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Action of calcium channel and beta-adrenergic blocking agents in bilayer lipid membranes

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The action of beta-adrenergic blockers (propranolol, exprenolol, metoprolol, sotalol, atenolol, timolol) and calcium-channel blockers (verapamil, diatiazem) on the electrical properties and fluidity of bilayer lipid membranes (BLM and liposomes) has been investigated. When antibiotic ionophore substances were used as a probe, the electrical measurements showed that many of the drugs inhibited the cation transport across the membrane facilitated by the mobile carrier valinomycin, while having no significant effect on the cation transport through channels formed by gramicidin. The ability of the drugs to decrease the carrier-dependent membrane conductance was correlated to their partition into the lipid bilayer and the magnitude of transmembrane potential induced by them. In the TEMPO ESR spectral measurements, a number of beta-adrenergic and calcium blockers showed the fluidizing effect on liposomes composed of different lipids. The drug concentration required for a detectable change in TEMPO spectra parameter (*f*) was rather high (0.01 M verapamil), and the variation of pH from 6.5 to 3.0 did not affect the fluidizing effect of the drugs.

Introduction

Beta-adrenergic blockers and calcium-channel blockers are two types of effective cardiovascular drugs which are widely used today in treating a wide variety of heart diseases such as angina, arrhythmias and hypertension. The precise mechanism of the action of these drugs in molecular terms remains obscure. It is known that beta-adrenergic blockers act in two ways. In addition to the specific effect in which beta-blockers bind to beta-adrenergic receptor directly to prevent the target cells from the stimulation of neurotransmitters [1], the drug molecules partition into the bulk lipid matrix and decrease ion flux through the interaction with membrane lipids [2]. These membrane lipid effects were suggested to account for the pacemaker and negative iontropic properties of beta-blockers [3].

Calcium blockers are thought to combat heart diseases primarily by preventing Ca^{2+} entry through the excited myocardiac membranes [4]. The potency, specificity, and subtle structural-activity relation of Ca^{2+} blockers imply that their pharmacological function is more relevant to the specific binding to recognition sites at or near Ca^{2+} channels, but many calcium blockers so far examined are appreciably membrane active agents, and it is possible that at least some Ca^{2+} blockers also function by physico-chemical interaction with the cell membranes. The molecular architectures of the 'Ca^{2+} blockers' have no outstanding common features, except that all possess a bulk hydrophobic mass with a secondary or tertiary nitrogen. A wide spectrum of chemical structures usually means a lack of stereospecificity in the drug's action, and it may be indicative of the interaction of the drugs with membrane lipids rather than with specific proteins. There have been some reports that the depression of Ca^{2+} uptake by

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calcium blockers may result from the general perturbation of the membrane lipids [5]. Galenhofen and Hermstein [6] observed two mechanisms in the depression of Ca^{2+} transport by methyl-verapamil (D600): (1) the depression due to a specific protein at low drug concentration, and (2) the depression due to the lipid effect at high drugs' concentrations. Recently, Erdreich and Rahamimoff [7] found that verapamil affected Na^{+}-Ca^{2+} antiport system derived from heart sarcolemmal vesicles. The inhibition of Ca^{2+} uptake by verapamil could be reversed by adding an excess of PC, and so it is possible that the drug acts in the lipid phase and not just on the transporting molecules.

Since the interaction with membrane lipids may represent an aspect of the action of the heart drugs, insight into the mechanism of the drug's action may be obtained by the model membrane studies. The changes in the electrical properties and fluidity of the membranes are usually known as the most common effects, with important consequences for a wide variety of membrane processes when a drug interacts with the lipid bilayer. These perturbations have been investigated using the artificial model membrane - planar bilayer lipid membrane (BLM) and lipid microvesicles (liposomes). Schlieper and Medda [8] found that several beta-adrenergic blockers induced the large transmembrane potential on the model membrane. Shen et al. [9] observed that the magnitudes of the potentials induced by beta blockers and calcium blockers were correlated with the lipid-solubility and the membrane effects of the drugs. The fluidizing effect of propranolol, a representative of beta-blockers, on liposome has been shown using different techniques; like electron spin resonance (ESR) [10], fluorescence [11] and differential scanning calorimetry (DSC) [12], and yet such investigation has not been extended much to other beta-blockers and calcium blockers. In the present experiments, we used the antibiotic ionophores to probe the effects of the two groups of drugs on the membrane transport on bilayer lipid membrane and studied their influence on the membrane fluidity of liposomes using TEMPO ESR spectra measurement. The experimental results showed (a) many of the drugs inhibited the carrier-mediated cation transport but not the channel-mediated transport through bilayer lipid membranes. This inhibition is mainly due to an electrical perturbation of the membrane. (b) Many of the drugs increased the membrane fluidity regardless of the original physical state of the membrane lipids. The fluidizing effects are correlated to the lipophilicities and membrane effect of the drugs. These facts suggest that the heart drugs induce several alterations of physicochemical properties of the membrane through their interaction with lipids. This lipid effect may be involved in, or may influence some of the biological and pharmacological action of the drugs directly, or through lipid-protein interaction.

