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Inhibition by Valinomycin of Atractyloside Binding to the Membrane-Bound ADP/ATP Carrier: Counteracting Effect of Cations

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Abstract

The atractyloside binding capacity of rat heart mitochondria, but not the binding affinity, was markedly decreased by preincubation of the mitochondria with valinomycin in isotonic KCl medium. Maximum inhibition was attained with 5 ng of valinomycin per mg of mitochondrial protein; it corresponded to a 40% decrease of the atractyloside binding capacity. The inhibitory effect of valinomycin was maximal between pH 7.0 and 7.5. It was more marked for heart mitochondria than for liver mitochondria. Valinomycin inhibition of atractyloside binding to heart mitochondria was counteracted by nigericin and FCCP, by sublytic concentrations of cationic surfactants such as cetyltrimethylammonium bromide, and by low concentrations of trivalent and divalent metal ions at acidic pH's still compatible with atractyloside binding, i.e., down to pH 5.5; trivalent metal ions were more effective than divalent metal ions. The effect of valinomycin was also counteracted by exceedingly high concentrations of K^+ (more than 300 mM), resulting in a substantial increase in the ionic strength. These results were discussed in terms of the relation between the atractyloside binding capacity of the inner mitochondrial membrane and the surface potential of this membrane.

Key Words: ADP/ATP carrier; atractyloside; valinomycin; surface potential; mitochondria; ionic surfactants.

Introduction

It was reported recently (Michejda and Vignais, 1981) that the binding capacity of mitochondria for atractyloside, a specific inhibitor of ADP/ATP transport, is significantly decreased in KCl-supplemented medium by valino-

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mycin, and restored upon addition of nigericin or FCCP.[3] This puzzling effect of valinomycin has been studied in more detail, and the conditions of its reversion have been analyzed. Data which document this new aspect of the reactivity of the membrane-bound ADP/ATP carrier are reported here.

Materials and Methods

Rat heart mitochondria were isolated by the method of Tyler and Gonze (1976). [^{3}H]Atractyloside was prepared as described by Brandolin *et al.* (1974). CTAB and CPB were obtained from Serva, Heidelberg, and BTC from Franconyx, Lyon. Valinomycin was from Calbiochem, and oligomycin and carboxyatractyloside were from Boehringer.

[^{3}H]Atractyloside binding assays were carried out routinely in standard KCl medium consisting of 125 mM KCl buffered with 10 mM MOPS, pH 7.2, and supplemented with 5 mM succinate and 1 μg oligomycin/mg protein for maximum development of the protonmotive force. The sucrose medium consisted of 220 mM sucrose, 10 mM MOPS, 5 mM succinate, and 1 μg oligomycin/mg protein, pH 7.2. The pH was adjusted to 7.2 by addition of Tris-base. The conditions of incubation are detailed in the legends to the figures. After incubation, mitochondria were collected by centrifugation. The pellet was digested by 1 ml formamide at 180°C, and the ^{3}H radioactivity was counted by scintillation.

Membrane potential was monitored with 3-3′-dipropylthiodicarbocyanine, using an MPF IIA spectrofluorometer; excitation and emission wavelengths were at 620 and 670 nm, respectively (Waggoner *et al.*, 1977). The cuvette assay, thermostated at 20°C, contained 3 ml of a medium consisting of 200 mM sucrose, 30 mM KCl, 10 mM MOPS, 4 mM succinate, and 5 μM dipropylthiodicarbocyanine, final pH 7.2. For ΔpH determination, we used the same medium buffered by 10 mM MES, pH 6.5, replacing dipropylthiodicarbocyanine by 2 μM fluorescein; a slightly acidic pH was required for accurate measurement of ΔpH (Thomas *et al.*, 1979). Measurements of ΔpH were performed with a dual-wavelength spectrophotometer, using 464–490 nm as wavelength couple. In all cases, we routinely used 1 mg of mitochondrial protein in 3 ml of medium. Rat heart mitochondria responded to succinate oxidation by developing ΔpH and $\Delta\psi$ immediately, which could be cancelled by nigericin and valinomycin, respectively.

