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THE POSSIBLE INVOLVEMENT OF THE PHOSPHOLIPID PHASE OF MEMBRANES IN MEDIATING THE EFFECTS OF VERAPAMIL ON Ca^{2+} TRANSPORT

ANAT ERDREICH and HANNAH RAHAMIMOFF
Department of Biochemistry, Hebrew University–Hadassah Medical School, Jerusalem 91010, Israel

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Abstract—The effect of verapamil in a model system of A23187-induced Ca^{2+}-uptake into liposomes was studied. This was done in order to separate the effects of verapamil on the lipid phase of membranes from its effects on membraneous proteins. In the absence of A23187, the liposomes exhibited a very low Ca^{2+} permeability, which did not change with addition of verapamil. Creation of a valinomycin-induced negative inside membrane potential combined with increased membrane permeability to Ca^{2+} (A23187), increased Ca^{2+}-entry fivefold and more. Addition of verapamil under these conditions led to a further increase in Ca^{2+} entry. The negative inside polarization of the liposomes' membrane (as estimated from [^{3}H]TPP^+ uptake) was not affected by verapamil. [^{3}H] Verapamil bound specifically to native synaptic plasma membranes with a K_d = 87.4 nM ± 21.5 (SD) and B_{max} = 2.19 pmol/mg protein ± 0.92 (SD). Specific binding to the liposomes could not be demonstrated. High nonspecific binding of up to about 20% of the total verapamil in the external solution was observed (3.8 pmoles [^{3}H]verapamil/mg phospholipid when 30 nM verapamil was used and 50 nmoles/mg phospholipid when 200 μM [^{3}H] verapamil was used). The high nonspecific binding of verapamil to the liposomes had no detectable effect on the fluidity of their membrane, as seen in fluorescence–anisotropy studies with the fluorescent probe DPH.

Verapamil, a widely used antianginal and antiarrhythmic drug, is classically considered to be a Ca^{2+} channel blocker [1, 2]. Different studies showed that cardiac tissue [3, 4] and skeletal muscle [5] have specific binding sites for verapamil and its derivatives. The K_d values found range between 0.9 and 17.4 nM with B_{max} between 0.13–13.6 pmol/mg protein. In addition to its effects on the Ca^{2+} channel in contractile tissue, there are a large number of reports in the literature in which other effects of verapamil were shown. These include inhibition of catecholamine release from adrenal medulla [6], inhibition of Na^+-dependent Ca^{2+} uptake and Na^+-dependent GABA uptake in synaptic plasma membrane vesicles [7, 8], inhibition of Na^+ channels [9, 10], and even an antagonistic action on 5HT-receptors in platelets [11]. In view of the lipophilic properties of verapamil, and the variety of different effects it is reported to have, a possible explanation for its diverse actions could involve some nonspecific effects mediated by the lipid phase of cell membranes. This possibility is also quite relevant especially in neuronal tissue since the concentrations of verapamil required to inhibit the voltage-dependent Ca^{2+} influx into synaptosomes [12, 13] are at least two orders of magnitude higher than those required to inhibit voltage dependent Ca^{2+} channels in skeletal muscle transverse tubules [14] and cardiac tissue [15]. Also, there is increasing evidence that certain groups of drugs (general anaesthetics, local anaesthetics, barbiturates, alcohols) exert their effects via actions on the membrane fluidity [16]. In order to examine this possibility, a model system of the lipid phase of the membrane comprising of protein-free liposomes was developed.

Reported here are the effects of verapamil on A23187-mediated Ca^{2+}-entry, [^{3}H]TPP^+* uptake in liposomes, and its binding to synaptic plasma membranes and liposomes.

MATERIALS AND METHODS

Preparation of liposomes. Liposomes were prepared from soybean or brain [17] phospholipids by dialysis. Purified asolectin [18] (soybean phospholipid) was solubilized in the desired "in" medium with addition of 2% cholate. The solution was dialyzed employing dialysis tubing (Visking 1$\frac{8}{32}$", Medical International, London) against a 1000-fold excess of the same "in" solution, but in the absence of cholate, at 4°. The external dialysis solution was changed twice during the next 40 hr. Liposomes were collected and kept at 4° for up to 1 month. As could be seen from electron micrographs (not shown), resulting liposomes were unilamellar, with a diameter of 35–200 nm (55% were between 40–60 nm). These liposomes were used in all experiments except in the measurements of polarized fluorescence (Fig. 3).

