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Perturbing effects of carvedilol on a model membrane system: Role of lipophilicity and chemical structure

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Abstract

Carvedilol, a β-adrenergic blocker used to treat cardiovascular diseases, protects cell membranes from lipid peroxidative damage. Previous studies suggested the drug resides in a non-polar environment and partitions into cell membranes, perturbing their fluidity. Here differential scanning calorimetry (DSC) and fluorescence spectroscopy were applied to further investigate interactions of carvedilol with a liposome model. Results indicate the association is relatively unaffected by pH or temperature, but could be sensitive to liposome composition. The drug's carbazole group plays the dominant role in bilayer perturbation. Compared with other β-blockers examined, carvedilol produced the strongest liposome DSC perturbation. Locations of carbazole and carvedilol in the liposome were determined using depth-dependent fluorescent probes. Both compounds are situated in the middle of the bilayer, consistent with strong hydrophobic interactions. This combination of high lipophilicity and specific chemical structure appear required for carvedilol's novel antioxidant activity, and may enhance cardioprotection.

Keywords: Carvedilol; β-blocker; Antioxidant; Liposome; Calorimetry; Fluorescence

1. Introduction

β-adrenoceptor antagonists block α-1 and α-2 receptors, which are found in the smooth muscle cells in walls of the arteries and heart. Most β-blockers are classified as antihypertensive drugs, inhibiting vasoconstriction and heart rate increase. A third-generation β-blocker of current interest is carvedilol ({1-carbazolyl-(4)-oxy]-3-[2-methoxyphenoxyethylamino] propanol-(2)}), a multiple action antihypertensive agent with both β-adrenergic blocking and vasodilating activities. As a commercial product (Kredex®, Coreg®, Dilatrend®), carvedilol is used for treatment of mild to moderate hypertension as well as for chronic unstable angina and congestive heart failure [1]. Of particular interest, carvedilol has been identified as having novel antioxidant properties distinct from its β-blocking capability that may contribute to its therapeutic effects [2].

The interactions of β-blockers with biological membranes, as well as with less complex liposome models, can be probed with various biophysical techniques to yield information regarding determinants of their pharmacological efficacy [3–6]. In previous studies utilizing differential scanning calorimetry (DSC) and fluorescence spectroscopy, it was proposed that carvedilol penetrates the lipid bilayer and is situated in a non-polar environment [7,8]. In the present study, factors that could influence the association of carvedilol with the bilayer were investigated, including drug chemical structure, pH, liposome composition, and temperature. DSC was used to monitor the effects of carvedilol and structurally related compounds on liposome thermal behavior. The DSC characteristics of the liposome model in the presence of carvedilol were also compared with those produced by other β-blocker drugs. Additionally, the drug location in the bilayer was studied with depth-dependent fluorescent probes, allowing assessment of the energy transfer between drug and probe. The experimental

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results are discussed with respect to carvedilol's antioxidant capability and potential therapeutic benefits.

2. Materials and methods

2.1. Chemicals

Carvedilol (I), a metabolite of carvedilol, (II); and a non-carbazole carvedilol fragment analog, (III), were obtained from GlaxoSmithKline. Carbazole (IV) was obtained from Aldrich Chemicals (Milwaukee, WI). The structures of carvedilol and related compounds are shown in Fig. 1. The phospholipids dimyristoylphosphatidylcholine (DMPC) and the sodium salt of dimyristoylphosphatidylglycerol (DMPG), as well as the compound stearylamine (SA), were obtained from Avanti (Pelham, AL), stored at −20 °C, and used without further purification. The fluorophores 2-(9-anthroyloxy) stearic acid (2-AS), 3-(9-anthroyloxy) stearic acid (3-AS), 6-(9-anthroyloxy) stearic acid (6-AS), 9-(9- anthroyloxy) stearic acid (9-AS), and 12-(9-anthroyloxy) stearic acid (12-AS) were obtained from Molecular Probes (Eugene, OR). The β-blocker drugs propranolol, timolol, pindolol, and atenolol were obtained from Sigma (St. Louis, MO), and used without further purification. All other chemicals were reagent grade.

2.2. Preparation of liposomes

The procedure used for preparing the liposomes was similar to that described in the literature [9]. Predetermined amounts of the liposome constituents with or without the drug were dissolved in chloroform; unless otherwise stated, liposomes in a molar ratio of 90% DMPC and 10% DMPG were prepared. Carvedilol was first dissolved in methanol and added to the chloroform solutions. In studies utilizing AS fluorescent probes, the probes were added to the liposome suspension from a stock solution of ethanol. The liposome solutions were vortexed and the solvent was evaporated with a stream of nitrogen, producing a thin white film in the test tube. The tubes were stored in a vacuum desiccator overnight to remove any traces of solvent. Samples were then rehydrated with 2 ml of phosphate-buffered saline (PBS), pH 7.4, and incubated at 37 °C for 10 min, to allow hydration of the lipids above their phase transition temperatures. 0.05 M sodium acetate and sodium carbonate buffer solutions were used for studies carried out at pH 5 and 10, respectively.

