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Mitochondrial Permeability to Ions Induced by Dibenzodioxines and Dibenzofuranes. A Simple Method to Measure Ion Permeability in Membranes

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Abstract

This paper proposes a method to demonstrate that 123789 Dibenzodioxine and 2378 Dibenzofurane enhance membrane permeability to the ions in mitochondria from rat liver.

The procedure can be employed:

i) as a general method to assess the enhancement of membrane permeability by toxic compounds,
ii) to study the decrease in conductivity of carrier-systems induced by inhibitors of transport, and
iii) to study the behaviour of ion-carrier systems which are potentially dependent on biological membranes.

Keywords: mitochondria, dibenzodioxines, dibenzofuranes

Introduction

Many papers report the interaction of toxic compounds such as DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl)-ethane) and PCBs (polichlorinated biphenyls) with mitochondrial and synthetic membranes [1-5]. In mitochondria, the most significant effect is an enhancement of the state 4 respiratory rate, which can be detected by measuring the rate of oxygen consumption. This effect is generally due to an uncoupling effect. Following Mitchell's chemiosmotic hypothesis, an uncoupler is a chemical compound, generally a weak acid, which enhances the permeability to protons of the mitochondrial membrane, and therefore stimulates the rate of oxygen consumption. Not only DDT and PCBs show this behaviour; in this paper we have verified that also dioxines (DD) and dibenzofuranes (DF) stimulate the respiratory rate, but like DDT and PCBs, DD and DF do not possess an acidic group. We hypothesize that the stimulation of the respiratory rate observed in the presence of DDT, PCBs, DD and DF is due to a more general enhancement of permeability of the membrane to all ions, including protons. In this paper we provide evidence that the above-mentioned compounds also enhance membrane permeability to other ions such as potassium, displaying a detergent-like action. Toxicological consequences of the action of these compound may be very dramatic, since such behaviour is not restricted to the mitochondrial membrane, but should affect all membranes. Therefore, this behaviour could contribute to an understanding of the molecular toxicity mechanism of DD and DF compounds and explain the wide range of toxic effects observed in whole organisms [6, 7]. Furthermore, the method that we introduce to assess the enhancement in permeability induced by DD and DF can be proposed as a general method to measure ion conductivity in membranes.

Experimental Procedures

Rat liver mitochondria were prepared according to standard methods [8]. Mitochondria were freshly prepared and were used the same day.

The rate of potassium efflux was measured with a selective electrode (Beckmann) thermostatically kept at 25°C in a glass vessel with magnetic stirring. The recording apparatus was a radiometer pHmeter connected to a Texas Instruments recorder.

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The mitochondria (1 mg/ml) were resuspended in a medium containing 0.24 M Sucrose, 10 mM Tris-buffer pH 7.4, 2μM Rotenone, and 2 mM Succinate.

Protein content of mitochondrial preparation was determined using the Lowry method [9].

All chemicals were of analytical grade.

Rotenone, Valinomycin (dissolved in Ethanol) and 2,4 Dinitrophenol were purchased from Sigma.

123789 Dibenzodioxine and 2378 Dibenzofurane were the gift of prof. A. Orio (Dipartimento di Scienze Ambientali, Universitá di Venezia).

Results and Discussion

In mitochondria, metabolite substrates arising from the Krebs cycle are oxidized by molecular oxygen. The oxidation occurs through a sequence of redox couples, the Respiratory Chain (RC). The electron flow in the RC is stoichiometrically coupled by a pump mechanism to a proton extrusion and, since the mitochondrial membrane is not permeable to the protons, energy is stored, as Mitchell's Chemiosmotic Hypothesys suggests [10], as ΔpH (alcaline in the matrix) and ΔΦ (negative in the matrix). As a consequence of ΔpH and ΔΦ induced by the proton pump, a slow proton reuptake through a specific Proton Channel is responsible for ATP synthesis. This "proton leak" is the limiting step in the RC and, in steady state, the respiratory rate is calculated from the slope of the curve of oxygen concentration against time. In this condition, named State 4, if an uncoupler (i.e. a proton carrier) is added to respiring mitochondria, the respiratory rate is enhanced up to a maximum value. The enhancement is due to the fact that in the presence of an uncoupler, the electrochemical forces which oppose proton extrusion, ΔpH and ΔΦ, are collapsed. If State 4 experiments are performed in the presence of DD (123789 Dibenzodioxine) or DF (2378 Dibenzofurane), the respiratory rate is enhanced and the enhancement is proportional to the concentration of DD and DF (Fig.1). The same effect is obtained by an uncoupler, 2,4 Dinitrophenol (DNP) or by DDT [2] and PCBs [5]. However, unlike uncouplers, DD and DF (and DDT and PCBs) do not possess an acidic group, which is essential for the uncoupling mechanism [10].

