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CHOLESTEROL AS ACTIVATOR OF ADP-ATP EXCHANGE IN RECONSTITUTED LIPOSOMES AND IN MITOCHONDRIA

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The influence of cholesterol on ADP-ATP exchange activity was measured in the reconstituted system, submitochondrial (sonic) particles and mitoplasts (isolated inner mitochondrial membranes). In the reconstituted system, cholesterol markedly enhanced the nucleotide-uptake rate, when added to membranes of various compositions i.e., pure phosphatidylcholine, phosphatidylcholine/phosphatidylethanolamine mixtures and crude egg yolk phospholipids. The stimulation was linearly dependent on the amount of incorporated cholesterol up to 7–13% added sterol, depending on the type of phospholipids. Cholesterol influenced neither the amount of actively reconstituted carrier proteins nor the affinity of the carrier towards nucleotides nor the breakpoint of temperature dependence in the Arrhenius plot. The stimulation could be correlated with an increase in the molecular activity of the carrier protein. The influence of cholesterol was also measured in the natural environment of the carrier protien, i.e., the inner mitochondrial membrane. Both with submitochondrial particles from beef heart and especially with mitoplasts from rat liver, incorporation of cholesterol by fusion with sterol-containing liposomes led to a stimulation of ADP-ATP exchange activity, comparable to the effect in the reconstituted system. These results are discussed in relation to the absence of cholesterol in the inner mitochondrial membrane and in the view of the generally accepted ordering effect of cholesterol on phospholipid bilayers.

Introduction

The physical state of a lipid membrane is determined by a variety of factors, such as temperature, fatty acid and phospholipid head-group composition, cholesterol and sphingomyelin content, and the presence of membrane proteins. The state of the membrane, on the other hand, influences the function and the activity of proteins embedded in the phospholipid bilayer. This kind of protein-phospholipid interaction is apparently more important [1,2] than specific interactions of one protein with a single phospholipid species, which are reported only for a few cases, e.g., β-hydroxybutyrate dehydrogenase [3] and cytochrome oxidase [4] and which are still under discussion [2].

The protein to be dealt with in this work, the adenine nucleotide carrier of the inner mitochondrial membrane, undergoes at least one major conformational change during its catalytic cycle of ADP/ATP transport [5–10]. As mentioned above, cholesterol is one of the important factors which can determine the properties of the lipid bilayer and thus presumably also the function of many integral proteins [11]. Cholesterol is known to cause ordering of phospholipid molecules above their respective gel-to-liquid-crystal

Abbreviations: PE, phosphatidylethanolamine; PC, phosphatidylcholine; Tricine, *N*-tris(hydroxymethyl)methylglycine; Mops, 4-morpholinepropanesulfonic acid.

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phase transition temperature [12,13]. The activity of the ADP/ATP carrier should be modulated by the order of the surrounding membrane. Taking into consideration the significant conformational changes of the carrier protein which are necessary for its function and the ordering effect of cholesterol on the phospholipid membrane, one would predict a decrease in exchange rate when the cholesterol content of the bilayer is increased.

Despite this fact, a stimulation of exchange activity was found upon mild cholesterol enrichment of the phospholipid membrane. Recently, Yuli et al. [14] observed transport activation by cholesterol for glucose transport in cell membranes. This was explained by an increase in the number of actively operating sites. In this work a completely different mechanism will be shown. Cholesterol enhances the activity of the adenine nucleotide carrier both in the reconstituted system and in the mitochondrial inner membrane directly by increasing its molecular activity.

Materials and Methods

Materials

The sources of the chemicals were as follows: Triton X-100, cholesterol, 7-dehydrocholesterol and cholesterol oleate – Sigma; carboxyatractylate and nucleotides – Boehringer Mannheim; radioactive nucleotides – New England Nuclear; Dowex 1-X8 – Fluka; Sephadex – Pharmacia; Biosil HA – Bio-Rad. Bongkrekate was a gift from Professor W. Berends (Delft). All other chemicals were of analytical grade. Hydroxyapatite was prepared as described previously [15].