[3]Abbreviations used: CTAB: Cetyltrimethylammonium bromide; CPB: Cetylpyridinium bromide; BTC: Myristyldimethylbenzylammonium chloride; Na Dod SO_4: Sodium dodecyl sulfate; MOPS: 3(*N*-Morpholino)propane sulfonic acid; MES: 2(*N*-Morpholino)ethane sulfonic acid; DTT: Dithiothreitol; FCCP: Carbonylcyanide *p*-trifluoromethoxyphenylhydrazone; NEM: *N*-ethylmaleimide.

Adenine nucleotides were determined in neutralized perchloric extracts as described by Adam (1963a, b).

Results

Parameters of the Inhibition of [^{3}H]Atractyloside Binding by Valinomycin

In accordance with previous findings (Vignais, 1976), the control curve of [^{3}H]atractyloside binding to rat heart mitochondria (Fig. 1A) shows a saturation plateau corresponding to 1.0–1.1 nmol of bound [^{3}H]atractyloside per mg protein and a K_d value of 20–30 nM. In the experiment of Fig. 1A, the KCl medium was aerated and supplemented with succinate and oligomycin (see Materials and Methods). A 7-min preincubation at 25°C of the mitochondria with 30 ng of valinomycin per mg protein resulted in a 40%

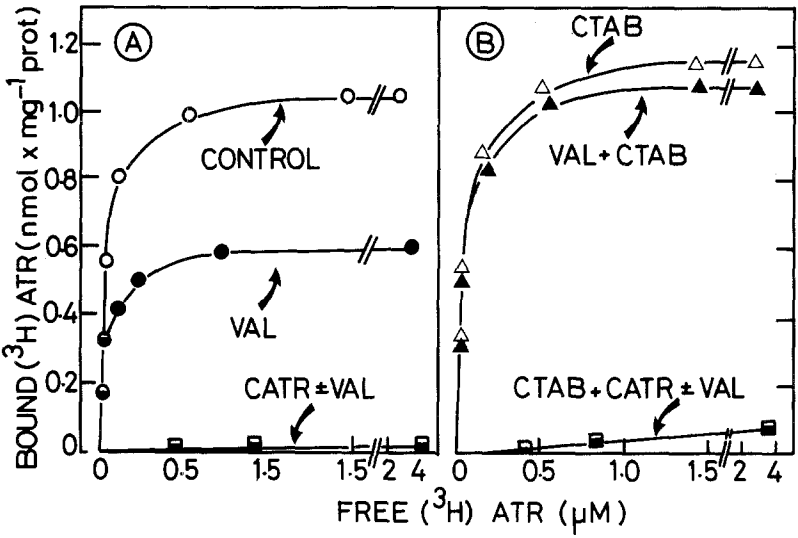

Fig. 1. Inhibitory effect of valinomycin on the binding of [^{3}H]atractyloside (ATR) by rat heart mitochondria. (A) Rat heart mitochondria (1 mg protein) were preincubated in centrifuge tubes at 25°C for 7 min in 2.5 ml of standard KCl medium under constant aeration in the presence or absence of valinomycin (30 ng/mg protein). Then [^{3}H]atractyloside was added at increasing concentrations and allowed to incubate for 15 min at 25°C to ensure full equilibration between bound and free [^{3}H]atractyloside. The incubation was terminated by centrifugation, and the radioactivity of the pellet was determined. Chase of the specifically bound [^{3}H]atractyloside was achieved by addition of 5 μM carboxyatractyloside. (B) Same conditions as in (A), except that CTAB was added to the standard medium at the final concentration of 30 μM prior to mitochondria.