We have thus considered the possibility that DD and DF could induce an increase of the respiratory rate through a different mechanism, i.e. by increasing mitochondrial permeability to all ions. In the experiments which follow we show that, indeed, DD and DF cause both a potassium and a proton efflux from mitochondria. Since mitochondria are not permeable to the protons and to potassium, this result

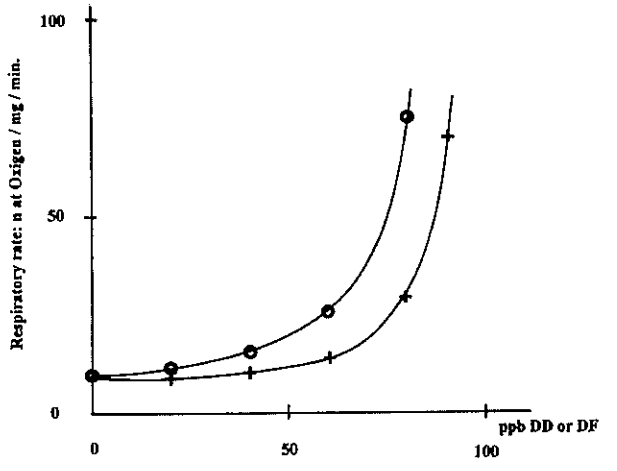


Fig.1. Respiratory rate stimulation induced by increasing concentrations of DD (o) or DF(+). The same effect is obtained by adding increasing amounts of 2,4 Dinitrophenol (DNP) to respiring mitochondria.
Medium and conditions: mitochondria (1 mg/ml) are resuspended in a medium (2 ml) containing Sucrose 0.25 M, 10 mM Tris buffer pH 7.4, 2 μM Rotenone and 2 mM Succinate. At 25°C in this condition, oxygen concentration in the solution is 240 n atm. oxygen/ml.