The liposomal solutions were then vortexed for 3 min; the incubation/vortex process was repeated twice. The resulting multilamellar vesicles (MLVs) were used for DSC studies as well as for the preparation of small unilamellar vesicles (SUVs). The latter, intended for use in the fluorescence binding studies, were created by sonication of the MLV solutions using a high bath sonicator (Model G112SP, General Laboratory Supplies Co., Hicksville, NY). A model PCS-4700 photon correlation spectrometer (Malvern Instruments, Southborough, MA) equipped with a Spectra Physics Argon laser was used to determine the average size of the liposomes and to ensure the batch-to-batch reproducibility.

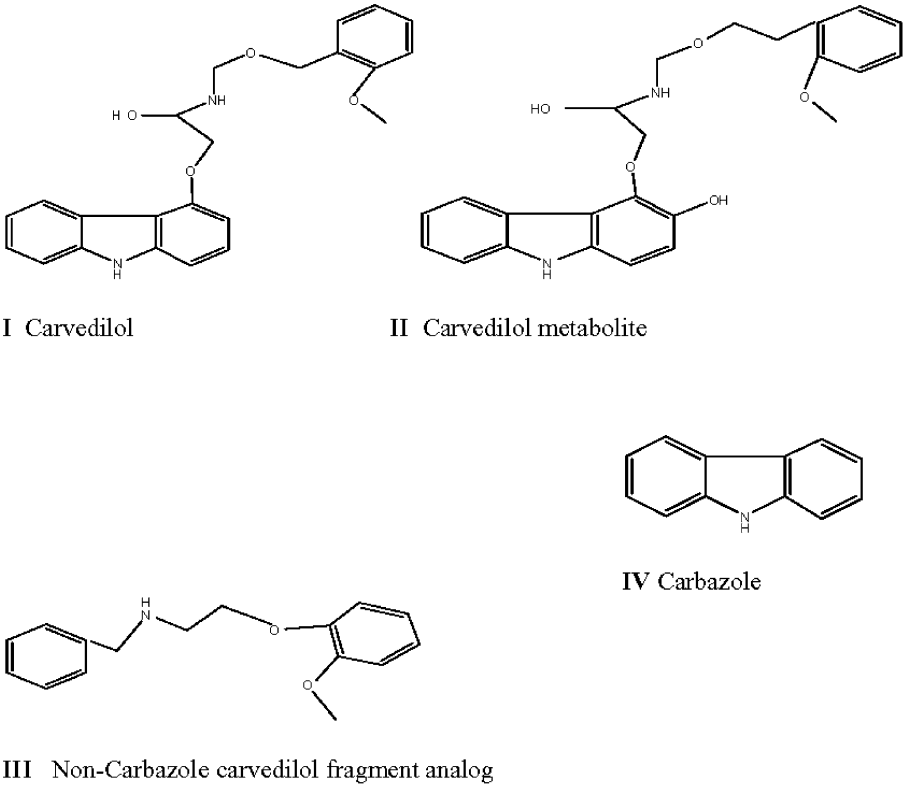


Fig. 1. Structures of carvedilol and related compounds I–IV.

2.3. Differential scanning calorimetry

DSC studies were carried out with a Microcal MC-2 Microcalorimeter (Amherst, MA). Samples were scanned from 5 to 40 °C at a scan rate of 20 °C/h using PBS in the reference cell. After the initial heating and cooling, samples were rescanned to check reversibility. Data were analyzed using ORIGIN software provided by Microcal. The drug, liposome, and fluorescent probe concentrations are as stated in the text and figure captions.

2.4. Determination of drug–liposome binding

Fluorescence experiments were carried out using a Model LS-100 Luminescence Spectrophotometer (Photon Technology International, South Brunswick, NJ). All measurements were carried out in 1 cm thermostatted cuvettes. For the drug–liposome binding studies, the emission spectra of the supernatant remaining after centrifugation were recorded from 335 to 375 nm, with the excitation wavelength at 285 nm and monochromator slits set at 3 nm. For the energy transfer studies in the absence of probe, emission spectra were recorded from 335 to 400 nm; here the excitation wavelength was set at 325 nm in order to minimize liposome interference occurring when carvedilol is excited at lower wavelengths.

For drug–liposome binding studies, DMPC/DMPG solutions containing a fixed total drug concentration of 5 μM and with lipid concentrations between 0.35 and 3.0 mM were prepared as outlined previously in Section 2.2. The resulting solutions were incubated at 37 °C for 2 h and transferred to micro-ultracentrifuge tubes. Separation of the liposomes from the aqueous phase was achieved with a Model TL-100 Ultracentrifuge (Beckman Instruments, Fullerton, CA) at 50,000 rpm with rotor model TLA 100.2 for 2 h at room temperature. The drug concentration in the supernatant was determined from its intrinsic fluorescence; the amount of drug bound was calculated from the difference between the fluorescence of the initial 5 μM drug solution and the measured fluorescence of the supernatant after centrifugation. The binding constant K was analyzed using a double reciprocal plot of the data. A plot of 1/(fraction bound) vs. 1/[lipid concentration] yields a straight line of slope $1/K$; therefore $1/\text{slope} = K$.