Liposomes for polarized fluorescence studies were prepared from phosphatidycholine by sonication (Head Systems model 350, Ultrasonics Inc.) at 4°. The liposomes were centrifuged at 130,000 *g* for

* Abbreviations used: TPP^+, tetraphenylphosphonium bromide; DPH, 1,6-diphenyl-hexa-1,3,5-triene; SPM, synaptic plasma membrane; BSA, bovine serum albumin.

30 min, to sediment the larger liposomes, and the supernatant (containing the smaller liposomes) was collected and kept at 4°.

In the fluorescent studies, phosphatidylcholine was solubilized in the presence of the fluorescent probe DPH (1,6-diphenyl-hexa-1,3,5-triene) with a molar lipid:DPH ratio of 1000:1, and in the presence or absence of 200 μM verapamil.

$^{45}Ca^{2+}$ and [^{3}H]TPP^{+} uptake studies. Uptake studies were performed by dilution of 3 μl liposomes (30–90 μg phospholipid) containing the appropriate "in" solution into 97 μl of an uptake solution containing $^{45}Ca^{2+}$ or [^{3}H]TPP^{+} as described in detail in the legends to the relevent tables and figures.

The uptake reaction was terminated by passing the entire reaction mixture through a Dowex-50 minicolumn [19], which separated intraliposomal from extraliposomal Ca^{2+} (or [^{3}H]TPP^{+}).

The $^{45}Ca^{2+}$ or [^{3}H]TPP^{+} content of the liposomes was determined by counting the samples in a 40% Lumax solution in a liquid scintillation counter.

[^{3}H] verapamil binding. (1) [^{3}H] verapamil binding to liposomes was studied by dilution of 3 μl liposomes into 97 μl of an external medium containing [^{3}H] verapamil as described in the legends to the relevant tables. The reaction was stopped by gel filtration through a Sephadex G-50-80 minicolumn in order to separate intraliposomal and extraliposomal [^{3}H] verapamil: 1 ml tuberculin syringes were filled with pre-swollen Sephadex and centrifuged at 1000 *g* for 2.5 min. The entire reaction mixture (with the liposomes) was injected carefully into the center of the shrunken Sephadex bed. Repeating the centrifugation under exactly the same conditions resulted in separation of the liposomes in the excluded volume.

[^{3}H] verapamil content of the liposomes was determined by counting aliquots of the solution excluded from the minicolumn in 25% Lumax in a liquid scintillation counter.

(2) [^{3}H] verapamil binding to SPM vesicles was done according to the method described by Ruth *et al.* [4], with some modifications. Three-microlitre SPM vesicles (30 μg protein), which were prepared as described in detail previously [7], were diluted into 97 μl of medium containing 50 mM Tris–HCl buffer pH 7.4, 5 mM EGTA, 0.1% BSA and 1–25 nM (0.01–0.25 μCi) [^{3}H] verapamil. Verapamil concentration was adjusted by addition of unlabeled verapamil (0–500 nM). Nonspecific binding was determined in the presence of 10 μM unlabeled verapamil. The incubation was performed at 4° and terminated after 30 min by pipetting the entire reactin mixture on to a GF/C filter. The filters were washed rapidly with 2 × 4 ml ice-cold wash solution (100 mM NaCl, 5 mM Tris HCl pH 7.4, 0.1% BSA), dried and counted in 25% Lumax solution in a liquid scintillation counter.

Polarized fluorescence studies. Changes in liposome membrane fluidity were determined by measuring polarized fluorescence [20].

The probe used was DPH, which was introduced into the membrane as described in "preparation of liposomes".

Equilibrium fluorescence studies were carried out in a spectrofluorometer (Perkin-Elmer MPF 44A with 150-xenon power supply) in which the measuring chamber was connected to a bath circulation system (Haake FK) to obtain the desired temperature. The spectrofluorometer was connected to an L-shaped polarization unit with KSW38 polarizers (Polarex; E. Kasemann).