Materials and Methods

I. Bilayer lipid membrane experiment

The membrane forming solution consisted of 5% natural phosphatidylcholine and 1.0% oxidized cholesterol in *n*-decane. The procedure for oxidation of cholesterol followed that of Tien et al. [14]. All the drugs, including beta blockers and calcium blockers, are the generous gifts of manufacturers. Antibiotics ionophore substances were purchased from Sigma Co. The drug stock was prepared by dissolving a drug in the electrolyte solution buffered with 5 mM Hepes (pH 6.5 except for that described specially), which was used as the bulk solution bathing the membrane, and the pH of the stock was readjusted so that the pH of bathing solution was kept constant after the addition of the drug. Ionophore substances were dissolved in 99% ethanol. The volume of the antibiotics stock added was less than 0.2% of the total volume of the bulk solution to avoid the perturbation of bilayer lipid membrane due to ethanol.

The bilayer lipid membranes was formed by the standard technique [14,15] on the small hole of a Teflon cup. The bilayer lipid membrane separated two bathing solutions, in which a pair of calomel electrodes with salt bridges were submerged. The electrode in the Teflon beaker (designated as inside) was connected to the measurement instruments. The electrode in the outer chamber (outside) was connected to virtual ground, serving as a reference electrode. The steady-state current (I_m) through the membrane was measured using a low impedance picoammeter (Keithley 417) while a

voltage (V_m) was applied. The membrane conductance (G_m) was calculated from the current-voltage (I-V) curve recorded by an x-y recorder. The membrane capacitance (C_m) was measured with a low level capacitance meter (ICF/Electronic Model 1-6). To ensure that I_m and C_m had reached a stable value, they were measured at 15 min after the addition of chemicals. All the measurements were performed at room temperature (22 ± 1°C) and were repeated two or three times.

II. ESR experiment

The lipid dispersions used in ESR measurement were prepared by mechanically shaking. The tissue lipid preparation was extracted from fresh rat heart by following a modification of Folch's procedure [14]. All lipids were dissolved in double-distilled and molecular sieve (M-514, Type 4A)-treated chloroform. The resulting solution was dried under a stream of argon for 4 h and in a vacuum for another 4 h. The dried lipids were added into 0.1 M phosphate buffer containing 0.2 mg/ml spin label compound TEMPO and the final lipid concentration was 8%. The mixture was dispersed by shaking for 5 min using a vortex rotamixer. Drugs were added into the resulting milky dispersion and the sample was left overnight at the ambient temperature for the equilibrium.

Spin label measurements were carried out on a Varian E112 X-band spectrometer with a quartz flat cuvette as the sample tube. A 100 kHz field modulation and the detection unit was used for detection purposes. The temperature was controlled by a nitrogen stream flowing through a Varian variable controller, and was monitored with a calibrated thermocouple attached to an Omega digital meter with an accuracy of ±0.2 Cdeg. The sample was allowed to equilibrate in the machine for 5–7 min before each measurement.