2.5. Fluorescence energy transfer experiments

For the fluorescent energy transfer (FRET) experiments, SUV liposomes were prepared as described previously, but with the added incorporation of one member of the family of the of n-(9-anthroyloxy) stearic acid (AS) probes ($n = 2, 3, 6, 9, 12$). In the absence of carvedilol or carbazole, probe emission was scanned from 400 to 625 nm with the excitation wavelength set at the absorption maximum of the probe, 360 nm. When drug was present, probe emission was scanned in the range of 335–625 nm, with the probe excitation wavelength set at 325 nm; probe fluorescence emission was measured at 470 nm. In these experiments, the AS probes served as the acceptor species, while carvedilol or carbazole acted as the donor; the acceptor concentration was varied while the donor concentration remained constant. Enhancement of AS fluorescence at 470 nm served as the indicator of FRET between drug and liposome probe.

3. Results

3.1. DSC studies of carvedilol–liposome interactions

In the absence of carvedilol, MLVs containing DMPC/DMPG in a 9:1 molar ratio exhibit a DSC pre-transition event at 15 °C, Fig. 2-a. This feature has been associated variously with non-specific head group interactions, or with a change in head group orientation [10]. The sharper, more prominent endotherm ca. 24 °C is the main melting transition (T_m), characteristic of the DMPC/DMPG liposome in the absence of drug. This thermal event represents the gel to liquid crystalline transition of the bilayer, the point at which the alkyl chains become disordered and the membrane fluidity increases. Changes in the liposome DSC endotherms occurring in the presence of carvedilol are shown in Fig. 2. As more carvedilol is introduced, the liposome pre-transition disappears, the T_m is decreased, and the main melting endotherm broadens. However, the enthalpy value of the main phase transition (ΔH_m) is essentially unaffected by the drug's presence and remains close to that of the pure DMPC/DMPG (9:1) liposome, ca. 4.5 kcal/mol, throughout the drug concentration range. At the highest carvedilol concentration studied, Fig. 2-d, there is evidence of asymmetry in the liposome melting endotherm which may reflect the onset of phase separation.

Calorimetric data on liposome behavior with carvedilol-related compounds were also generated. In particular, DSC data for carbazole (IV), the non-carbazole carvedilol fragment analog (III), and a carvedilol metabolite (II) were obtained as a function of their concentrations in the same lipid bilayer. The effects on liposome thermal behavior due to increasing concentrations of these carvedilol-related compounds are

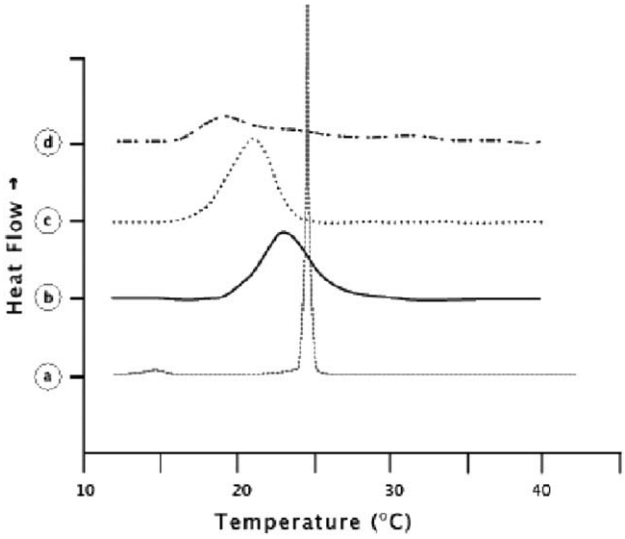


Fig. 2. DSC thermograms of DMPC/DMPG liposomes, 2.95 mM, as a function of carvedilol concentration: a) no drug, b) 50 μM, c) 100 μM, d) 200 μM.

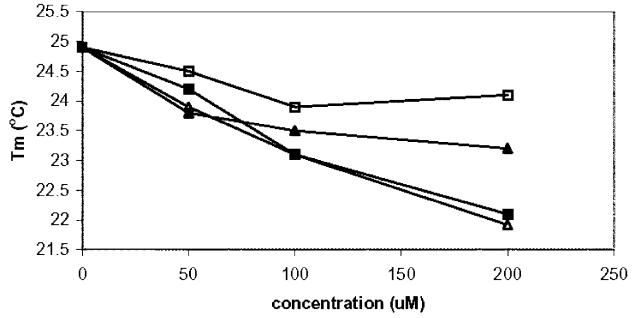


Fig. 3. T_m values of DMPC/DMPG liposomes, 2.95 mM, with carvedilol and structurally related compounds (I–IV) at drug concentrations of zero, 50, 100, and 200 μM. (□): III, (▲): IV, (■): I, (△): II.

shown graphically in Fig. 3. The extent of observed decrease in liposome T_m can be summarized as carvedilol metabolite (II)=carvedilol (I)>carbazole (IV)>non-carbazole carvedilol fragment analog (III). Carvedilol and its metabolite thus had the greatest effect on the bilayer; i.e., they caused the greatest depression of the liposome T_m as their concentrations increased. The least effect on liposome thermal properties was observed with the non-carbazole carvedilol fragment analog.