The temperature in the fluorometer chamber was monitored by a thermistor probe (Yellow Springs Instrument Co. Inc.; YSI-423) connected to a YSI thermometer (Model 425C).

Phospholipid content was determined according to the method of Ames [21]. All experiments were repeated several times with different preparations and all data points are averages of duplicate or triplicate determinations. Zero-time measurements were subtracted from all results.

Verapamil and D600 were a kind gift from Professor Dr. D. Lenke, Knoll AG. $^{45}CaCl_2$ and [^{3}H] verapamil were purchased from New England Nuclear. [^{3}H] TPP^{+} was purchased from the Nuclear Research Center, Negev, Israel. All biochemicals were analytical grade, and purchased from Sigma, Israel. Lumax was purchased from Lumac B.V. (The Netherlands).

RESULTS

Ca^{2+} *influx into liposomes*

Liposomes prepared from soybean phospholipids, possess only a limited Ca^{2+}-permeability.

Table 1 shows an experiment in which liposomes loaded with K-phosphate buffer (pH 7.4) and diluted into an isoosmotic sucrose medium containing $^{45}CaCl_2$, take up only 0.43 nmoles Ca^{2+}/mg phospholipid/10 min. When the membrane permeability for Ca^{2+} is increased by the addition of the Ca^{2+}-ionophore A23187, Ca^{2+} entry somewhat increases and reaches 2.29 nmoles/mg phospholipid/10 min. Addition of valinomycin induces K^{+} efflux and creates a negative-inside polarization of the membrane. Consequently, Ca^{2+} entry is increased further. This negative inside membrane polarization dependent Ca^{2+} entry is observed, however, only in the presence of A23187.

Effect of verapamil on Ca^{2+}*-influx*

Addition of verapamil to the external Ca^{2+}-containing sucrose solution (Table 1), in the absence of Ca^{2+} ionophore A23187, causes only a small change in Ca^{2+} influx, even in the presence of valinomycin. Addition of both A23187 and verapamil in the external medium increases Ca^{2+} entry more than 17-fold. In six other experiments the increase in Ca^{2+} entry in the presence of both the Ca^{2+} ionophore A23187 and verapamil was always at least sixfold higher than the Ca^{2+} entry obtained in their absence. This increase in A23187-induced Ca^{2+} entry by verapamil can be further enhanced by the creation of a negative inside membrane polarization—addition of valinomycin to the K^{+} loaded liposomes. Thus, under conditions of increased Ca^{2+} permeability and increased Ca^{2+} entry, addition of verapamil leads to a further increase in Ca^{2+} content of liposomes. In six different experiments the increase in Ca^{2+} entry in the presence of added A23187, valinomycin and verapamil was between two- and sixfold of the entry

Table 1. Ca^{2+} entry into liposomes: Effect of verapamil and A23187

Additions	Ca^{2+} entry nmol/mg phospholipid/10 min				
	No addition	Verapamil	A23187	Verapamil +A23187	Verapamil +A23187 +Triton
No valinomycin	0.43	0.70	2.29	7.68	0.03
Valinomycin	0.46	0.79	3.75	20.82	0.02

Three-microlitre liposomes (30–90 μg soybean phospholipids) containing 0.15 M K-phosphate buffer pH 7.4 were diluted into 97 μl external solution containing 0.3 M sucrose, 10 mM K-phosphate buffer pH 7.4 and 50 μM $^{45}CaCl_2$ (about 0.1 μCi) with 1 μM A23187 and/or 200 μM verapamil and/or 20 μM valinomycin. After 10 min, 90 μl of this mixture were loaded on a Dowex mini-column, and the reaction was terminated as described in Methods. Ca^{2+} entry in the presence of verapamil and A23187 was measured also following 1% Triton X100 addition prior to loading the reaction mixture on the Dowex mini column.

