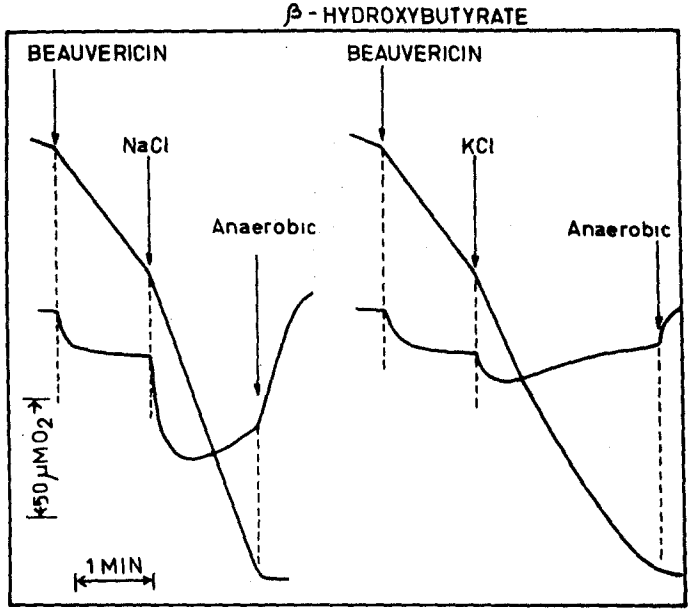

Figure 1C. Effect of beauvericin on β-hydroxybutyrate oxidation by rat liver mitochondria in the presence of K^+ or Na^+. Reaction mixture: 0.7 mg/ml of mitochondrial protein, 0.15 M sucrose, 0.003 M triethanolamine-HCl, pH 7.4, 0.01 M D-L-β-hydroxybutyrate (triethanolamine salt), and where indicated: 0.008 M KCl or NaCl and 13 x 10^{-6} M beauvericin. Final volume: 5.0 ml. Temperature: 25° C.

It is also worth noting that the antibiotic's discrimination between Na^+ and K^+ observed with β-hydroxybutyrate (Fig. 1C) resembles that of succinate, but it is less apparent ($Na^+/K^+ \cong 2$).

(b) Influence of phosphate on the K^+/Na^+ discrimination of beauvericin in intact mitochondria. The variations in the K^+/Na^+ selectivity of beauvericin imposed by phosphate upon mitochondria oxidizing different substrates is illustrated in Fig. 2. The overall effect of phosphate appears to be an enhancement of the rate of oxygen consumption with those cations which are ineffective in releasing the respiration of beauvericin supplemented mitochondria in the absence of phosphate. Thus, when the data are expressed as percent enhancement of respiration in the presence of phosphate, *versus* in its absence, an apparent inversion of the K^+/Na^+ discrimination of the antibiotic is observed both with succinate and glutamate plus malate, being $K^+ > Na^+$ with succinate and $Na^+ > K^+$ with glutamate plus malate whereas no significant differences were detected for the case of β-hydroxybutyrate. However, it should be noted that an inhibitory effect of phosphate on