To better understand the role of drug ionization in the carvedilol–liposome association, the medium pH was varied from pH 5 to 10. Altering pH in this range will affect the charge state of carvedilol, which has a pKa of 8.0 [11]: at pH 7.4, the drug has a net positive charge, and becomes even more positive at pH 5. In contrast, carvedilol is uncharged at pH 10. Because the DMPC/DMPG (9:1) model system has a net negative charge, it is possible that there might be a difference in the carvedilol–liposome interaction related to the drug's ionization state. However, that does not seem be the case here: although T_m decreased and the melting endotherm broadened upon the incorporation of carvedilol at all three pH conditions, their DSC perturbing effect on the membrane model was essentially the same, i.e., the extent of perturbation appears to be independent of solution pH.

3.2. Carvedilol–liposome binding

A more quantitative assessment of pH effects was carried out using the fluorescence binding assay described previously. Carvedilol contains the moiety carbazole, which has an intrinsic fluorescence spectrum resulting in emission maxima at 345 and 355 nm when excited near its absorption maximum of 325 nm [12]. As previously demonstrated [7], carvedilol fluorescence can be used to measure the amount of drug associated with the liposome. The fluorescence intensity of the supernatant following centrifugation of the drug–liposome samples will decrease compared with the original carvedilol fluorescence if some of the total drug remains associated with the liposome. In the case of carvedilol, double reciprocal plots of the bound drug fraction versus liposome concentration yielded binding constant values of 2500, 2000, and 2000 M^{-1} for the solutions at pH 5, 7.4, and 10, respectively. Results from the binding studies are thus consistent with the corresponding DSC results, i.e., the carvedilol–liposome binding appears relatively insensitive to pH, being only slightly enhanced when the drug is totally protonated.

In addition to varying solution pH, small changes in the lipid composition of the bilayer were made alter polarity of the liposome head group. The DMPC/DMPG 9:1 system, which was used for most of our experiments, carries a net negative charge due to the DMPG component. To make the net charge zero, a bilayer was prepared using only DMPC. DSC thermograms of the two liposome systems with and without carvedilol give very similar results (not shown); their T_m values are essentially identical. Incorporation of 100 μM carvedilol into either bilayer results in the same T_m depression and endotherm broadening. However, in the case of incorporation of positively charged stearylamine (SA), the observed thermal behavior of the drug–liposome system was much more visibly affected. Addition of SA increased the T_m of the resulting liposome compared with pure DMPC or DMPC/DMPG. Upon increasing the carvedilol concentration at pH 7.4, where the

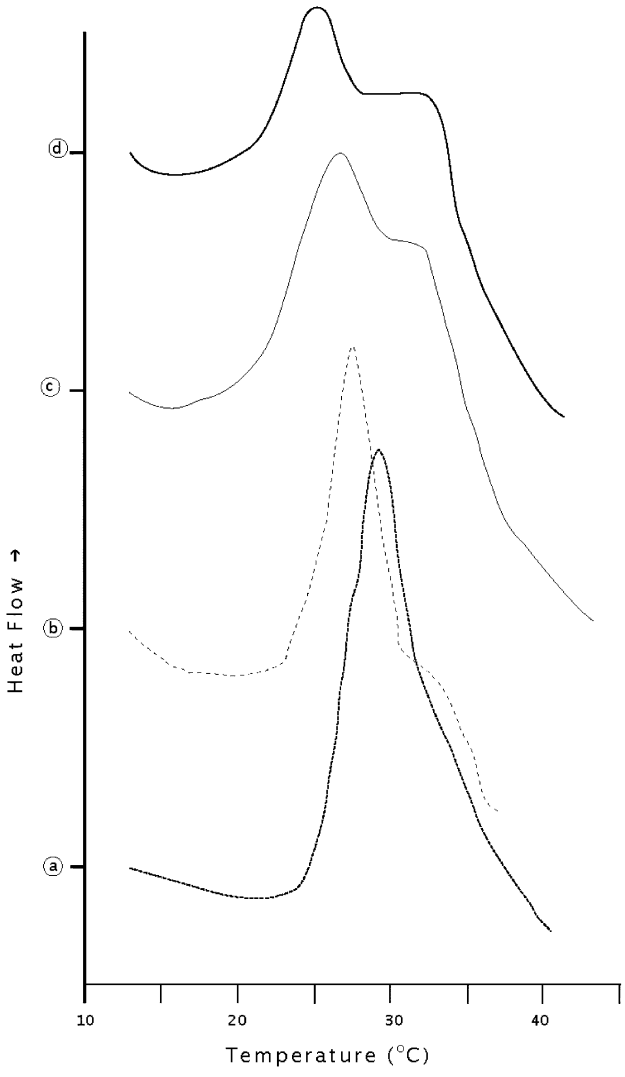


Fig. 4. DSC thermograms of DMPC liposomes containing 20 mol% SA as a function of carvedilol concentration. a) No drug, b) 50 μM, c) 100 μM, d) 200 μM.