Result

I. Bilayer lipid membrane experiments

The effect of drugs on valinomycin-mediated K^+ transport. The addition of 10^{-8} M valinomycin into 0.1 M KCl buffer solution (5 mM Hepes, pH 6.5) increased the conductance of bilayer lipid membranes by 2–3 orders (from 10^{-9} $\Omega^{-1} \cdot cm^{-1}$ to 10^{-7} to 10^{-6} $\Omega^{-1} \cdot cm^{-1}$). When a certain amount of propranolol was introduced, the membrane current was decreased. Over the range of 10^{-4} to 10^{-2} M, the membrane conductance, G_{M^+}, in log scale appeared as a linear function of propranolol concentration. At the higher concentrations, the saturation of the drug's effect was observed. The maximum reduction of G_{K^+} caused by propranolol was generally about 10-fold. In other words, the membrane conductance could not be restored by the addition of propranolol to its original level before the addition of valinomycin . The absolute concentration of propranolol for blocking K^+ transport is about 10^{-4} M, 2–3 orders higher than concentrations affecting the specific proteins.

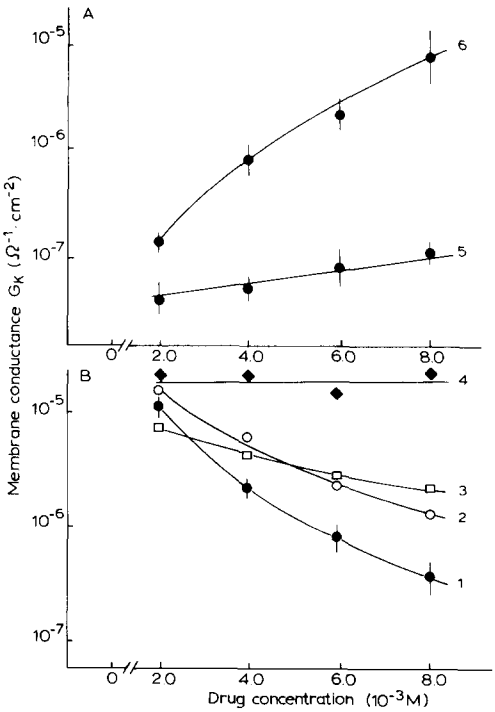

Fig. 1. Effect of heart drugs on the diffusions of valinomycin-K^+ (curves 1–4), $Ca(A23187)_2$) (curve 5) and 2,4-dinitrophenol $H(DNP)_2^-$ (curve 6) through bilayer lipid membranes. The drugs tested were propranolol (●), verapamil (○), metoprolol (□) and sotalol (◆).

Several beta-blockers and calcium blockers were tested using valinomycin as a probe (Fig. 1). The ability of the drugs to depress K^+ conductance of bilayer lipid membranes was found to be parallel

TABLE I

THE ABILITY OF VARIOUS DRUGS TO INHIBIT VALINOMYCIN-MEDIATED K^+ CONDUCTANCE, AND THE COMPARISON OF THE ABILITY WITH DRUG'S LIPOPHILICITY AND INDUCED POTENTIALS

G_1 is bilayer lipid membranes K^+ conductance in 0.001 M drugs and G_0 is K^+ conductance in the absence of the drugs. P_{corr} is the corrected partition coefficient of a drug in octanol/water system. The values of P_{corr} are calculated from pK_a and log P_{app} quoted from (a) Wang and Lien [28], (b) Harada et al. [29], (c) Lullmann and Wehling [30], and (d) Hellenbrecht et al. [31].

Drug	G_0/G_1	log P_{corr}	Induced potential (mV) on PC + PE + oxidized cholesterol bilayer lipid membranes
Propranolol [a]	9.20	3.29	−53
Verapamil [c]	5.60	2.74	−45
Oxprenolol [b]	4.30	2.17	−48
Metoprolol [a]	2.50	2.04	−38
Sotalol [d]	0.88	0.60	−5
Atenolol [a]	0.82	0.43	−9.5