Determinations

Protein was determined by the method of Lowry et al. in the presence of 1% SDS [16] and phosphorus was estimated by the method of Chen et al. [17]. Cholesterol content was measured according to the procedure of Zlatkis et al. [18] after extraction of the samples with chloroform. Monoamine oxidase activity was determined as described by Tabor et al. [19].

Isolation, reconstitution and assay of ADP-ATP exchange

The carrier protein was isolated by hydroxyapatite chromatography in a batch procedure with Triton X-100 as described previously [15]. Liposomes were prepared by sonication of phospholipids in a Branson sonifier [20]. The carrier protein was incorporated into liposomes [21] and the ADP/ATP translocation activity was reconstituted by a freeze-thaw procedure [22] and a second sonication [15,23]. The sonication buffer consisted of 100 mM Na_2SO_4, 20 mM Tricine, pH 8.0, 10 mM ATP. Adenine nucleotide exchange in the forward direction was measured according to Ref. 15 and the exchange velocities were calculated as described in Ref. 24. The amount of active carrier sites and the molecular activity were determined essentially as described in Ref. 15.

Isolation and purification of phospholipids

Egg yolk phospholipids were isolated according to Ref. 25. Phospholipids were separated on silicic acid with a stepwise gradient of chloroform/methanol [26]. Neutral lipids were chromatographically separated on silicic acid using a gradient of hexane/ether [27].

Submitochondrial particles

Submitochondrial particles were prepared from beef heart mitochondria as described earlier [28]. Fusion of submitochondrial particles with liposomes was first carried out as described for mitoplasts by Madden et al. [29] or by Schneider et al. [30]. After both procedures, however, the sonic particles had lost their nucleotide-transport activity. The fusion procedure therefore had to be modified as follows: The sonic particles were incubated with liposomes in a weight ratio of 1 : 5 (mitochondrial protein/liposomal lipid) for 10 min, without change of osmolarity or pH in 175 mM sucrose, 5 mM sodium phosphate, 10 mM ATP, 5 mM $MgCl_2$, 20 mM Mops, pH 6.8. This mixture was then frozen in liquid nitrogen and slowly thawed. After sonication in a Branson sonifier with microtip, the nonvesicular material was spun down in a Sorvall centrifuge (15 000 rpm, 15 min, SS 34 rotor). The submitochondrial particles were washed and isolated by two ultracentrifugation runs at 105 000 × g, for 30 min, and resuspended in the same medium as that described above, without ATP. 'Back exchange' in these particles was measured with a bongkrekate stop method described by Klingenberg [28].

Mitoplasts

Rat liver mitochondria were prepared as described previously [31]. Removal of the outer membrane and purification of the inner membrane fraction (mitoplasts) were carried out by means of a controlled digitonin incubation [32]. In order to obtain mitoplasts active in ADP-ATP exchange, the amount of digitonin to be added to the rat liver mitochondria for removal of the desired fraction of outer membranes had to be tested separately before every experiment. Not more than 85% of the outer membrane could be removed without complete loss of exchange activity; therefore, the amount of digitonin had to be reduced to about 0.12–0.14 g/g protein. The liposome preparation and fusion procedure was similar to the method of Schneider et al. [30] with the following essential modifications: External osmolarity was lowered only to 40% during the fusion process. The incubation was performed at 4°C for 2 h; during this period the mitoplasts were supplied with ATP (2 mM every 30 min), succinate (2 mM every 30 min) and oxygen. They were then washed twice in a Sorvall centrifuge (10 min, 11 000 rpm, SS 34 rotor). Forward exchange was carried out as described by Pfaff et al. [33] for mitochondria. Analyses of the mitoplasts for incorporated phospholipids and cholesterol were performed after separation on a sucrose density gradient as described by Schneider et al. [30].

Results

Reconstituted system

The transport activity of the reconstituted ADP/ATP carrier when incorporated into crude egg yolk phospholipid liposomes turned out to be always significantly higher than that of liposomes composed of purified egg yolk PC and PE and mixed in the original ratio found in egg yolk. In order to detect a possible stimulating component, egg yolk lipids were separated on silicic acid with hexane/ether for neutral lipids and with chloroform/methanol for phospholipids. Liposomes were prepared by adding variable amounts of the different column fractions to a constant PC/PE = 5 : 1 mixture. After reconstitution, a possible effect of these fractions on the ADP-ATP exchange was studied. Several substances both in the phospholipid fraction (lysophospholipids) and in the neutral lipid fraction (free fatty acids, triacylglycerols) inhibited the exchange (data not shown). On the other hand, the only fractions which stimulated reconstituted exchange activity were those containing cholesterol.