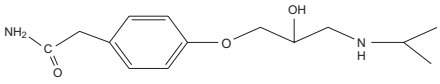
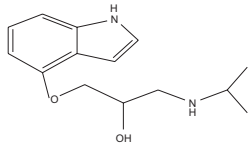
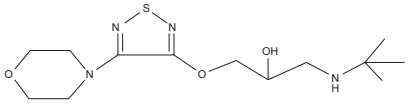
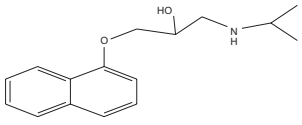
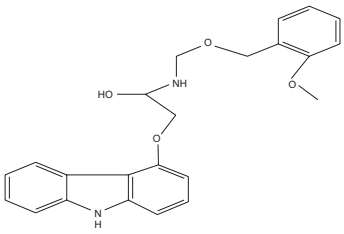
drug is positively charged, the T_m of the DMPC/SA liposome first increased slightly while the endotherm appeared to split, as shown in Fig. 4.

Since the calorimetric studies show that carvedilol lowers the T_m and broadens the melting endotherm of the DMPC/DMPG bilayer in its gel state, it was also of interest to determine whether the drug bound more strongly to the bilayer above its T_m, where the membrane is expected to be more fluid. The carvedilol–liposome binding constants determined by fluorescence gave values of 3270 and 4110 M^{-1}, at 20 and 37 °C, respectively, suggesting that carvedilol interacts to only a slightly greater extent above the liposome T_m than below it.

3.3. DSC studies of liposomes with other β-blockers

The interactions of liposomes with other β-blockers, i.e., propranolol, pindolol, timolol, and atenolol, the structures of which are shown in Table 1, were examined for comparison with carvedilol. The effects of each β-blocker on the DSC behavior of the DMPC/DMPG model are illustrated in Fig. 5. Of the β-blockers examined, only carvedilol and propranolol show significant ability to broaden the melting endotherm and to lower T_m; note that both endotherms exhibit asymmetry at the highest drug concentration, Fig. 5-a. Furthermore, carvedilol has a greater effect on the liposome T_m at higher drug concentrations, Fig. 5-b.

Table 1
Structures of β-blockers and corresponding calculated partition coefficient (log *P*) values [19]

Beta-blocker	log *P* [19]
	Atenolol 0.33
	Pindolol 1.86
	Timolol 1.06
	Propranolol 3.00
	Carvedilol 3.91

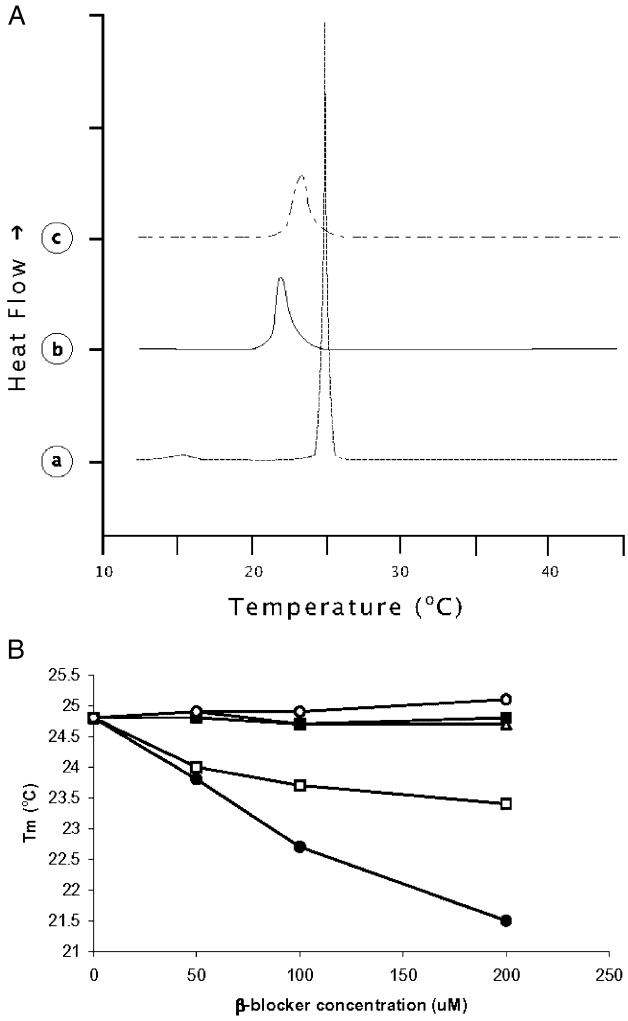


Fig. 5. A. DSC thermograms of DMPC/DMPG liposomes, 2.95 mM with selected β-blockers. a) Liposome alone, b) with carvedilol, 100 μM, c) with propranolol, 100 μM. B. T_m values of DMPC/DMPG liposomes, 2.95 mM, with different β-blockers at concentrations of zero, 50, 100, and 200 μM. (>): atenolol, (■): timolol, (△): pindolol, (□): propranolol, (⋆): carvedilol.