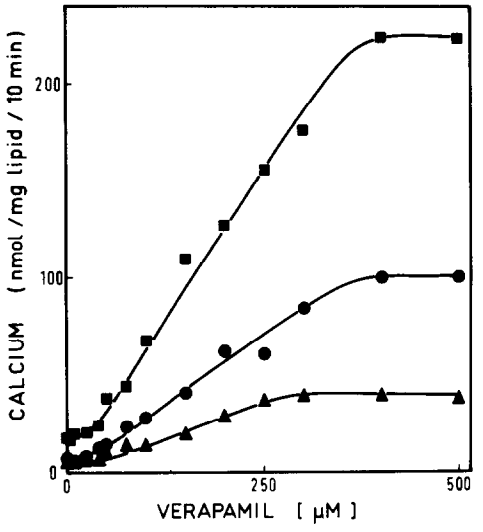

Fig. 1. Effect of verapamil on Ca^{2+} entry into liposomes. Three-microlitre liposomes (30–60 μg soybean phospholipids) containing 0.15 M K-phosphate buffer pH 7.4 and 10 mM Tris–HCl pH 7.4 were diluted into 97 μl external solution containing 0.3 M sucrose, 10 mM Tris–HCl pH 7.4, 20 μM valinomycin, 1 μM A23187, between 0 and 500 μM verapamil and 25 μM (▲) or 50 μM (●) or 100 μM (■) $^{45}CaCl_2$ (about 0.1 μCi). The reaction was terminated after 10 min by loading 90 μl of this mixture on a Dowex mini-column and washed as described in Materials and Methods.

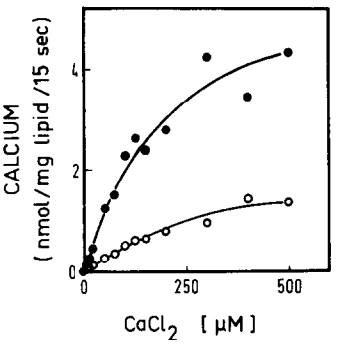

Fig. 2. Effect of $[Ca^{2+}]$ on Ca^{2+} entry into liposomes. Three-microlitre liposomes (30–60 μg soybean phospholipids) containing 0.15 M K-phosphate buffer pH 7.4 and 10 mM Tris–HCl pH 7.4 were diluted into 97 μl of external solution containing 0.3 M sucrose, 10 mM Tris–HCl pH 7.4, 20 μM valinomycin, and varying concentrations of $^{45}CaCl_2$ (about 0.1 μCi), in the presence (●) or absence (○) of 200 μM verapamil. The reaction was terminated after 15 sec as described in detail in Materials and Methods.

in the absence of valinomycin. This additional increase in the Ca^{2+} content of the liposomes is probably not an increase in the binding to the phospholipids, since addition of Triton X-100 (1%) releases all the Ca^{2+} from the liposomes (Table 1).

The effect of verapamil on the A23187 induced-negative inside membrane polarization dependent Ca^{2+} influx is concentration-dependent. This can be seen from the results of the experiments presented in Fig. 1. Three phases of the effects of verapamil on Ca^{2+} entry are displayed: Up to about 50 μM verapamil, only a small rise in Ca^{2+} entry is observed. Between 50–300 μM verapamil a steeper slope is obtained, and between 300–500 μM verapamil a plateau is reached. This type of concentration-dependence was observed with three different Ca^{2+} concentrations studied (25 μM, 50 μM and 100 μM).

The effect of external Ca^{2+} concentration on the initial rate of Ca^{2+} entry into liposomes is shown in Fig. 2. In this experiment Ca^{2+} entry was induced in the presence of the Ca^{2+} ionophore A23187 and negative inside membrane polarization. The initial rate of Ca^{2+} entry increased with increasing Ca^{2+} concentrations (open circles). Addition of verapamil (closed circles) led to an even higher increase in the rate of Ca^{2+} influx.