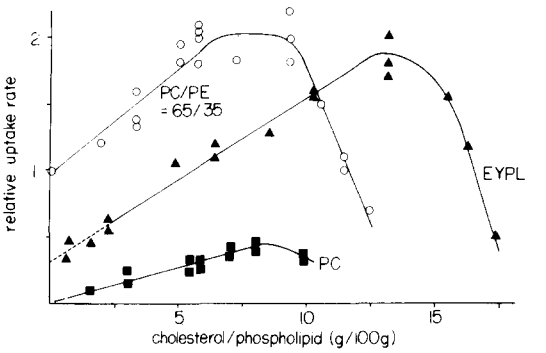

Fig. 1. Enhancement of reconstituted ADP-ATP exchange by cholesterol. The adenine nucleotide carrier was reconstituted in liposomes with phospholipid compositions as indicated in the figure. The exchange velocities were measured at 22°C and were normalized to the exchange rate = 1.0 with liposomes from PE/PE + PC = 0.355. The absolute value for this conditions in several experiments was 1.5–2.3 mmol/g per min. The nucleotide concentrations were 5 mM ATP inside and 0.1 mM ATP outside the vesicles. The exchange rates were calculated as described in Ref. 24. The dashed line, extending the data for egg yolk phospholipids (EYPL) to low concentrations of cholesterol, represents experiments with liposomes composed of egg yolk phospholipids which had been cholesterol depleted by repeated acetone precipitation.

The enhancement of the reconstituted ADP-ATP exchange by cholesterol incorporated in liposomal membranes with different phospholipid compositions is shown in Fig. 1. In all cases, cholesterol markedly stimulated the transport activity when added to pure PC, PC/PE mixtures and crude egg yolk phospholipids. The isolated crude egg yolk phospholipids consistent mainly of about 80% PC, 17% PE and 1.5–2% cholesterol. A further confirmation of these results is demonstrated by the reduced exchange rates in liposomes with egg yolk phospholipids that have been partially depleted of their natural cholesterol content by repeated acetone precipitation (Fig. 1). The different intercepts on the y-axis of this figure are explained by the dependence of the reconstituted ADP-ATP exchange on PE as has been shown earlier [34]. Surprisingly, even with lecithin, which

does not permit ADP-ATP exchange at all in the reconstituted system [34], cholesterol was able to stimulate the transport rate to a considerable extent.

When the cholesterol content was raised to more than 7–13%, depending on the phospholipid composition of the membrane, the reconstituted adenine nucleotide exchange decreases. This, however, was partially due to instability of liposomes with higher amounts of incorporated cholesterol. Especially in the case of pure PC, liposomes seemed to become leaky for nucleotides after the repeated column chromatography procedures necessary for reconstitution and exchange assay.

Egg yolk contains not only cholesterol, but also 7-dehydrocholesterol and cholesterol esters. Both these substances were also tested in the reconstituted system. The stimulation by 7-dehydrocholesterol was somewhat lower as compared to cholesterol, whereas cholesterol oleate seemed to have little or no effect (data not shown).

For the elucidation of transport stimulation it is necessary to decide between two possible explanations, as pointed out earlier [15,35]. Either the increase in activity is due to an increasing amount of active or functionally incorporated carrier molecules, or it is caused by a stimulated molecular activity of every single protein. This question was solved by correlation of titratable carboxyatractylate-binding sites in the reconstituted system with a corresponding measurement of residual exchange activity [15]. It can be seen in Table I that cholesterol definitely enhanced the turnover number of the reconstituted carrier proteins, but did not significantly influence the amount of actively reconstituted carrier molecules. Furthermore, incorporation of cholesterol did not drastically change the transport affinity constant K_m. The small decrease in the apparent K_m might be due to the fact that the rate constants of the transport steps which were influenced by cholesterol are also included in the transport affinity constant K_m [24].