3.4. Fluorescence energy transfer studies

To gain additional understanding of carvedilol's location with respect to the bilayer, fluorescence energy transfer (FRET) was applied. Here carvedilol acts as the donor, utilizing its intrinsic fluorescence. While liposomes do not naturally fluorescence, various fluorescent compounds can be introduced as bilayer probes; in this case, different n-AS probes were used, and served as the acceptor species. The AS probes are distinguished by their location in the bilayer, i.e., 2-AS is nearest the head group, while 12-AS penetrates the most deeply into the hydrophobic region.

The purpose of these experiments is to assess the position of carvedilol (donor) in the bilayer from the ability of a given AS probe (acceptor) to quench donor fluorescence. However, certain experimental criteria must be satisfied to obtain meaningful results. First, it is necessary to establish the maximum amount of drug that can be incorporated into the bilayer. Our previous fluorescence binding studies [7] showed

that in order to insure complete incorporation of carbazole or carvedilol into the liposome, the liposome to drug concentration ratio must be >50; this condition was used in all FRET experiments.

It is also important to confirm that the probe is completely incorporated into the liposome. Therefore, AS fluorescence was studied as a function of the lipid/probe concentration. It was found that all of the probes are completely incorporated into the liposome above a lipid/probe ratio of 20. Thus, a lipid/probe ratio above 20 was used in all experiments, insuring that the observed fluorescence originated from the lipid bilayers and not from the aqueous solution.

Finally, it necessary to investigate whether all of the probes partition in the bilayer to the same extent, and to determine whether their fluorescence properties are the same as in a homogeneous solution such as methanol. Control experiments