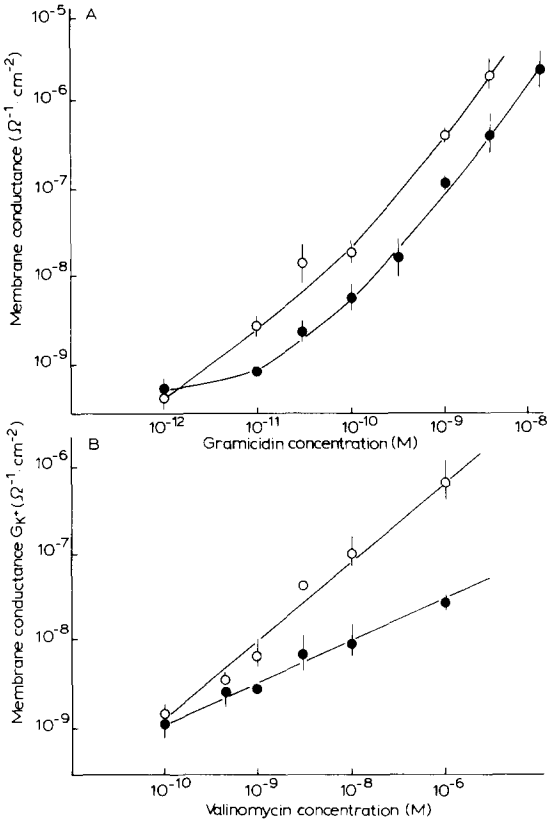

Fig. 2. Dependence of bilayer lipid membranes K^+ conductance on gramicidin A (A) and valinomycin (B) concentration: in the presence of 0.001 M propranolol (●) and in the absence of propranolol (○).

to their lipid effect. To clear this, valinomycin-dependent G_{K^+} was measured in the absence and presence of the drugs and designed as G_0 and G_1, respectively. The ratio G_0/G_1 serves as an indication of the ability of a drug to inhibit G_{K^+}. The comparison (Table I) confirms the correlation between G_0/G_1 and the lipophilicities and induced transmembrane potentials of the drugs.

When the experiment was repeated with 2,4-dinitrophenol, which travels through the membrane in a negatively charged form HA_2^- [16], the diffusion of 2,4-dinitrophenol through the membrane was accelerated by propranolol (Fig. 1). On the other hand, propranolol showed no significant effect on the Ca^{2+} transport mediated by A23187, which carries Ca^{2+} across the membrane in an electrically neutral form, $Ca(A23187)_2$ [17].

Effect of drugs on gramicidin A-mediated K^+ and Na^+ transport. Beta-blockers and Ca^{2+} blockers behaved quite differently on gramicidin A-modified bilayer lipid membrane. Of all the drugs tested, only propranolol was able to inhibit gramicidin-facilitated K^+ and Na^+ transport, but its effect was much smaller than that in the valinomycin experiment. None of the rest of the drugs showed an effect upon gramicidin-dependent G_{K^+} and G_{Na^+}, irrespective of their effects on valinomycins function.

Ion transport at various ionophore concentration. The difference in the effect of the drugs on the two ionophore transports was also shown by measuring the membrane conductance at varying ionophore concentration in the absence and presence of the drugs. When gramicidin was applied, G_{K^+} changed in the same pattern with the variation of gramicidin concentration from 10^{-11} to 10^{-8} M, regardless of the presence of propranolol (Fig. 2). In both cases, G_{K^+} tended to increase with the same increment corresponding to a given quantity of the ionophore. This can be clearly seen from the parallel curves, implying that the trans-

port function of gramicidin is independent of the drug. At a given concentration of gramicidin, G_{K^+} is lower in the presence of propranolol, but the difference is not significant. This may be explained by the interference of the drug on the translocation of a small portion of ions through the lipid phase. The two curves looked quite different when valinomycin was used (Fig. 2). They rose with different slipes. G_{K^+} changed much more slowly when propranolol was applied.

Influence of ionic strength on the drug's action. The inhibition of drugs on valinomycin-facilitated K^+ transport is dependent upon ionic strength, and it is depressed greatly at high salt concentration. When valinomycin and drugs were administered at a fixed level, the ability of the drugs (e.g., propranolol and verapamil) to block the K^+ transport was reversely proportional to K^+ concentration. When we kept K^+ concentration constant (10^{-3} M) and changed the ionic strength of the bulk solution by varying the Na^+ concentration, the same antagonizing phenomenon was observed (Fig. 3).

II. ESR experiment

Effect of drugs on TEMPO ESR spectra. The interaction of the drugs with the membrane lipids was investigated using TEMPO (2,2,6,6-tetramethyl-piperdine-1-oxyl) as the probe. TEMPO spectral parameter (f), the ratio of hydrophobic component (H) and hydrophilic component (P) ($f = H/(H+P)$), is used as a measure of the fraction of the spin label dissolved in the lipid bilayer. One can note that at room temperature, the lipid phase peak (H) of natural phosphatidylcholine (plant) becomes more pronounced in the presence of some drugs, indicating increases in the membrane fluidity (Fig. 4). Changes in f induced by the drugs were also related to the ability of the drugs to interact with membrane lipids. Propranolol and verapamil enhanced (f) values about 25–30%. The increase induced by metoprolol was smaller (13%), and sotolol and timolol showed little effect on the lipid fluidity (Table II). Ca^{2+} blockers (verapamil and diatiazem) appeared to have a stronger effect on membrane fluidity than would be expected from their partition coefficients, compared to those of beta-blockers. This may be due to their lower pK, and chemical structures different from those of the latter.