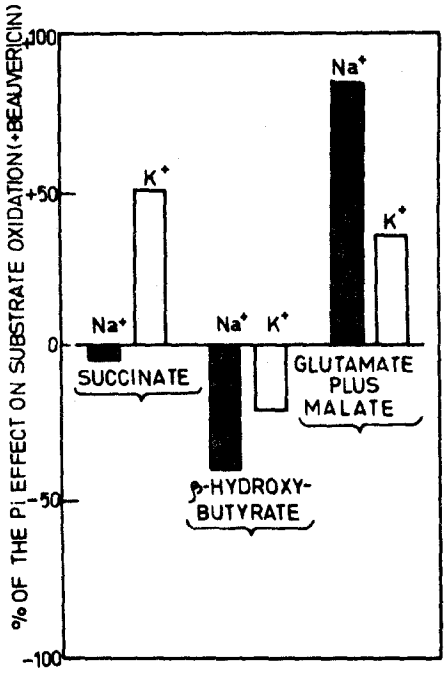

Figure 2. Effect of inorganic phosphate on the Na^+-K^+ discrimination of beauvericin in intact mitochondria oxidizing succinate, β-hydroxybutyrate or glutamate + malate. Reaction mixture: The mitochondrial protein concentration is: 0.4 mg/ml, 1 mg/ml and 0.7 mg/ml for succinate glutamate + malate and β-hydroxybutyrate oxidation, respectively, 0.15 M sucrose, 0.003 M triethanolamine-HCl, pH 7.4, 0.004 M NaCl or KCl, 0.005 M inorganic phosphate, 13×10^{-6} M beauvericin and where indicated: 0.002 M succinate, 0.01 M glutamate + 0.01 M malate, and 0.01 M β-hydroxybutyrate (triethanolamine-salts). In the experiments with succinate rotenone was included in the mixture at a final concentration of 2 μg/ml. Final volume: 5.0 ml. Temperature: 25° C.

the beauvericin-induced enhancement of the respiratory rate is only observed with β-hydroxybutyrate.

Ionic Selectivity of Beauvericin in Submitochondrial Particles

In order to gain further insight into the mechanism of the variable ionic discrimination of beauvericin in intact mitochondria, a study of the effects of the ionophore [16] on submitochondrial particles was undertaken.

Lee and Ernster [17] have shown that oligomycin induces an inhibition of respiration in submitochondrial particles which is released

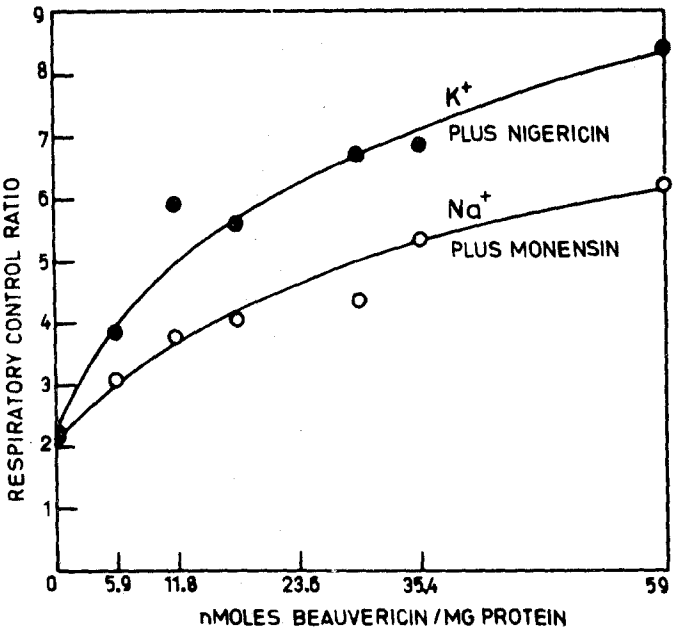

Figure 3. Combined effect of beauvericin and carboxylic antibiotics on the oligomycin-induced respiratory control of submitochondrial particles oxidizing NADH. Reaction mixture: 0.1 mg/ml of submitochondrial particle protein, 0.25 M sucrose, 0.002 M Tris-Acetate, pH 7.4, 1 μg/ml oligomycin, 1.5 mM NADH, and where indicated: 0.02 M KCl or NaCl, 1.27 nmoles/mg protein of nigericin, 5.5 nmoles/mg protein of nonensin A. Final volume: 5.0 ml. Temperature: 25° C.

by uncouplers. Montal, Chance and Lee [18] have shown that a combination of a carboxylic ionophore and valinomycin in the presence of K^+ released the oligomycin-induced respiratory control. Hence, the effect of beauvericin and alkali metal cations on the oligomycin-induced respiratory control of submitochondrial particles was studied.

The oligomycin-inhibited respiration is released by beauvericin only in the presence of a carboxylic ionophore and an alkali metal cation. Thus, in order to test the K^+/Na^+ discrimination of beauvericin, the particles were supplemented with saturating concentrations of either of the carboxylic antibiotics nigericin, which has greater affinity for K^+ over Na^+ [16, 19, 20], or monensin A-which exhibits greater affinity for Na^+ over K^+ [20, 21].