decrease of their binding capacity for [^{3}H]atractyloside, but not of their binding affinity. As shown in Table I, the effect of valinomycin was clearly expressed in respiring heart mitochondria suspended in standard KCl medium, but not in sucrose medium. With isotonic mixtures of sucrose and KCl, the valinomycin effect was clearly expressed from 30 to 150 mM KCl. At hypertonic concentrations of KCl (>300 mM), inhibition by valinomycin tended to disappear. No effect of valinomycin was observed with heart mitochondria whose respiration had been inhibited by KCN. In the absence of valinomycin, the [^{3}H] atractyloside binding capacity of rat heart mitochondria was virtually the same, i.e., 1.0–1.1 nmol per mg protein, whatever the conditions of incubation (KCl or sucrose medium, respiring or nonrespiring mitochondria). In other words, the respiratory state of the heart mitochondria and the ionic strength of the medium did not influence atractyloside binding *per se* but rather the inhibitory effect of valinomycin on atractyloside binding. This inhibitory effect of valinomycin developed in time. Maximal inhibition was attained after 6–7 min at 25°C at pH 7.2; the half maximal effect required 2 min of contact with mitochondria, a period of time much longer than that required for abolition of membrane potential by valinomycin. This suggests that the effect of valinomycin on atractyloside binding is not directly due to the decrease in membrane potential, but to some membrane events dependent on membrane potential. As illustrated by the dose–effect curve of Fig. 2, only part of the total atractyloside binding capacity of heart mitochondria (about 40%) was susceptible to valinomycin. The plateau of inhibition

Table I. Inhibitory Effect of Valinomycin on [^{3}H)-ATR Binding to Rat Heart Mitochondria. Dependence on the Ionic Strength of the Medium and the Respiratory Activity of Mitochondria[a]

Medium	Additions	Bound [^{3}H]-ATR (nmol/mg protein)
KCl medium	None	1.03
	Valinomycin	0.62
	Nigericin	1.13
	Valinomycin + nigericin	1.08
	FCCP	1.00
	KCN	1.10
	KCN + valinomycin	1.07
Sucrose medium	none, or valinomycin or nigericin	1.10 ± 0.05

[a]Rat heart mitochondria (1 mg protein) were preincubated in centrifuge tubes at 25°C for 7 min in 2.5 ml of KCl medium or sucrose medium, pH 7.2 (cf. Materials and Methods), supplemented as indicated with 30 ng valinomycin, 500 ng nigericin, 1 μM FCCP, or 1 mM KCN. Then [^{3}H]atractyloside was added at the final concentration of 2 μM, and binding was determined as described in the legend to Fig. 1.

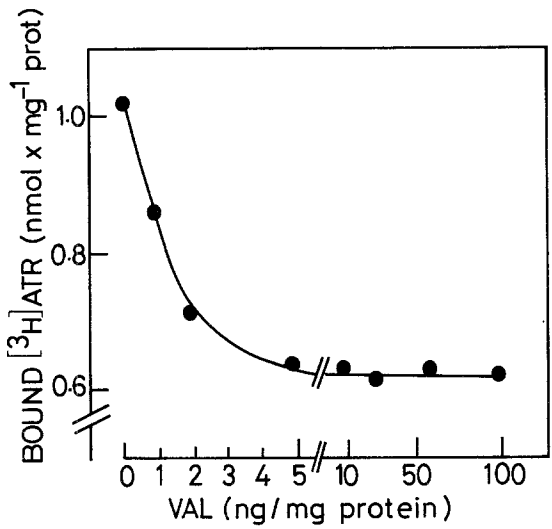

Fig. 2. Effect of increasing concentrations of valinomycin (VAL) on the [^{3}H]atractyloside (ATR) binding capacity of rat heart mitochondria. Same conditions as in Fig. 1, except that [^{3}H]atractyloside was used at the final concentration of 2 μM, and that increasing concentrations of valinomycin, up to 100 ng per mg protein, were used.

was obtained with 5 ng valinomycin per mg protein, and the half maximal effect required between 1 and 2 ng valinomycin/mg protein. Inhibition of [^{3}H]atractyloside binding by valinomycin was more marked for rat heart mitochondria than for rat liver mitochondria.

Relief of the Inhibitory Effect of Valinomycin by Uncoupling or Acidic pH

The data in Table I show that nigericin which had no effect *per se* on atractyloside binding could oppose the inhibition of atractyloside binding by valinomycin. The same observation holds for FCCP, indicating that uncoupling conditions, i.e., valinomycin plus nigericin, or FCCP fully restore the binding capacity of heart mitochondria for atractyloside.