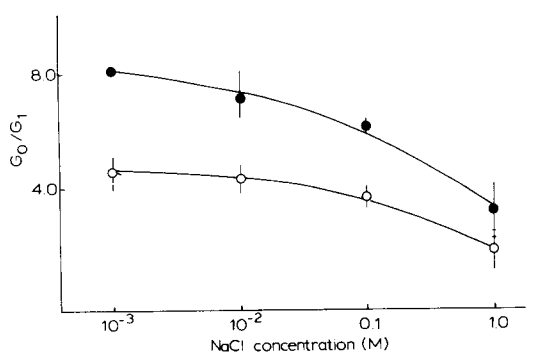

Fig. 3. The effect of electrolyte concentration on the ability of drugs to block valinomycin ($1.0 \cdot 10^{-8}$ M)-mediated K^+ transport. G_0, the membrane K^+ conductance in the absence of propranolol. G_1, membrane conductance at 0.001 M propranolol (●) or verapamil (○). K^+ concentration in the bulk solution was fixed at 0.001 M.

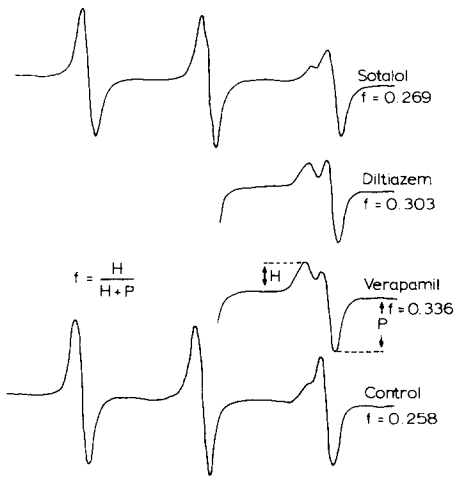

Fig. 4. Effect of various drugs on partitioning of TEMPO in natural phosphatidylcholine liposomes. TEMPO ESR spectra were measured at 22.0 (±0.2°C). The concentration of drugs was 0.1 M.

Temperature scanning measurement. The temperature scanning spectra of spin label can provide more general information about the physical state of the membrane. Different lipids, namely, synthetic dimyristoyl-PC (DMPC), natural phosphatidylcholine (plant) and heart lipid extracts were used in this experiment. DMPC liposomes showed an abrupt phase transition over a narrow temperature range (< 5 Cdeg. (Fig. 5). The pres-

TABLE II

CHANGES IN TEMPO SPECTRAL PARAMETER (f) INDUCED BY VARIOUS DRUGS ON NATURAL PHOSPHATIDYLCHOLINE

Drug concentration was 0.10 M. (f) was measured at 22.0°C (±0.2°C), $(f) = ((f_{drug} - f_{cont})/f_{cont}) \times 100\%$. ($f$) values in the table were the average from two repeats + standard deviation of the mean.

Drug	f	Δf (%)	log P_{app}	Induced potential (mV) on PC + PE + oxidized cholesterol bilayer lipid membranes
Control	0.263 ± 0.005			
Verapamil	0.336	28	2.74	−45
Propranolol	0.332 ± 0.002	26	3.29	−53
Diatiazem	0.303 ± 0.010	15		−37
Metoprolol	0.297 ± 0.004	13	2.04	−38
Sotalol	0.279 ± 0.010	6	0.60	−5
Timolol	0.254 ± 0.017	−3.5		

ence of verapamil did not broaden the transition, but it decreased the transition temperature (T_t) greatly, shifting T_t to 12.5°C from 22.5°C at 0.1 M. Another prominent effect was the disappearance of the pretransition region of DMPC. This may be explained by the interaction between the lipid polar head and the cationic part of verapamil.