In order to correlate an effect of cholesterol on the phospholipid bilayer [12,13] with the ADP-ATP exchange activity, the temperature dependence of reconstituted adenine nucleotide exchange was measured (Fig. 2). The observed break point around 20°C was not markedly influenced by low amounts of cholesterol (up to 9%) used to stimulate the reconstituted transport. The activation energy of adenine nucleotide transport, however, was somewhat decreased by added cholesterol. These results have been confirmed with other phospholipid compositions (data not shown). In all cases, added cholesterol up to 10% did not show a pronounced effect on the temperature dependence of the reconstituted ADP-ATP exchange.

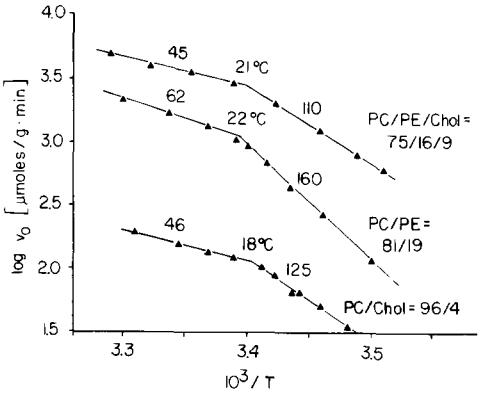

Fig. 2. Arrhenius plot of the exchange rates in different phospholipids. The ADP/ATP carrier protein was reconstituted in liposomes with phospholipid composition as indicated in the figure. Conditions for the transport assay were the same as in Fig. 1. The values above the straight lines denote the activation energies in kJ.

TABLE I

INFLUENCE OF CHOLESTEROL ON THE RECONSTITUTED ADENINE NUCLEOTIDE EXCHANGE

Experimental conditions were as described in Fig. 1. For determination of the transport affinity constants see Ref. 24. Measurement of the active carrier fraction and calculation of molecular activity are described in the text and in Ref. 15. The lipid composition is given in weight ratios.

Lipid composition (PC/PE/cholesterol)	Molecular activity (min^{-1})	Fraction of active carriers (%)	K_m (μM)
73:27:0	890	8.2	22
77:21:2	1825	7.9	17
70:18:12	3625	7.4	13
100:0:0	16	5.9	62
95:0:5	175	7.0	54

Inner mitochondrial membrane

Although there are well described methods for the incorporation of external phospholipids and cholesterol into the inner mitochondrial membrane [29,30], it proved difficult with these procedures to obtain inner membrane vesicles that still contain an active adenine nucleotide exchange system and/or are not leaky for nucleotides.

Experiments in which liposomes were incubated with intact or swollen mitochondria did not lead to any conclusive results. Since submitochondrial particles are rather stable and can be loaded with high amounts of nucleotides, the effect of incorporated cholesterol was next tested with these sonic particles. Only one of several tested methods (see Materials and Methods) was successful. Submitochondrial particles were incubated with liposomes and subjected to a freeze-thaw procedure. After washing and loading with labeled ATP, the efflux of nucleotides was measured [28]. As shown in Fig. 3, the back-exchange rate substantially increased when the particles were exposed to cholesterol-containing liposomes. The amount of incorporated cholesterol, however, was very low

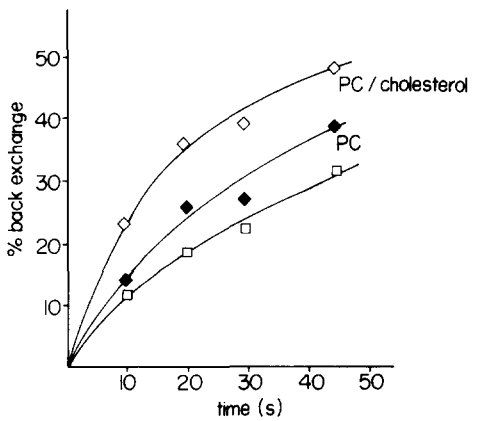

Fig. 3. Influence of incorporated cholesterol on the ADP-ATP exchange in sonic particles. The exchange on beef heart submitochondrial particles was measured as back exchange at 7°C. Either no liposomes were added (□———□), or submitochondrial particles were fused with pure PC liposomes (◆———◆) or with liposomes consisting of (PC/cholesterol = 3:1 (◇———◇). The leakage rates were: 34% (untreated), 47% (+PC) and 59% (+PC/cholesterol). The amount of cholesterol (μg cholesterol/mg phospholipid) in the three different preparations could be estimated to 1.2 (untreated), 1.0 (+PC) and 7 (+PC/cholesterol).