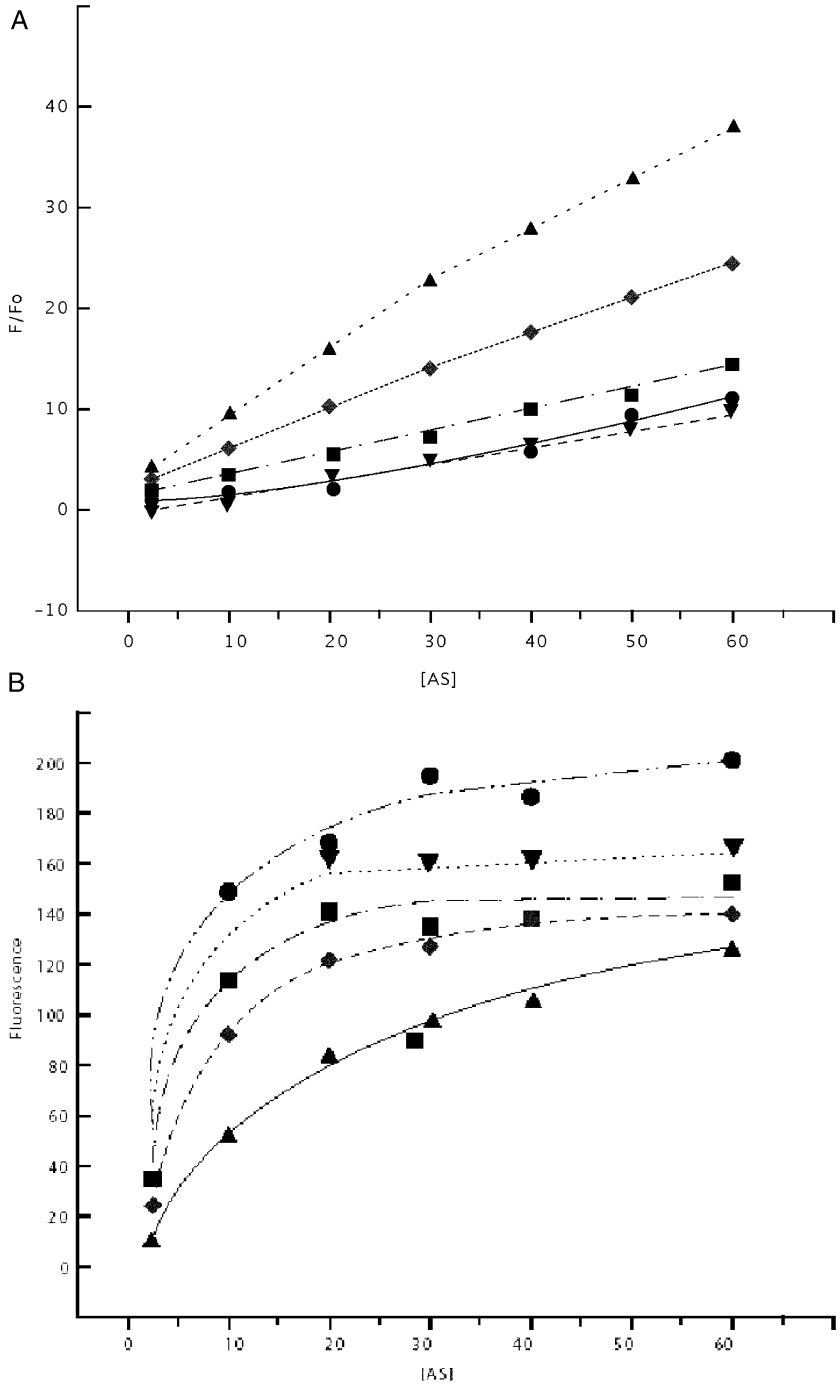


Fig. 6. A. Fluorescence enhancement of AS probes with carbazole present, in methanol. F_0 is AS fluorescence in the absence of carbazole; F is measured AS fluorescence with carbazole present. Excitation wavelength: 280 nm; emission wavelength: 470 nm; carbazole concentration, 4.8 μM. (▲): 2-AS, (♦): 3-AS, (■): 6-AS, (▼): 9-AS, (⋆): 12-AS. B. Fluorescence enhancement of AS probes with carbazole present, in aqueous DMPC/DMPG solution. *Y*-axis gives AS intensity in arbitrary units. Other conditions and labels are as stated for a.

for the quenching of carbazole by a series of AS probes in methanol are shown in Fig. 6A; carvedilol quenching (not shown) is essentially identical. Although probes 6, 9, and 12 act similarly in methanol, probes 2 and 3 are quenched more effectively. In contrast, Fig. 6B illustrates the behavior of carbazole and AS probes in the DMPC/DMPG liposome environment; carvedilol behavior (not shown) is very similar albeit with slightly lower fluorescence intensity values. For both compounds, the observed quenching efficiency of the AS probes in the liposome was in the order of 12>9>6>3>2. While it must be taken into account that the AS probes do not all quench with the same efficiency in a homogeneous solution such as methanol, the effect of applying this correction would actually make the observed quenching order in the bilayer even more pronounced.

4. Discussion

DSC has been well established as an indicator of drug interactions with the lipid bilayer. Of particular significance is the temperature at which the gel to liquid crystalline transition takes place, T_m. Solutes penetrating the bilayer generally cause a decrease in T_m, along with noticeable transition peak broadening [13]. A strong interaction between drug and liposome will likely be indicated by a greater decrease in T_m; in some instances this can be correlated with drug potency [14]. The lack of change in ΔH_m concomitant with the observed T_m lowering and endotherm broadening is also significant. This can be interpreted in terms of the drug forming an ideal solution with the liposome, consistent with deep penetration into the bilayer [15].

The present DSC data suggest that carvedilol fluidizes the liposome bilayer, and that the carbazole moiety plays the dominant role in this interaction. The carvedilol fragment analog lacking a carbazole group (III) represents the most hydrophilic portion of the molecule, and the DSC results indicate that this portion of the drug is probably excluded from the hydrophobic region of the liposome bilayer. Carvedilol (I) and its metabolite (II), which are structurally very similar, have approximately the same effect on the bilayer melting transition; this perturbation is greater than that observed for carbazole (IV), which is also hydrophobic but smaller in size.

It is possible that both hydrophobic interactions within the bilayer and hydrophilic interactions involving the bilayer headgroups could contribute to carvedilol's interaction with the DMPC/DMPG liposomes. However, both DSC and fluorescence binding studies indicate that altering the drug pH has little effect on the carvedilol–liposome interactions. This is a contrast to other cationic amphiphiles such as propranolol, where pH clearly can modulate the extent of liposome association [16]. Electrostatic effects would thus appear to be less important to the carvedilol–liposome association, and more influenced by hydrophobic effects.

In addition to pH, the effect of liposome composition was explored. There is considerable evidence that many cationic amphiphiles, including β-blockers, interact preferentially with anionic liposomes, and the extent of such interactions can be manifested in the DSC behavior [17]. In the case of carvedilol, replacing the relatively small amount of negatively charged DPPG component with DMPC did not have an obvious effect on liposome DSC behavior. On the other hand, adding a small amount of a positively charged SA to the liposome model causes some striking differences in thermal behavior. In the absence of carvedilol, the liposome T_m is increased, consistent with rigidification of the bilayer due to the presence of SA [18]. Furthermore, upon addition of carvedilol, two distinct endotherms are observed, which may signify the existence of separate domains. While interactions between carvedilol and the liposome appear to be dominated by hydrophobic effects, the addition of SA would presumably weaken any existing interactions of protonated drug with the liposome surface due to charge repulsion. The ability of SA to rigidify the bilayer is also expected to make drug penetration more difficult.

The relatively weak dependence of carvedilol–liposome binding on temperature suggests the drug effect is similar in either the gel or liquid crystalline state of the liposome model. This is a contrast to other compounds such as propranolol, which is reported to have a markedly greater association with DMPC liposomes in the liquid crystalline phase [3].