When the scanning measurement was repeated with natural phosphatidylcholine liposomes, there was no sharp change in (f) parameter from 0 to 60°C suggesting no distinct phase transition induced by verapamil (Fig. 5). The lack of sharp transition is not surprising if we consider the fact that natural phosphatidylcholine consists of chemically various species with different (T_c) due to the heterogeneity in the degree of saturation and the length of hydrocarbon chains. This heterogeneity conferred upon the membrane a broad transition, in which the solid phase and liquid crystalline phase coexisted. In this experiment, (H) peak appeared at a rather low temperature, indicating that a portion of the hydrocarbon chains was already melted. The transition seemed to be finished at about 40°C, beyond which (f) did not change significantly. Due to the lack of a discriminable transition, we could not detect the decrease in T_t caused by verapamil on natural phosphatidylcholine liposomes. However, the scanning experiment demonstrated clearly that verapamil tends to increase the membrane fluidity over a wide temperature range. Verapamil also fluidized the liposome prepared from rat heart lipid extracts, and, in this case, the phase transition became even broader.

Effects of drug concentration and pH. In the

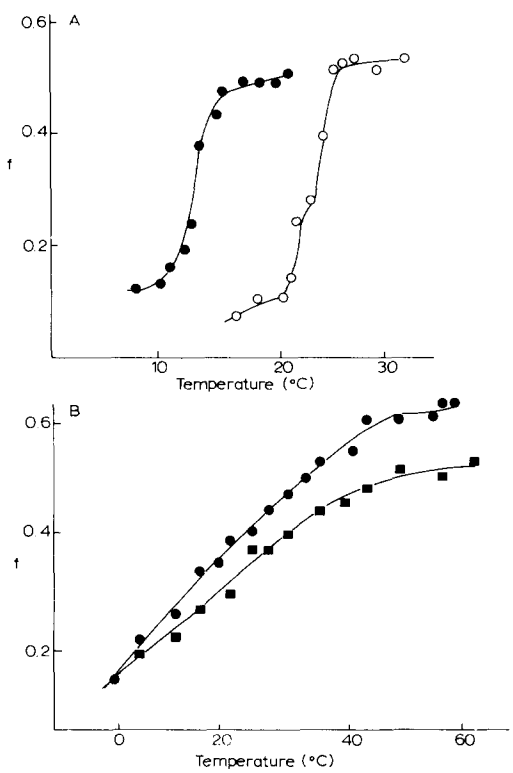

Fig. 5. TEMPO spectral parameter (f) as a function of temperature. (f) was measured at 0.10 M verapamil (●) and in the control (○) on DMPC (A); and at 0.10 M verapamil (●) and in the control (■) on natural liposomes (B).

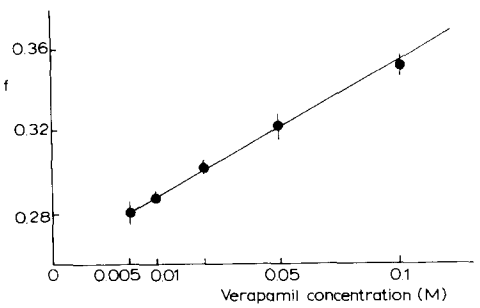

Fig. 6. TEMPO spectral parameter (f) as a function c verapamil concentration. TEMPO spectra were measured at 22°C.

dose-response experiment (Fig. 6), the membrane fluidity increased rapidly in an exponential manner with the increase of the drug's concentration from 0.01 to 0.1 M. Below 0.01 M no change in spin label spectra was detected. The measurement was not extended to concentrations higher than 0.1 M because of the limited solubility of the drug in aqueous solution.

On the other hand, the effect of verapamil on the membrane fluidity was not influenced by varying pH from 6.5 to 3.0 (Fig. 7). The (f) began to rise at pH 3.0, but this was a pure pH effect, independent of the drug, as demonstrated by the control. This is probably due to the ionization of the phosphate group of PC in the acidic media [18]. The more significant pH range is around the