In Fig. 3 is illustrated a plot of increasing concentrations of beauvericin versus the respiratory control ratio RCR (the ratio of the respiration rate in the presence and absence of beauvericin) of submitochondrial particles oxidizing NADH in media supplemented with

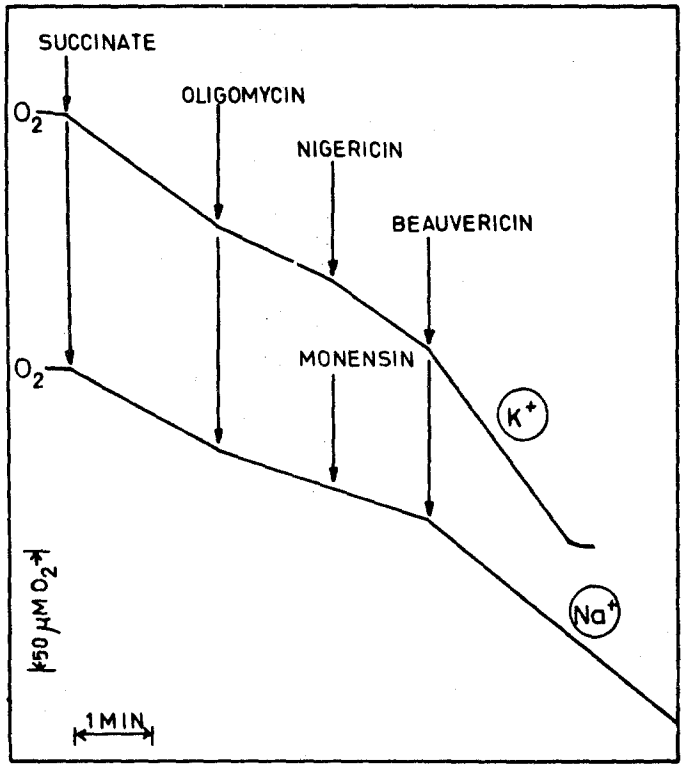

Figure 4. Combined effect of beauvericin and carboxylic antibiotics on the oligomycin-induced respiratory control of submitochondrial particles oxidizing succinate. Reaction mixture: 0.1 mg/ml of submitochondrial particle protein, 0.25 M sucrose, 0.002 M Tris-Acetate, pH 7.4, 0.006 M succinate (triethanolamine-salt), and where indicated 0.02 M KCl or NaCl, 1.0 μg/ml oligomycin, 1.27 nmoles/mg protein of nigericin, 5.5 nmoles/mg protein of monensin A, and 6.8×10^{-6} M beauvericin. The particles were preincubated with 2.0 mM succinate for 30 min at 37° C prior to the assay. Final volume: 5.0 ml. Temperature: 25° C.

either K^+ or Na^+. It is clear that all over the range of concentrations of beauvericin studied, the uncoupling effect of the ionophore is more effective in the K^+ than in the Na^+ medium. This K^+/Na^+ discrimination is also maintained when succinate is used as the respiratory substrate (Fig. 4), in contrast to what is observed in intact mitochondria (see Fig. 1).

Papa [22] and Cockrell and Racker [23] observed that the combination of NH_4Cl and valinomycin uncoupled submitochondrial particles whereas neither NH_4Cl nor valinomycin did so alone.

As shown in Fig. 5, release of respiratory control can also be obtained with a combination of 13×10^{-6} M beauvericin and NH_4Cl.