The pH dependence of the inhibition of atractyloside binding by valinomycin is illustrated in Fig. 3. In this experiment, [^{3}H]atractyloside was added at a saturating concentration. Maximal inhibition was found between pH 7.0 and 7.5. At slightly acid pH's, still compatible with atractyloside binding, i.e., between pH 7.0 and 5.5, valinomycin inhibition was decreased or even abolished. It is noteworthy that at pH's higher than 7.5, there was a slight decline in the amount of [^{3}H]atractyloside bound at saturation. Whether this

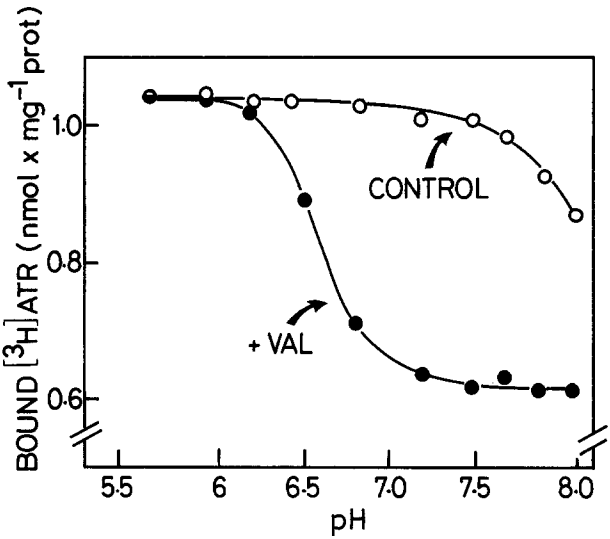

Fig. 3. Effect of pH on the inhibition by valinomycin (VAL) of the [^{3}H]atractyloside (ATR) binding capacity of rat heart mitochondria. Same conditions as in Fig. 1, except that the KCl medium was buffered by a mixture of MES and MOPS, each buffer being used at the final concentration of 10 mM. The final concentration of [^{3}H]atractyloside was 2 μM.

is due to an effect of the alkaline pH on some ionizable groups of the ADP/ATP carrier protein or to an adverse effect on the structure of the mitochondrial membrane is not known.

Relief of the Inhibitory Effect of Valinomycin by Cationic Surfactants

A plausible explanation of the inhibition of atractyloside binding by valinomycin is that valinomycin alters the surface charge of the mitochondrial membrane and thereby modifies the topography of the atractyloside site in the membrane-bound ADP/ATP carrier. If this were the case, one can expect that addition of appropriate charged compounds will cancel the inhibitory effect of valinomycin. To test this hypothesis, the effect of cationic and anionic surfactants on valinomycin inhibition was tested. When used at the final concentration of 30 μM, CTAB was indeed able to fully restore the atractyloside binding capacity of heart mitochondria (Fig. 1B). The specificity of this restoring effect was attested by the fact that the restored [^{3}H]atractyloside binding was abolished by carboxyatractyloside, another specific inhibitor of the ADP/ATP carrier, which binds to the carrier with an even higher affinity than atractyloside (Vignais, 1976).

Routinely CTAB was added at the same time as valinomycin. However,

CTAB was still effective when added after the mitochondria had been in contact for 2 min with valinomycin. At CTAB concentrations higher than 30 μM, the amount of bound [^{3}H]atractyloside at equilibrium continued to increase, even in the presence of valinomycin (Fig. 4A); this extrabinding was, however, no longer susceptible to inhibition by carboxyatractyloside, indicating nonspecific binding of [^{3}H]atractyloside. A possible explanation is that CTAB, at high concentrations, forms micelles which can entrap [^{3}H]atractyloside and stick to mitochondria. This explanation, however, is not fully satisfactory since the critical micelle concentration of CTAB is reported to be as high as 400 μM (De Vendittis *et al.*, 1981).

Two other cationic surfactants, CPB and BTC, were tested (Fig. 4B, C). Like CTAB, below a threshold concentration of 30 μM, BTC and CPB restored specifically the atractyloside binding capacity of heart mitochondria treated by valinomycin. At concentrations of BTC and CPB higher than 30 μM, nonspecific binding of [^{3}H]atractyloside was revealed by lack of inhibition by carboxyatractyloside.