(see Fig. 3). When the procedure was modified to achieve higher cholesterol incorporation, the exchange activity was lost, presumably due to leakiness of the particles for nucleotides. Under the conditions described above, there also still remained severe difficulties with nucleotide leakage which increased from 34% (without liposomes) to 59% (cholesterol-containing liposomes).

In order to confirm these results using a third experimental object, i.e., mitoplasts, the methods for incorporating phospholipids into mitochondrial inner membranes (mitoplasts) had to be extensively modified. After the original fusion procedures [29;30], the treated mitoplasts do not show any measurable nucleotide exchange. It was necessary to reduce both the amount of digitonin and the osmotic stress (see Materials and Methods). The incubation with liposomes had to be carried out at 4°C instead of at room temperature and the mitoplasts were supplied with nucleotides, substrate and oxygen during the incubation period. When cholesterol was incorporated under these conditions, the mitoplasts had functionally active ADP-ATP exchange, which at 4°C (Table II) corresponds to 5–15% of the activity in beef heart mitochondria at this temperature [5]. The amount of incorporated cholesterol was highest when liposomes were prepared from soybean phospholipid (asolectin). With egg yolk phospholipid, pure of PC or PC/PE mixtures, substantially less cholesterol was incorporated (data not shown).

In these experiments the exchange was measured as 'forward exchange', i.e., as nucleotide uptake, since the mitoplasts do not survive the additional centrifugation steps necessary for loading and washing the vesicles for back exchange. When the mitoplasts, treated with liposomes, were subjected to sucrose gradient centrifugation [30], substantial amounts of additional incorporated phospholipids and cholesterol could be detected in the inner mitochondrial membrane (Table II).

The effect of cholesterol enrichment on the adenine nucleotide exchange in mitoplasts is shown in Fig. 4. From these kinetic data the initial uptake rates can be calculated (Table II). First, a decrease in nucleotide exchange activity, due to the fusion procedure with liposomes, can be observed (A → B). After fusion with cholesterol-rich liposomes, however, the exchange activity was

TABLE II

CHOLESTEROL INCORPORATION INTO MITOPLASTS AND ACTIVITY OF ADP-ATP EXCHANGE

Four typical experiments were selected. The exchange rates (v_0) and the relative exchange rates ($v_{0,rel}$) are calculated according to Ref. 24. In the case of relative rates, 100% means uptake after 5 min. The liposome-treated mitoplasts were separated on sucrose gradients as described in Materials and Methods [30]. The residual monoamine oxidase activity, phospholipid and cholesterol content were determined from the combined lower fractions of the sucrose gradient which correspond to 'band 3' and 'pellet' [30], since the highly lipid-enriched fractions ('band 1+2') do not show nucleotide uptake. The three different experimental conditions are A (no liposomes added), B (addition of asolectin liposomes) and C (addition of liposomes consisting of asolectin/cholesterol = 4 : 1).

		Expt. No.			
		1	2	3	4
Monoamine oxidase	A	62	38	18	–
(U/mg, in % of rat	B	58	35	17	14
liver mitochondria)	C	60	37	16	12
Phospholipid	A	0.3	0.22	0.24	–
(g/g protein)	B	0.6	0.37	–	–
	C	0.65	0.39	0.36	–
Cholesterol	A	3	2	2	2
(mg/g protein)	B	2	2	–	1.5
	C	43	16	25	74
Exchange (v_0)	A	9.6	3.25	2.4	–
(μmol/g per min)	B	3.2	1.85	1.3	1.2
	C	3.8	2.25	2.1	2.5
Exchange ($v_{0,rel}$)	A	475	234	180	–
(% exchange/min)	B	330	165	102	78
	C	540	208	195	215