In native SPM vesicles the inhibitory effects of verapamil on Ca^{2+} uptake were "side-dependent" [7]. Introduction of 200 μM verapamil from the external side of the membrane had a larger inhibitory effect than when it was introduced into the vesicles. When verapamil was introduced from both sides simultaneously, the inhibitory effect was even more

Table 2. [^{3}H] TPP^+ entry into liposomes

		[^{3}H] TPP^+ uptake nmol/mg phospholipid/60 min			
		No addition	Verapamil	A23187	Verapamil +A23187
No addition	—	0.32	0.31	0.32	0.23
	Valinomycin	7.55	7.25	5.54	6.98
$CaCl_2$	—	0.41	0.31	0.35	0.29
	Valinomycin	6.32	7.11	5.25	5.07

Three-microlitre liposomes (30–60 μg soybean phospholipids) containing 0.15 M K-phosphate pH 7.4 were diluted into 97 μl external solution containing 0.3 M sucrose, 10 mM K-phosphate buffer pH 7.4 and 17.6 μM [^{3}H] TPP^+ (about 10 nCi), with addition of 50 μM $CaCl_2$ and/or 200 μM verapamil and/or 1 μM A23187 and/or 20 μM valinomycin. The reaction was terminated after 60 min as described in detail in Materials and Methods and in Table I.

pronounced. The "side-dependent" effects of verapamil were also studied in the A23187-induced Ca^{2+} entry in the liposome model. In this case, no significant "side-dependent" differences could be detected (results not shown).

Effect of verapamil on [^{3}H] *TPP$^+$ entry*

In SPM and sarcolemmal vesicles, addition of verapamil led to a considerable decrease in the negative inside membrane potential [8, 22]. In order to examine whether verapamil had an effect on the membrane polarization in the liposome model, we studied its effects on the [^{3}H] TPP^+ uptake. The distribution of the lipophilic cation TPP^+ across the liposomes' membrane at equilibrium reflects the membrane polarization. In the experiment shown in Table 2, when K-phosphate loaded liposomes are diluted into an external solution containing valinomycin, a large [^{3}H] TPP^+ influx is observed. In the absence of valinomycin, only a very small amount of [^{3}H] TPP^+ is taken up by the liposomes. The addition of Ca^{2+} and/or verapamil and/or A23187 has only a minor effect on the [^{3}H] TPP^+ content of the liposomes (Table 2). A similar pattern of results was obtained in four different liposome preparations. In these experiments, [^{3}H] TPP^+ entry in the presence of valinomycin was between 10 and 50-fold higher than in its absence.

In the experiments presented so far it was shown that A23187 induced Ca^{2+} entry into liposomes is increased by verapamil. Verapamil also retains its effect when Ca^{2+} entry is increased in the presence of a negative inside membrane polarization which is created by valinomycin. Membrane polarization itself, as measured from the distribution of [^{3}H] TPP^+, was almost unaffected by verapamil.

Binding of verapamil to SPM and liposomes

In order to study whether synaptic plasma membranes have specific binding sites for verapamil, [^{3}H] verapamil binding was measured in native membranes and in liposomes.

K_d and B_{max} values were obtained from Scatchard plots derived from saturable specific binding curves to native SPM (see Materials and Methods). The average values calculated from five different experiments were : K_d = 87.4 nM ± 21.9 (SD) and B_{max} = 2.19 pmol/mg protein ± 0.92 (SD).

[^{3}H] verapamil binding to liposomes was investigated as well (Table 3). Specific binding could not be demonstrated in the presence of 30 nM [^{3}H] verapamil, and the nonspecific binding at this concentration was 3.8 pmoles [^{3}H] verapamil/mg phospholipid (not shown). When the concentration of verapamil was increased to 200 μM a nonspecific binding of approximately 50 nmol verapamil/mg phospholipid was observed. This binding did not change with addition of Triton X-100 (Table 3). Addition of valinomycin and/or A23187 and/or Ca^{2+} did not change the amount of [^{3}H] verapamil associated with the phospholipid membrane (Table 3). This pattern was consistently observed in four different experiments performed, although the initial amount of [^{3}H] verapamil that bound to the liposomes varied between 30 and 120 nmols/mg phospholipid.