In contrast to the cationic surfactants, the anionic surfactant Na Dod SO_4 used at sublytic concentrations exerted some inhibition on the [^{3}H]atrac-

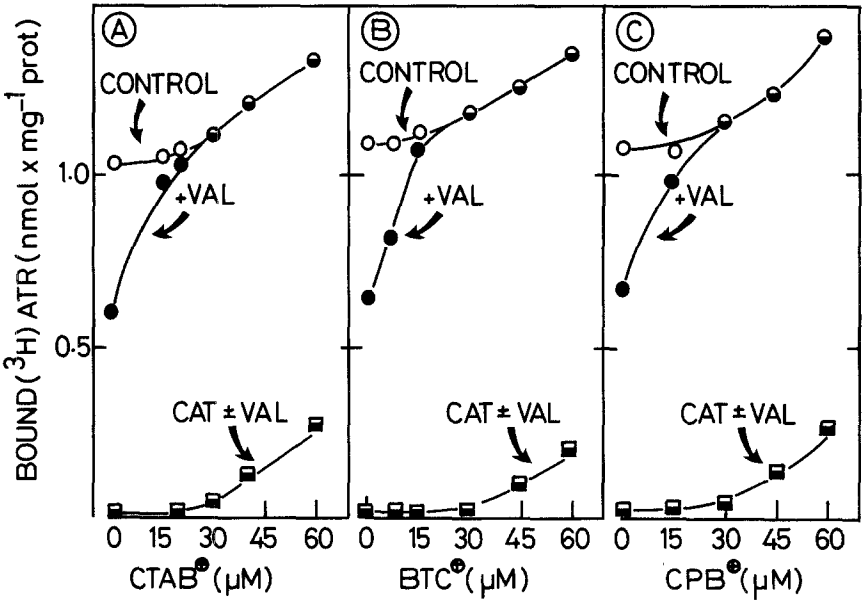

Fig. 4. Comparison of the antagonistic effects of different cationic surfactants on the [^{3}H]atractyloside (ATR) binding capacity of rat heart mitochondria. Same conditions as in Fig. 1. The cationic surfactants CTAB, BTC, and CPB were added at increasing concentrations in the standard KCl medium. After 5-min incubation at 25°C, [^{3}H]atractyloside was added at the final concentration of 2 μM. Other conditions of incubation as in Fig. 1.

tyloside binding capacity of mitochondria. This inhibition was completely removed by CTAB and the other cationic surfactants added at the same concentration as Na Dod SO_4, most likely by charge neutralization.

Effect of Cationic Surfactants on the Membrane Potential and the pH Gradient Generated by Respiration

Since valinomycin collapses the membrane potential in respiring mitochondria, it was interesting to check whether CTAB, CPB, and BTC, which antagonized the inhibitory effect of valinomycin on atractyloside binding, were also able to interfere with valinomycin on the membrane potential.

At 30 μM, CTAB, CPB, and BTC in the absence of valinomycin caused a significant decrease (20 to 40%) of the membrane potential (not shown). A concentration of valinomycin as low as 10 ng per mg protein was enough to collapse the membrane potential; this collapsing effect of valinomycin was not counteracted by the cationic surfactants. Likewise, addition of 30 μM CTAB, CPB, or BTC in the absence of nigericin resulted in a slight decrease (about 20%) of the pH gradient generated by respiration; abolition of the pH gradient by nigericin was not modified by the cationic surfactants. These data suggest that it is not the changes in membrane potential, nor in pH gradient, but related electric processes such as modification of the surface charge density of the inner mitochondrial membrane, that may be responsible for the inhibition of atractyloside binding by valinomycin and for the restoration of the atractyloside binding capacity by cationic surfactants.

Effect of Valinomycin and Cationic Surfactants on the Release of Internal Adenine Nucleotides

Besides the putative surface charge modification caused by valinomycin, another parameter that may be of importance in the effect of valinomycin is the release of internal adenine nucleotides. When rat heart mitochondria is incubated with 30 ng valinomycin per mg protein in aerobiosis for 7 to 10 min, 50 to 60% of the internal adenine nucleotide pool is released, most of them under the form of AMP (90%). When used at sublytic concentrations, CTAB, CPB, and BTC had no effect on the valinomycin-induced release of internal adenine nucleotides. There is therefore no apparent relationship between the changes in the atractyloside binding capacity of heart mitochondria induced by valinomycin and the cationic surfactants, and the size of the internal pool of adenine nucleotides.