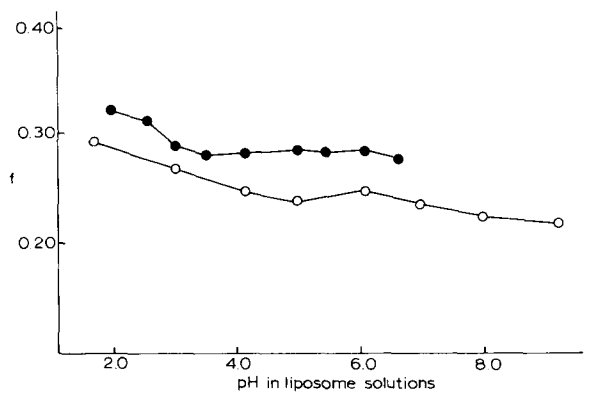

Fig. 7. TEMPO spectral parameter (f) at various pH values of natural phosphatidylcholine liposome solutions. (f) was measured at 0.10 M verapamil (●) and in the control (○). The experimental temperature was 22°C.

pK of the drugs (8.45 for verapamil), where one would expect to observe some interesting things. But it is very difficult to do the spin label measurement at such high pH since verapamil solubility in water decreases dramatically around pH 6.5.

Discussion

In the bilayer lipid membrane experiment, the cation transport through the bilayer lipid membranes is inhibited by beta-adrenergic blockers and calcium blockers. The primary event of the inhibition occurs at the lipid level, owing to the following experimental facts: (1) The drugs only act on the mobile, carrier-mediated cation transport, while having little effect on the counterpart facilitation by channel-forming mediator. (2) There is a correlation between the ability of the drugs to decrease the carrier-dependent membrane conductance and their interaction with membrane lipids. (3) The concentration of the drugs for blocking the ion translocation is of the same order as that for perturbing lipid bilayers, but 2–3 orders higher than that for affecting proteins specifically. From the fact that both beta-adrenergic blockers and calcium blockers induce the transmembrane potential, it can directly ascribe the inhibition of carrier transport to the drug-induced change in electrostatic properties of the membrane: the elimination of negative charge and development of the transmembrane potential make it unlikely for the positively-charged valinomycin-K^+ complex to enter into the lipid bilayer and travel through it. A change in the membrane conductance may be due to some other factors, such as dielectric and fluidity of the membrane [19], but this seems not the case in this investigation.

The involvement of the membrane dielectric in the alteration of the membrane permeability seems to be ruled out by capacitance measurement in which the capacitance was only slightly increased (generally 10% to 20%) by the addition of drugs, irrespective of their capacities of blocking K^+ conductance (Table III).

The membrane permeability is also linked with the membrane fluidity. A permeant moves with more difficulty in a frozen lipid area. It is easy to deduce that the fluidity-associated membrane conductance is independent of the charge state of a

TABLE III

BILAYER LIPID MEMBRANES CAPACITANCE C_m MEASURED AT 0.001 M VARIOUS DRUGS

The capacitance was measured using a low level capacitance meter. The membrane solution used was the usual one: PC + PE + oxidized cholesterol + PS and bathing solution was 5 mM KCl (pH 7.0).

Drug	C_0 (capacitance in control, 10^{-9} F)	C_1 (capacitance at 10^{-3} M drug, 10^{-9} F)
Verapamil	3.8	4.0
Diatiazem	4.2	5.6
Propranolol	3.5	4.2
Oxprenolol	5.5	5.7
Metoprolol	5.0	5.5
Timolol	5.0	4.0
Sotalol	4.0	4.2
Atenolol	4.8	5.2

carrier [19]. The positive permeant (G^+), negative permeant (G^-) and neutral permeant (G) are not discriminated by a change in fluidity, so that the change of the membrane conductance for G^+, G^- and G always follows in the same direction with an alteration of membrane fluidity. This differs from the potential-associated membrane conductance for which G^+ and G^- conductance change in the opposite direction, and a decrease in G^+ conductance is always accompanied by an increase in G^- conductance. Another difference between the two types of permeability change, is that the fluidity-associated one is not sensitive to the ionic strength of aqueous solution. In our experiment, the diffusion of the negative species $HDNP_2^-$ was accelerated, while valinomycin-K^+ transport was slowed down in the presence of the heart drugs. The third diffusion species, an electrically neutral complex $Ca(A23187)_2$, was not influenced by the drugs. It was also found that the inhibitory effect of the drugs on the carrier's transport function was diminished at the high salt concentration. Therefore, it is likely that the electrostatic perturbation, rather than the fluidity change of the membrane, is the major factor responsible for the carrier-mediated transport.