Effect of verapamil on the fluidity of the membrane

One possibility to explain the inhibitory effects of verapamil on both the Ca^{2+} influx and the [^{3}H] TPP^+ uptake of native sarcolemmal and SPM vesicles [8, 22] could be related to the effects of verapamil exerted on the fluidity of the membrane. In order to explore this possibility, verapamil-induced changes in membrane fluidity were measured by the method of polarized fluorescence in phosphatidylcholine liposomes. We have used phosphatidylcholine liposomes instead of soybean phospholipid liposomes used previously, since changes in phase transitions occur at well-defined temperatures [23]. This was done by measuring the difference in the polarization of fluorescence of DPH (used as a probe) in the presence or absence of verapamil. Figure 3 describes the fluorescence anisotropy of the probe (r) as measured from the steady state fluorescence polarization at temperatures between 18° and 42° in the presence and absence of verapamil. Verapamil itself has no intrinsic fluorescence, thus the changes measured reflect changes in DPH fluorescence only. It can be seen (Fig. 3) that no difference is obtained in the anisotropy in the presence (closed circles) and

Table 3. [^{3}H] verapamil binding to liposomes

	[^{3}H] verapamil bound nmol/mg phospholipid				
	No addition	Valinomycin	A23187	Valinomycin +A23187	2% Triton
No addition	42.4	55.2	42.5	49.7	48.1
$CaCl_2$	40.1	47.8	49.2	54.6	48.4

Three-microlitre liposomes (30–60 μg soybean phospholipids) containing 0.15 M K-phosphate buffer (pH 7.4) were diluted into 97 μl external solution containing 0.3 M sucrose, 10 mM K-phosphate buffer pH 7.4, and 200 μM [^{3}H] verapamil (about 50 nCi) with 50 μM $CaCl_2$ and/or 1 μM A23187 and/or 20 μM valinomycin. The reaction was terminated after 10 min on Sephadex G-50-80 mini-columns as described in Materials and Methods.

in the absence (open circles) of added verapamil. Consequently, the effects of verapamil can probably not be ascribed to an effect on membrane fluidity.

DISCUSSION

In this work we studied the effects of verapamil in liposomes which served as a protein-free simplified model system of the lipid membrane.

In SPM and sarcolemmal vesicles [8, 22], Ca^{2+} entry can be increased by creating a negative inside membrane potential. Addition of the Ca^{2+} ionophore A23187 to native vesicles under these conditions led to further increase in the Ca^{2+} content of the vesicles, presumably since equilibrium has not yet been reached [22]. Addition of verapamil to native vesicles led to a concentration-dependent decrease in Ca^{2+} content in parallel with a decrease in the negative inside membrane potential.

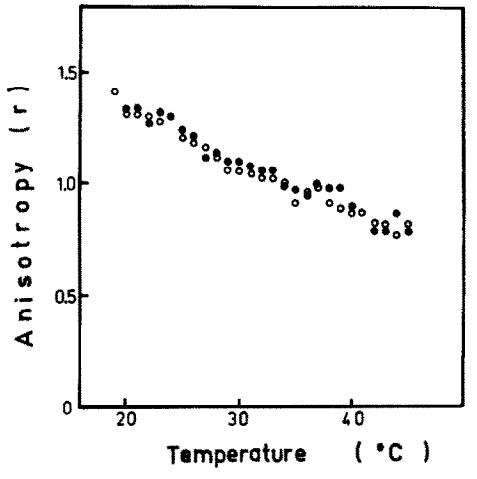

Fig. 3. Effect of verapamil on the steady state fluorescence anisotropy of DPH. The polarized fluorescence of DPH, which was excited at 360 nm, was measured at 430 nm in a medium of phosphatidylcholine liposomes (8 mg/ml) prepared with DPH (see Materials and Methods), and suspended in 0.3 M sucrose, 10 mM Tris–HCl pH 7.4, 50 μM $CaCl_2$, with 200 μM verapamil (●) or without it (○). Anisotropy (r) was calculated as follows: $r = (I_\parallel - I_\perp)/(I_\parallel + 2I_\perp)$ where r is the anisotropy of the probe, $I_\parallel$ is the vertical component of the fluorescence excited by a vertical polarized light (parallel component), and $I_\perp$ is the horizontal component of the above fluorescence (perpendicular component). The expression $F = I_\parallel + 2I_\perp$ is the total fluorescence for a rod-like molecule.