Counteraction of the Inhibitory Effect of Valinomycin by Metal Ions

In the following experiment, the chloride salts of the following metal ions were used: Mg^{2+}, Mn^{2+}, and La^{3+}. All of them were able to antagonize the

inhibition of atractyloside binding by valinomycin (Fig. 5). La^{3+} was the most active; it was fully effective at 1 mM, the half maximum effect being observed at 0.3 mM. Mn^{2+} and Mg^{2+} were less efficient than La^{3+}, the half maximal effects being obtained with 0.8 mM $MnCl_2$ and 4 mM $MgCl_2$. In all cases, the recovery of the atractyloside binding capacity can be considered as specific of the ADP/ATP carrier since it is abolished by carboxyatractyloside. It may be recalled (see first Section of Results) that valinomycin inhibition of atractyloside binding is also relieved by very high concentrations of K^+ salt (more than 300 mM).

As discussed by Barber (1980), the existence of a net negative charge in biological membranes produces an electrical potential in the aqueous phase immediately adjacent, which is responsible for attraction and accumulation of cations close to the membrane; this attraction is an exponential function of the valence of the cation, which can explain the higher efficiency of La^{3+} compared to Mn^{2+} and Mg^{2+}.

The Inhibitory Effects of NEM and Valinomycin on Atractyloside Binding to Respiring Mitochondria Are Nonrelated Processes

As shown by Vignais and Vignais (1972), NEM inhibits the atractyloside binding capacity of respiring mitochondria or mitochondria preincubated

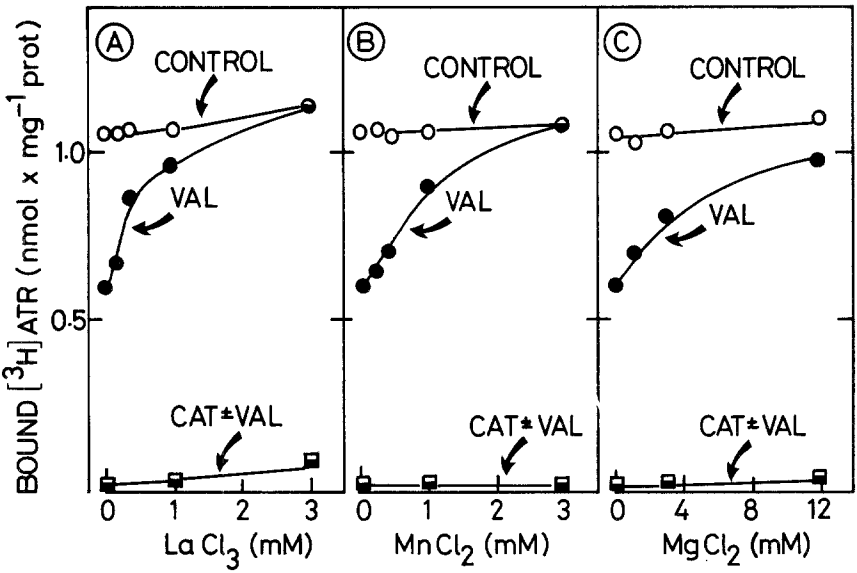

Fig. 5. Comparison of the antagonistic effects of metal ions on the [^{3}H]atractyloside (ATR) binding capacity of heart mitochondria. Same conditions of incubation as in Fig. 4, except that the cationic surfactants are replaced by the metal ions La^{3+}, Mn^{2+}, and Mg^{2+}.

with minute amounts of ADP by combining with strategic cysteine residue(s) (Vignais *et al.*, 1975); NEM inhibition is relieved by uncouplers. These findings led to the postulate that the protonmotive force developed by respiration could influence the accessibility of the membrane-bound ADP/ATP carrier to atractyloside (Vignais *et al.*, 1976). Based on the fact that nigericin counteracts the inhibitory effect of NEM on atractyloside binding by energized mitochondria, the component of the protonmotive force responsible for the inhibitory effect of NEM was identified with ΔpH (Michejda and Vignais, 1981). Since in the present experiments nigericin also counteracted the effect of valinomycin, it was necessary to determine whether a common mechanism or different mechanisms were involved in the inhibition of atractyloside binding by NEM and valinomycin. For this purpose, we preincubated the mitochondria with DTT to eliminate the effect of NEM, and with CTAB to eliminate the effect of valinomycin. As illustrated in Fig. 6, DTT preincubated with mitochondria afforded an efficient protection against inhibition of atractyloside binding by NEM, but not by valinomycin. On the