In the ESR experiment, a number of beta-blockers and Ca^{2+} blockers were found to increase the membrane fluidity. One doubt about the practical significance of this effect is the high drug concentration requirement, which was also reported elsewhere [20]. This problem is partially due to the relative nonsensitivity of ESR and the small proportion of the drugs incorporated into liposomes [21]. Spin label ESR usually needs a high concentration of the drug, therefore the dose-response relation in this experiment does not mean that less than 0.01 M of verapamil has no liquifying effect. In DSC [12] and fluorescence experiments [13] millimolar concentration of propranolol induced a decrease of a few degrees in T_t of DPPC liposome. The concentration of millimoles, of course, is still too high as compared with the usual plasma drug level, but the latter is not always likely to be a good indicator of the local drug concentration in the target tissues. Verapamil [22] and propranolol [23] were found to accumulate to a marked extent in their target tissues.

Not all researchers agree that the fluidizing effect mimics the real function of propranolol on the living membranes since the experimental data are conflicting and drug-induced decreases in fluidity were sometimes observed. One criticism is that most of the investigations have been performed by measuring T_t on the liposomes composed of a single type of synthetic phospholipid like dipalmitoyl-PC (DPPC) and dimyristoyl-PC (DMPC) which are characteristic of distinct phase transitions. Such model membranes do not represent well the biological membranes, which have coexistent liquid or liquid-solid functional states and undergo a phase transition over broad temperature ranges, because of the extreme diversity in constructional compositions. There have been few experiments done on such membranes and thus it is not clear how the heart drugs influence the fluidity of the membranes which are already in the liquid or liquid-solid coexistent state. Phadke et al. [20] reported opposite effects of propranolol on the membranes of different states: while liquifying the gel-state DPPC liposomes, the drug-stiffened egg PC, and egg PC plus cholesterol liposomes, which were known to be in the liquid-crystalline state. Surewicz and Leyko [24] reported that propranolol exerted a marked ordering effect on liquid-state bilayer prepared from certain phospholipids and thus they proposed that some drugs may affect the membrane order and liquidity quite differently, depending on the original physical

state of the membranes. In this model, propranolol acts as a modulator, like cholesterol and some intrinsic membrane proteins, adjusting the membrane fluidity to a critical point. They liquify the membrane if the fluidity is below the point, and impart rigidity to the fluid state of the hydrocarbon chains above it.

The interior of bilayer lipid membrane is liquid-like, owing to the existence of a small amount of organic solvent [25]. According to the 'modulator hypothesis', propranolol and other heart drugs should increase the degree of lipid order. However, our ESR experiment showed that in all the liposomes tested, regardless of their physical state, the membrane fluidity was increased by the drugs. The natural phosphatidylcholine, which was the very one used in the ionophore transport experiment, heart lipid liposomes were shown to be in the coexisted liquid-solid state, and their fluidity was increased by the drugs over a wide temperature range. The ESR results corroborate the idea that the inhibition of the carrier-mediated cation translocation through the membrane is mainly caused by the electrical effect rather than the fluidity effect of the drugs. Theoretically, the fluidizing of the membrane by these drugs may influence the transport process, but it was negligible since the diffusion of neutral species, Ca $(A23187)_2$, were not accelerated by it.

The fluidity change induced by the heart drugs does not contribute much of the blockade of the carrier transport in bilayer lipid membrane but this does not exclude the possibility of this mechanism by which the drugs act in the living membranes in vivo. Thayer et al. [26] reported that verapamil and D600 impaired the binding function of their receptors through the perturbation of the membrane fluidity. There has been no evidence whether or not, and how, the fluidizing effect of beta-blockers and Ca^{2+} blockers involves their pharmacological functions, e.g., the blockade of Ca^{2+} uptake. One hypothesis about it is the annular transition model proposed by Lee [27] for interpreting the cation of local anesthetics. In this theory, the sodium channel is presumed to be a complex form of the pore protein and annulus lipids. The latter ar normally in gel state and the rigidity keeps the channel open. The addition of drugs triggers a change in lipids from the gel to a fluid state, causing the channel to relax into its most stable state with consequent closing of the pore. Whether or not a similar model can be used to explain partly the pharmacological function of the heart drugs is not known because of the lack of knowledge about the structure and properties of the channels and about the interaction between lipids and proteins.

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