In the liposome model the effects of verapamil are considerably different. The permeability of liposomes to Ca^{2+} in the absence of Ca^{2+} ionophore is very low, and is not affected by addition of verapamil (Table 1). As also in native vesicles, increased Ca^{2+} influx can be obtained by creation of an artificially imposed negative inside membrane potential in the presence of the Ca^{2+} ionophore A23187. Verapamil, however, under these conditions, led to a considerable increase in the Ca^{2+} content of the liposomes, unlike its inhibitory effect in the native vesicles.

Another major difference was that the effect of verapamil was not dependent on the side of the membrane from which verapamil was introduced. In native vesicles the inhibitory effect of verapamil on Ca^{2+} uptake was more pronounced when verapamil was introduced from the outer face of the vesicles' membranes as compared to their inner face [7]. When introduced from the inside and outside simultaneously, inhibition was even greater. Side-dependent effects of verapamil were reported also in guinea-pig myocytes [25]. One possibility to understand this difference could be related to the well-known asymmetric architecture of the phospholipids in native membranes as compared to the even phospholipid distribution observed in large unilammelar liposomes [24]. Alternatively, the side-dependent effects of verapamil could reflect its more specific effects mediated via membrane proteins, and therefore not be manifested in phospholipid vesicles.

The effects of verapamil on membrane potential, as manifested by considerable decrease in TPP^+ content in native vesicles, could not be reproduced in the liposome model system. In liposomes, verapamil did not alter significantly the polarization of the membrane. Similar distribution of [^{3}H] TPP^+ across the liposome membrane was obtained in the presence or in the absence of verapamil. This observation was repeated both when there was no membrane polarization, or when a negative inside membrane polarization occurred due to valinomycin-induced K^+ efflux.

It is unlikely that the depolarizing effects of verapamil observed in native membrane vesicles were due to translocation of the positively charged ver-

apamil molecule itself through the lipid membrane, since (1) the content of [^{3}H] verapamil in liposomes was found to be independent of the negative inside membrane polarization, created by valinomycin-induced K^+ efflux (Table 3); (2) the depolarizing effects of verapamil could not be reproduced in the liposome model; and (3) triton treatment of the liposomes did not release the [^{3}H] verapamil associated with the liposomes.

As in contractile tissues [3–5], native synaptic plasma membranes possess specific binding sites for verapamil. The binding constants measured with [^{3}H] verapamil are of similar order of magnitude as those in skeletal muscle and cardiac tissue [3–5]. This finding is especially interesting since the effect of verapamil on the voltage dependent Ca^{2+} fluxes could be demonstrated only at much higher concentrations [12–15].

In order to separate "protein related" specific binding sites from putative phospholipid binding sites, we have studied verapamil binding to liposomes as well. [^{3}H] verapamil bound nonspecifically to the liposomes. When the added verapamil concentration was 200 μM, the binding reached about 50 nmol verapamil/mg phospholipid. This corresponds to about 20% of the total verapamil present (4.5 nmol out of 20 nmol total). A similar proportion of verapamil binding was found when 30 nM verapamil was used (not shown). This high binding of verapamil to the lipid membrane could result in a large number of nonspecific effects on the lipid bilayer. This in turn may indirectly affect the transport properties of membranal proteins. For example, verapamil could alter the fluidity of the membrane. This possibility was investigated by studying the changes in the fluidity of phosphatidylcholine liposomes. Verapamil had no effect on the anisotropy (Fig. 3). One cannot rule out though, that in a biological membrane, where the lipid composition is diverse and distributed asymmetrically, local changes in verapamil induced fluidity may occur. This might, of course, indirectly affect the microenvironment of a membrane protein.

In view of all this, it seems that the inhibition of Ca^{2+}-uptake by verapamil, and the decrease in membrane potential observed in native membrane vesicles [7, 8, 22], cannot be explained by involvement of the membrane's lipid phase only. Thus, the diverse effects of verapamil observed in a large number of biological systems would probably have to involve specific interactions with membrane proteins as well.

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