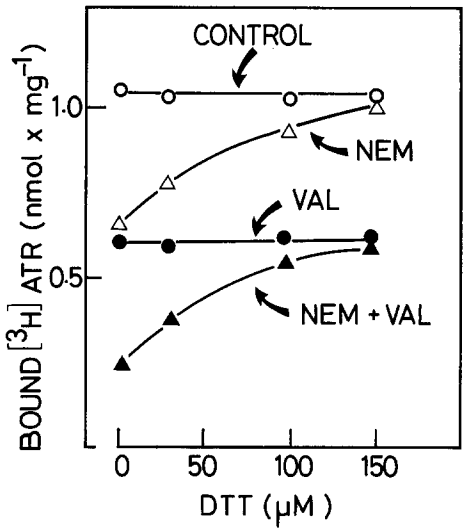

Fig. 6. Effect of DTT on the inhibition of [^{3}H]atractyloside (ATR) binding to rat heart mitochondria by valinomycin (VAL) and NEM. Same medium as in Fig. 1. DTT was added at the indicated concentrations prior to mitochondria. The mitochondria (1 mg protein) were allowed to incubate for 2 min at 25°C, followed by 150 μM NEM or 30 ng valinomycin, or both. Five minutes later, [^{3}H]atractyloside was added at the final concentration of 2 μM, and incubation was allowed to proceed as in Fig. 1.

other hand, a concentration of 30 μM CTAB that was sufficient to antagonize the effect of valinomycin was totally ineffective on NEM inhibition (not shown). These results indicate that NEM and valinomycin act by different mechanisms.

Discussion

In the present paper, we have described in some detail the curious inhibitory effect of valinomycin on the atractyloside binding capacity of heart mitochondria. A number of data to be discussed below have suggested that this effect could be due to changes in the surface potential of the inner mitochondrial membrane, resulting in masking of atractyloside binding sites in a fraction of the carrier units. This effect was explicit for KCl concentrations ranging from 30 to 150 mM. Above 300 mM KCl, inhibition by valinomycin tended to disappear. This dual effect of K^+ concentration may reside in the fact that below 150 mM KCl, inhibition of atractyloside binding by valinomycin is expressed through the valinomycin-K^+ complex without much effect of K^+ on the surface charge of the membrane, whereas at high KCl concentrations (more than 300 mM), the modification of the surface charge is prevalent. This view is consistent with the relieving effect of H^+ and di- and trivalent metal cations, and also monovalent cationic detergents, on the inhibition of atractyloside binding by valinomycin. The postulated relation between the surface charge of the inner mitochondrial membrane and the availability of the atractyloside site on the ADP/ATP carrier can be considered as another example of the well-known effect of the membrane environment on properties of membrane-bound enzymes. The kinetic parameters of many membrane-bound enzyme are in fact dependent on the fixed charges in the membranes. As discussed in the simple case of an enzyme immobilized in a polyelectrolyte gel (Goldstein *et al.*, 1964), the electrostatic field prevailing in the gel affects the distribution of the charged low-molecular-weigh substrates between the gel, i.e., in the microenvironment of the enzyme, and the bulk medium. There are many examples of enzymes immobilized in gels or bound to biological membranes where this electrostatic effect manifests itself by a modification of the K_M value or a shift of the pH activity profile (Goldstein, 1972; Goldstein *et al.*, 1964; Wharton *et al.*, 1968; Engasser and Horwarth, 1975; Maurel and Douzou, 1976; Douzou and Maurel, 1977; Remy *et al.*, 1978; Wojtczak and Nalecz, 1979; Ricard *et al.*, 1981). In the case of mitochondria, by different approaches including measurement of the electrophoretic mobility (Kamo *et al.*, 1976), binding of charged paramagnetic amphiphiles (Quintanilha and Packer, 1977a), or fluorescent probes (Aiuchi *et al.*, 1977), it has been shown that, upon respiration, i.e., when a protonmo-

tive force is generated, negative charges appear at the outer surface of the inner mitochondrial membrane; the resulting surface potential is, however, relatively small (< -20 mV) compared to the transmembrane potential ($\simeq 180$ mV). This negative surface potential is abolished by uncouplers (Quintanilha and Packer, 1977b); it is decreased by cationic surfactants such as CTAB and by acidic pH's (Wojtczak and Nalecz, 1979). Most likely, the added positively charged ions, by binding to negatively charged groups on the outer surface of the inner mitochondrial membrane, decrease the surface charge density of this membrane. The possibility that CTAB and other cationic surfactants may flip-flop in the perpendicular plane of the membrane is ruled out, as shown by experiments carried out with a spin-labeled derivative of CTAB (Quintanilha and Packer, 1977).