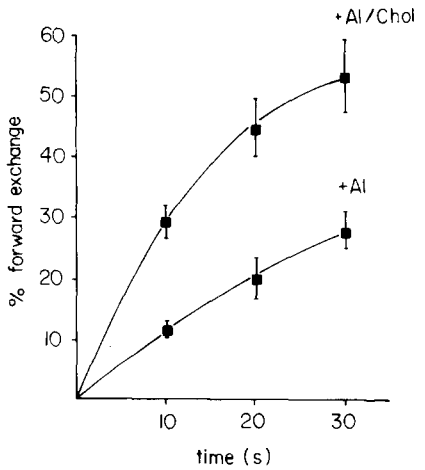

Fig. 4. Influence of cholesterol on the ADP-ATP exchange in mitoplasts. The exchange in rat liver mitoplasts was measured as forward exchange at 4°C. As indicated in the figure, AL denotes addition of asolectin liposomes, AL/Chol addition of liposomes consisting of an asolectin/cholesterol = 4 : 1 mixture. The data of this figure were used to calculate the exchange rates of Expt. No. 4 in Table II. The standard errors are calculated from four different kinetic stop values with the same mitoplasts preparation.

markedly increased as compared to that after addition of pure asolectin liposomes (B → C). The stimulation of ADP-ATP exchange by cholesterol can be demonstrated most conclusively when the relative exchange rates, as explained in Table II, are compared (Fig. 4). The enhanced leakage after treatment with liposomes is thereby eliminated in the calculations.

From the data of Table II it can further be concluded that: (a) although the uptake activity decreased with enhanced effectivity of outer membrane removal, as monitored by the residual monoamine oxidase, the relative stimulation by incorporated cholesterol (B → C) increase simultaneously; and (b) the relative stimulation effect of cholesterol was higher, the more cholesterol was incorporated into the membrane (cf. Expts. 2–4).

Discussion

There are only a few examples of a possible specific interaction of a single phospholipid species with a membrane protein [3,4]. However, the activity of membrane proteins is influenced by the composition and the physical state of the membrane in which it is embedded [1,2]. The adenine nucleotide carrier seems to be a very pronounced example of the latter case. The exchange activity of the reconstituted carrier protein is dependent on the composition of the phospholipid membrane [23,34,36,37]. Pure lecithin is a very poor environment for this protein; addition of PE proved necessary for its catalytic function. Negatively charged phospholipids also improve the carrier activity. It has been shown [34] that the dramatic influence of the surrounding lipids on the transport activity is apparently not due to specific protein-phospholipid interactions, but is related to the physical state of the membrane which can modulate the carrier activity.

Besides the phospholipid composition of the membrane there are other important parameters, one of which is the cholesterol content, that may influence the activity of the membrane proteins. It is demonstrated here that cholesterol has, in fact, a stimulating effect on the adenine nucleotide carrier activity. The transport of ADP and ATP in the reconstituted system is enhanced by increasing the content of cholesterol in membranes of various phospholipid compositions, i.e., pure PC, PC/PE mixtures and natural egg yolk phospholipids.

This stimulation is linearly dependent on the amount of cholesterol added and reaches a maximum when 7–13% cholesterol is present in the membrane. With higher amounts of cholesterol the exchange activity decreases, at least partially due to mechanical damage of the less stable cholesterol-rich vesicles during the reconstitution procedure.

The adenine nucleotide carrier is a good example for demonstrating that a reconstituted system is well suited for studying the influence of membrane composition on the activity of an integral membrane protein. Possible objections against the functional integrity of the carrier protein are not valid for these experiments, since it has been shown earlier that the reconstituted adenine nucleotide exchange shows identical properties as compared to mitochondria with respect to its binding [21], transport activity [15,23] and regulation phenomena [35]. Objections may, however, be raised concerning the correlation of these results with the physiological situation in mitochondria. When comparing influences of the membrane, we have to take into consideration that the phospholipid/protein ratio is very much increased in the reconstituted stystem and that there is always residual detergent (Triton X-100) present in the liposomal membrane.