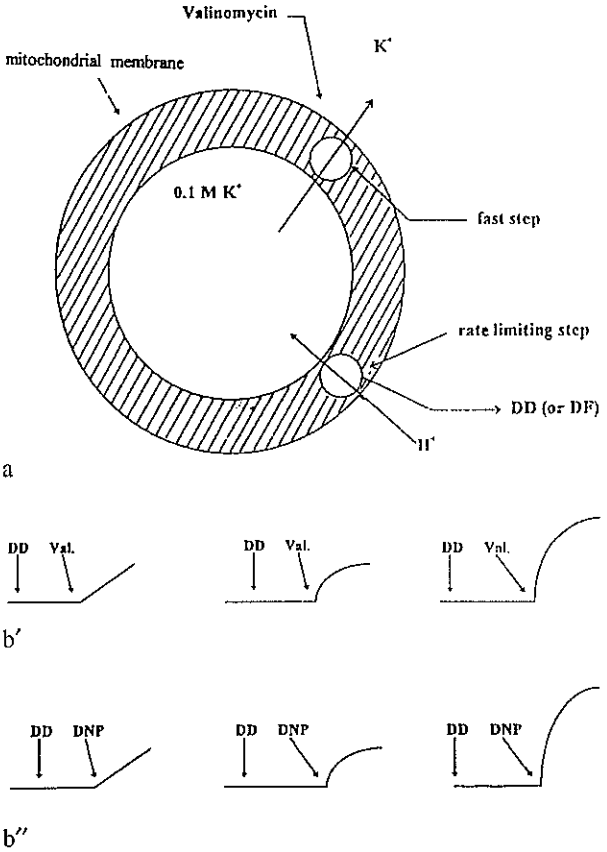


Fig.2 Schematic representation of potassium and proton transport in the mitochondrial membrane (Fig. 2a). In an excess of Valinomycin (2 nmoles/mg protein) and in the presence of DD (or DF) the potassium efflux measures the enhancement in proton conductance (rate of proton influx) induced by DD (or DF). Fig. 2b′ shows typical experiments in which increasing amounts of DD cause a corresponding increase in the rate of potassium efflux.
If the experiment is performed in an excess (100 mM) of DNP (and in the absence of Valinomycin) the potassium conductance (rate of potassium efflux) induced by DD (or DF) is measured, since in this condition this is the rate limiting step. Fig. 2b″ shows that in this condition, increasing amounts of DD enhance potassium conductance. Both kinds of experiments demonstrate that DD enhances proton and potassium conductance.
Figure (a) shows the mitochondrial membrane which in the internal aqueous phase contains 100 mM potassium. Mitochondria (1 mg/ml) are resuspended in a medium containing 0.25 M Sucrose, 2 mM $MgCl_2$, 10 mM Tris pH 7.4, 2 μM Rotenone, 20 mM KCl and 1 mM EDTA.

suggests that DD induce a detergent effect with consequent leakage of ions.

Since mitochondria in the matrix contain about 0.1 M potassium, if freshly prepared and non respiring mitochondria are resuspended in a potassium-free medium (or with a low potassium concentration) – (Fig. 2a), an outward potassium flow is observed (and monitored by a potassium selective electrode) in the presence of a potassium carrier and of a counterion entering the mitochondria to ensure charge balance. This kind of experiment can be carried out using an excess of Valinomycin, which allows potassium extrusion, in association with low concentrations of proton carriers (e.g. DNP). Conversely, if the same experiment is performed with an excess of proton carrier and low concentration of Valinomycin, the rate limiting step is potassium transport. The procedure, therefore, allows us to monitor the kinetics of potassium transport or that of proton transport, respectively, induced by the respective carriers.

The experiment of Fig. 2a has been performed using DD as a carrier of protons (instead of DNP). The experiment was started adding an excess of Valinomycin (2 nmoles/mg protein) to non respiring mitochondria containing various amounts of DD and measuring the initial rate of potassium efflux (Fig. 2b'). Since this potassium efflux is monitored only in the presence of DD, it is evident that DD behaves as DNP: as a proton carrier. In the experiment the rate of proton influx is the rate-limiting step since the rate of potassium efflux does not change if Valinomycin concentration is enhanced. In this condition the graph of Fig. 3a (+) reports the initial rate of potassium efflux which is enhanced by DD, at various DD concentrations.

If the same experiment as in Fig. 2a is performed withouth Valinomycin, but starting with an excess of DNP (100 μM), and in the presence of increasing amounts of DD (Fig 2b'), in this case the rate limiting step is the potassium conductance due to DD.

Fig. 3a (o) shows the initial rate of potassium efflux at various concentrations of DD, when potassium transport is the rate limiting step, thus measuring potassium conductance (o).

Analogously, Figs. 3b and 3c report the enhancement in potassium (o) and proton (+) conductance due to DF and DDT.

From the data in the Figures 3a, 3b and 3c, it is evident that DD, DF and DDT increase the membrane conductance to protons and to potassium ions in a similar manner. This behaviour is similar to that obtained with detergents, chemical compounds which increase the membrane permeability to all ions [11, 12].

We believe, therefore, that DD, DF and DDT induce a non specific "ion leak" in the mitochondrial membrane, probably due to a modification in the assembly and structure of the phospholypidic bilayer [1]. This behaviour explains the respiratory chain stimulation observed in mitochondria with these compounds (Fig. 1), which is similar to that obtained with DNP, a classical uncoupler, even if DD, DF and DDT do not possess an acidic group. Analogously, the same interpretation can be proposed to justify the enhancement in the respiratory rate and decrease in the rate of ATP synthesis observed in mitochondria in the presence of DDT [2] and PCBs [5].