The effect of cationic and anionic surfactants on the kinetic parameters of mitochondria-bound enzymes, possessing anionic substrates, has been carefully tested by Wojtczak and Nalecz (1979); typically, the K_M of these enzymes, but not the V_{max}, was modified, which is consistent with the increase or decrease of the local concentration of the substrates in the immediate vicinity of the membrane. In the specific case of ADP/ATP transport, there are several lines of evidence indicating that the ionic environment of the mitochondrial membrane controls the kinetics of transport: (1) ADP transport is stimulated by cations such as K^+; its inhibition by the lipophilic anion tetraphenylboron is overcome by Mg^{2+} (Meisner, 1971, 1973); (2) competitive inhibition of ADP transport by the negatively charged long-chain acylCoA's (Morel *et al.*, 1974) is relieved by positively charged surfactants such as CTAB (Duszynski and Wojctzak, 1974); (3) the affinity of mitochondria for carboxyatractyloside is higher in a KCl medium than in a neutral sucrose medium (Vignais, 1976). In the present work, the implication of electric interactions in the control of the binding capacity of atractyloside is strongly suggested by the relief of the valinomycin-induced inhibition of atractyloside binding by cations and H^+, and also by the higher efficiency of trivalent metal ions compared to divalent ones. An increase in H^+ or cations in the medium is expected to neutralize part of the negative charges randomly distributed on the surface of the inner mitochondrial membrane; it is also possible that a fraction of H^+ and cations binds to some anionic sites of the ADP/ATP carrier protein, exposed to the outside of the inner membrane.

In contrast to a number of membrane-bound enzymes for which the ionic environment modifies predominantly the affinity parameter, in the case of inhibition of atractyloside binding by valinomycin in heart mitochondria, it is not the binding affinity but the binding capacity which is modified. This all-or-none effect implies that the change is intrinsic to the ADP/ATP carrier protein, bearing, for example, on the topographical relationship between the carrier protein and the inner membrane in which the carrier is embedded.

What are the possible modifications to be envisaged? This could concern the conformation of the carrier protein or its degree of insertion in the lipid core of the membrane, thereby influencing the accessibility of atractyloside to its specific binding site. One may also imagine that, due to their ability to diffuse in the lateral plane of the membrane, the carrier units may be either dispersed or stacked, and that stacking is controlled by the surface charge density of the membrane; it is possible that, upon stacking, the atractyloside binding efficiency per carrier unit is decreased. This latter explanation deserves consideration, especially in view of the fact that only a fraction of the carrier units (40%) is susceptible to the inhibitory effect of valinomycin, as if an equilibrium exists, in the population of carrier units, between units competent and noncompetent for atractyloside binding.

It has been reported that, in the presence of valinomycin, respiring mitochondria take up rapidly and extensively K^+ ions and extrude H^+, this ion movement being competitively inhibited by Mg^{2+} (Ligeti and Fonyo, 1977). The Mn^{2+} effect was interpreted as the consequence of either a change in the fluidity of the membrane interfering with the mobility of the valinomycin-K^+ complex, or interference with the formation of the valinomycin-K^+ complex due to the binding of Mg^{2+} to the outer surface of the membrane. The latter explanation fits well with our results and suggests that cationic detergents and Mg^{2+} or other multivalent cations may work by a similar mechanism. Another related effect of cations is the restoration by Mg^{2+} of the membrane potential in mitochondria which had dropped due to Ca^{2+} accumulation (Toninello *et al.*, 1982); restoration by Mg^{2+} might well involve electrostatic interactions of Mg^{2+} with the negatively charged surface of the inner mitochondrial membrane.

Acknowledgments

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