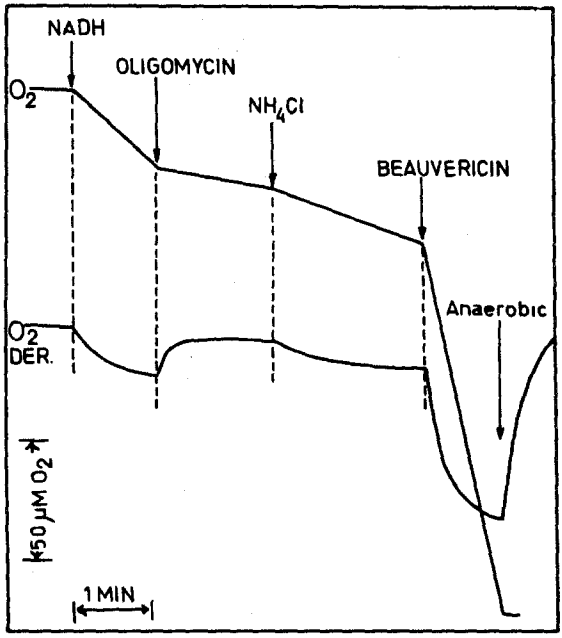

Figure 5. Combined effect of beauvericin and NH_4Cl on the oligomycin-induced respiratory control of submitochondrial particles. Reaction mixture: 0.1 mg/ml of submitochondrial particle protein, 0.25 M sucrose, 0.002 M Tris-Acetate, pH 7.4, and where indicated: 1.5 mM NADH, 1.0 µg/ml oligomycin, 0.01 M NH_4Cl, and 6.8×10^{-6} M beauvericin. Final volume: 5.0 ml. Temperature: 25° C.

Discussion

The evidence hereby presented indicates that the cyclic hexadepsipeptide antibiotic beauvericin exhibits ionophoretic properties in mitochondrial membranes analogous to those previously described for the valinomycin-like antibiotics (cf. Pressman [2], Chance and Montal [3]). A point of utmost importance that clearly emerges from our observations is the drastic modification of its apparent ionic selectivity observed when different substrate anions are oxidized by intact mitochondria. For instance, a $K^+ > Na^+$ discrimination is demonstrated when respiration is supported by glutamate plus malate (Fig. 1B, Table I) or glutamate [7] whereas an opposite selectivity is detected for the oxidation of succinate and β-hydroxybutyrate. Interestingly, the oxidative enzymes for the latter substrates are membrane bound whereas those for the former substrates are soluble in the mitochondrial matrix space [24].

An additional consideration is worth noting in assessing the role of

anions in the apparent ionic selectivity of beauvericin in intact mitochondrial membranes. It is now well accepted [3, 25-27] that uncoupling of mitochondria is associated to the simultaneous collapse of the membrane potential and pH gradient components of the respiration-generated electrochemical H^+ gradient. When succinate is the substrate, the beauvericin-mediated uncoupling effect in the presence of Na^+ suggests the existence of a Na^+/H^+ antiporter due to the lack of phosphate anion requirement to completely uncouple respiration. When Na^+ is replaced by K^+, uncoupling is obtained only in the presence of phosphate, indicating that if a K^+/H^+ antiporter exists, it is less active than the Na^+/H^+ exchanger; therefore, uncoupling requires the translocation of phosphoric acid in order to collapse the transmembrane pH gradient [28]. With glutamate or glutamate + malate the additional constraint of limited substrate anion translocation imposes a requirement for phosphate to evidence the beauvericin + Na^+-dependent uncoupling effect.

This proposal is further substantiated by the results obtained with submitochondrial particles where all the substrates are readily accessible to the dehydrogenases. In this case, a $K^+ > Na^+$ discrimination is exhibited by beauvericin regardless of the oxidizable substrate anion present. Furthermore, the fact the beauvericin and NH_4Cl completely uncouple the particles in the absence of any other ionophore strongly indicates that the most significant effect of beauvericin in the uncoupling mechanism is the mediation of an electrophoretic cation efflux. This collapse of the membrane potential is associated to the abolishment of the pH gradient by the internal protonation of the freely-diffusible NH_3 [23, 29].

It is relevant to emphasize that the antibiotics that exhibit a poor discrimination between K^+ and Na^+ in intact mitochondrial membranes show drastic modifications in their selectivity patterns dependent on he nature of the anionic species present. Such is the case for gramicidin [3], monazomycin [31] and beauvericin. This is in contrast to the negligible effect of anions on the ionic selectivity of the highly discriminative ionophores such as valinomycin [2], the enniatins [1] and the nonactin homologs [12, 32].

Finally, a word of caution is needed when evaluating ionic selectivity patterns of antibiotics in biological membranes where the intrinsic selectivity properties of the adsorbate can be significantly masked by several conditions such as the nature of the ionic environment, the presence of native or intrinsic translocators and the lipid composition of the membrane. In fact, data to be described elsewhere [33] indicate that the selectivity exhibited by beauvericin in submitochondrial particles is indeed the intrinsic selectivity of the antibiotic, as demonstrated in model lipid bilayers of the Mueller-Rudin type [34] of different composition.

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