Furthermore, our experiments can explain the proton efflux and the potassium leakage induced by DDT in mitochondria and (less) in erytrocytes [3].

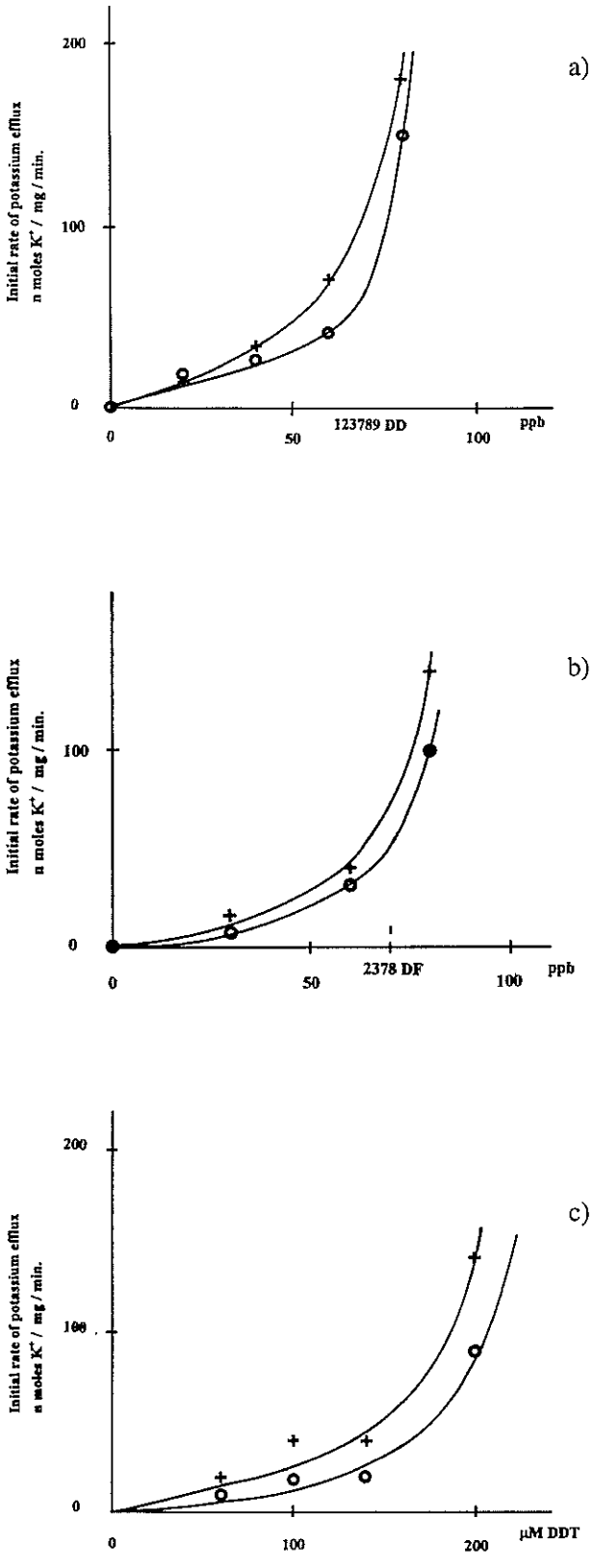


Fig.3 Proton (+) and potassium (o) conductance induced by increasing amounts of DD, Fig. 3a; DF, Fig. 3b; and DDT, Fig. 3c. Conductance is measured as the initial rate of potassium efflux by a selective potassium electrode. Proton conductance is measured in the presence of an excess of Valinomycin. Potassium conductance is measured in the presence of an excess of DNP.

In conclusion, we have demonstrated that DD and DF induce membrane permeability both to the protons and to potassium using the potassium gradient as a driving force. Our results indicate that these compounds behave as detergents. It can be hypothesized, therefore, that some toxic effects observed in living animals could be due to this behaviour [6, 7].

The procedure described can thus be proposed as a general method to measure the ion permeability of biological membranes induced by toxic compounds or to study the effects of inhibitors on carrier transport systems.

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