Therefore, in order to confirm a stimulation of ADP-ATP exchange by cholesterol also in the natural surrounding of the carrier protein, the sterol was incorporated into the inner mitochondrial membrane. Severe methodical problems restricted the amount of cholesterol added. Nevertheless, it could be demonstrated that incorporation of cholesterol increases the activity of the ADP/ATP carrier. Especially with mitoplasts, a correlation between the amount of incorporated cholesterol and the relative stimulation of transport activity could be shown (Fig. 4).

These results are surprising in two respects. Firstly, because the inner mitochondrial membrane contains practically no cholesterol [38]. Although there are indications of a close contact between the inner and outer membrane, which does contain cholesterol, consideration of an influence caused by the sterol from the outer membrane would be highly speculative, since it would require a lipid transfer from the outer to the inner membrane. Secondly, cholesterol is known to enhance the order of the lipid bilayer [12,13] and is therefore supposed to inhibit a process involving a large change in protein conformation [5–10]. Nevertheless, liposomal membranes containing cholesterol apparently provide a more suitable surrounding for the adenine nucleotide carrier than a membrane without this component.

How can this observation be explained? The simplest answer – a specific interaction of the carrier protein with cholesterol – seems very unlikely, not only because of the very low cholesterol content of the inner mitochondrial membrane, but also since the stimulation of transport activity is linearly dependent on the amount of incorporated cholesterol over a wide range (Fig. 1). Also, a

distinct effect on the order of the lipid bilayer influencing the carrier activity can be ruled out, since neither the temperature dependence of nucleotide exchange nor the break point around 20°C were markedly affected by cholesterol up to 10% (Fig. 2). On the contrary, this temperature break point seems rather to be an attribute of the reconstituted carrier protein than of the phospholipid membrane.

Another possible explanation for the exchange stimulation would be a cholesterol-induced asymmetry in the bilayer which consists of different phospholipids. This could be the case in the experiments with egg yolk phospholipids, PC/PE mixtures and natural membranes. However, since cholesterol increases ADP-ATP exchange also when pure PC is used in the reconstituted system, this explanation is ruled out as well.

Recently, a similar stimulatory effect of cholesterol on the glucose carrier activity in cell membranes was reported [14]. The authors explained their observations with the following scheme: Though cholesterol decreases the activity of every single carrier molecule by its ordering effect in the membrane, this is overcompensated by an increased fraction of operating carrier molecules, thus leading to the observed transport stimulation. In contrast to this explanation, it is shown here, however, that at least the reconstituted adenine nucleotide transport is stimulated by cholesterol due to a different mechanism. The molecular activity and not the amount of active carrier sites is increased. Thus, the effect of cholesterol as described in this paper can be directly compared to the stimulation of reconstituted nucleotide exchange by PE and negatively charged phospholipids [34], which also modulate the molecular activity and not the fraction of functionally active carriers.

It seems more likely that cholesterol does not directly affect the protein or its conformation, as discussed above, but that this sterol, when applied in low concentrations, has a specific influence on the physical state of the membrane. It has been reported that the interaction of cholesterol with a lipid bilayer may be different from its generally accepted ordering effect when it is present in the membrane at less than 10–15% [39–41]. Furthermore, cholesterol can destabilize the bilayer structure [42,43] and may, under special conditions, provoke inverse phase phenomena [42]. The interior of the membrane is also altered by cholesterol; this can be measured by a change in the dipolar potential of the membrane, so that the interior becomes more positive [44,45]. Recently, another parameter, the energy state (surface free energy) of the membrane, was introduced to describe the effect of particular membrane environments on integral membrane proteins. This was shown for acetylcholine receptor and matrix protein [46] which proved to be strictly dependent on the surface pressure of bilayers with different lipid compositions in which the proteins were reconstituted [47].

The somewhat surprising result of transport stimulation by cholesterol can also be rationalized in a more general view. When designing a membrane, nature has to compromise between the demands of many different proteins for different environments. Thus, the natural membrane, where these proteins are all embedded together, is not necessarily the optimal surrounding for a single protein of this particular membrane.

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