As can be seen from Table 1, the β-blockers chosen for comparison with carvedilol differ greatly in their partition coefficients [19], and the differences are manifested in liposome DSC effects. Carvedilol and propranolol are the most lipophilic and would thus be most likely to penetrate the bilayer, resulting in obvious alterations of liposome DSC behavior. The thermal behavior of liposomes treated with propranolol observed here agrees with that observed previously under similar conditions [3,4,6]. However, the DSC data also suggest that carvedilol behaves differently than propranolol, perhaps reflecting the former's higher partition coefficient. At lower drug concentrations, carvedilol and propranolol appear to fluidize the bilayer in a similar manner; however, as the drug concentration is increased, carvedilol produces a stronger effect. It has been shown that an increase in drug hydrophobicity can shift its dose-effect curve to lower molar ratios and enhance the lowering of T_m [20]. This could explain the stronger perturbing ability of carvedilol in the concentration range used here.

In contrast to carvedilol and propranolol, atenolol is hydrophilic, and is not expected to penetrate the bilayer. Timolol and pindolol fall into an intermediate range; while less hydrophobic than propranolol, they are more lipid-soluble than atenolol, and some evidence exists for their interactions with the bilayer. In the case of timolol, this interaction is significantly weaker than that of propranolol, the former requiring markedly higher concentrations of the drug to observe any kind of perturbing effect [3]. Also, the timolol–membrane association appears to be dissimilar and more complex than that of propranolol, with the two drugs positioned differently within the bilayer. With pindolol, there is no evidence of significant bilayer penetration in the drug concentration range employed here, in agreement with an earlier report [4]; much higher concentrations of pindolol are

required to observe any DSC changes. Although pindolol, like propranolol, has "membrane stabilizing" capability at high drug concentrations, that effect is clearly distinct from the membrane fluidizing effects demonstrated here by much lower concentrations of propranolol and carvedilol.

To gain a better understanding of carvedilol's location in the liposome, depth-dependent fluorescent probes were utilized. The probes were tagged with an anthroate fluorophore at various positions along the acyl chain, which allowed monitoring of fluorescence energy transfer (FRET) between drug and probe. The fluorescence properties of AS probes have been well characterized [21], and they have proven informative in locating drugs within lipid environments [22,23]. Moreover, energy transfer between AS probes and carbazole has been previously demonstrated [24]. Such energy transfer can be monitored either by the quenching of the donor [22], or by fluorescence enhancement of the acceptor [23]. For carvedilol and carbazole, the former approach proved to be less reliable; FRET was thus monitored by measuring the intensity enhancement of the acceptor (AS) at a fixed donor concentration. The FRET results obtained here with the depth-dependent AS probes appear consistent with the DSC results, suggesting carvedilol has strong bilayer penetration but weak interactions with head groups.

The antioxidant capability of β-blockers such as carvedilol has been linked to membrane stabilizing activity (MSA). This activity is characteristic only of certain β- blockers, with lipophilicity and β_1-blockade ability being key prerequisites [25]. The clinical relevance of MSA has been questioned in the past, in part because of the relatively high drug concentrations needed to produce a therapeutic effect. However, it has also been pointed out that lipophilic drugs may exist at high local concentrations within the membrane, allowing manifestation of some MSA effects.

Of the β-blockers studied here, only carvedilol and propranolol have demonstrated convincing concentration-dependent antioxidant capability in vitro and in vivo, with carvedilol appearing to be more potent [26,27]. Both compounds are very lipophilic and capable of perturbing the bilayer, which is reflected in DSC changes. Both compounds also produce hydroxylated metabolites with very potent antioxidant activities, which may confer in vivo benefits [8,28,29].

While lipophilicity facilitates compound perturbation of the bilayer, specific chemical structures are also required for optimal antioxidant potency. In the case of propranolol, this would involve the naphthalene ring; for carvedilol, that structure would be the indole-derived carbazole group, which has been linked to many neuroprotective effects [30]. While pindolol also contains an indole ring, it apparently lacks the requisite hydrophobicity of the other two drugs and fails to alter DSC behavior. Although carvedilol (I) and its metabolite (II) exhibit similar behavior in the DSC here, II is known to be a much more potent antioxidant [8]. Presumably this difference is due to the hydroxylation of the carbazole ring in the metabolite, which allows stabilization of the oxyl radical species [31]. X-ray scattering studies comparing I and II suggest that the latter is capable of additional bilayer perturbation [8] although this difference is not obvious in the concentration range of our DSC studies. Furthermore, there is new evidence that differential membrane interactions of I and II are involved in mitochondrial function [32]. On the other hand, previous studies with 4-hydroxy carbazole suggest that hydroxylation of the carbazole ring by itself is not enough to confer good antioxidant activity [7]; again, molecular size seems to be important for optimal effectiveness.

As an antioxidant, carvedilol's mechanism of action is not well understood, but it appears to be different from free radical scavengers such as α-tocopherol [33]. Whether the membrane stabilizing/antioxidant capability of β-blockers like carvedilol is directly linked to in vivo cardioprotective effects remains unclear at present. In vitro, however, the antioxidant capability of carvedilol and related structural metabolites seem to be an important contributor to the efficacy of this drug in cardioprotection. This capability appears to require strong hydrophobic interactions between the drug and lipid bilayer, as well as the presence of carbazole group in the drug. Additional studies involving carvedilol-related compounds and different types of phospholipids are expected to provide more details about these interactions.

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