Inhibition of Protein Kinase C by Cationic Amphiphiles†

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ABSTRACT: A large number of PKC inhibitors are positively charged. We evaluated the structural features of cationic amphiphiles which are necessary for inhibiting PKC. Many of these compounds were derivatives of cholesterol, which possesses a hydrophobic backbone which does not perturb hydrocarbon packing in membrane bilayers. In addition, they contain a tertiary or quaternary nitrogen functionality in the head group. All designed cholesterol-based amphiphiles inhibit PKC activity; the potency of the amphiphile correlates with the presence of positive charge. Quaternary ammonium amphiphiles are 10-fold more potent than their tertiary amine counterparts, generally inhibiting in the 10–60 μM range using the Triton mixed micelle assay. Aside from charge, factors such as the structure of the amine-containing head group, its length from the hydrocarbon moiety, or the number of amine groups on the amphiphile did not markedly influence inhibitor potency. In contrast, the hydrocarbon backbone did influence potency: cationic amphiphiles containing a steroid backbone were more potent inhibitors of PKC than their straight-chain analogues. Changing the nature of the hydrocarbon from a sterol to an alkyl group lowers the pK of the amine head group so that the straight-chain analogues are no longer cationic in the conditions in the PKC assay. The results of these studies suggest that a combination of positive charge and a bilayer-stabilizing structural characteristic provides a basis for the rational design of PKC inhibitors.

Protein kinase C (PKC) has gained considerable attention because it is thought to play a regulatory role in a number of diverse physiological events such as exocytosis, neurotransmission, growth, and differentiation (Nishizuka, 1986), as well as pathological processes such as tumor promotion (Housey et al., 1988; O'Brian et al., 1988; Persons et al., 1988; Weinstein, 1988), the P-glycoprotein type multidrug resistance (MDR)[1] (Fine et al., 1988; Posada et al., 1989; Chambers et al., 1990; Sato et al., 1990; Dong et al., 1990), and HIV entry into cells (Fields et al., 1988). As a consequence of PKC's involvement in a number of biological phenomena, there has been an interest in the study of the properties of currently available PKC inhibitors as well as efforts to design novel inhibitors (Hidaka & Hagiwara, 1987; O'Brian et al., 1988; Weinstein, 1988; Gescher & Dale, 1989; Marasco et al., 1990; O'Brian et al., 1990).

PKC is a family of structurally related protein kinases, many of which are dependent on Ca^{2+} and phospholipid for activity. Although the enzyme can be found in the cytosol or bound to the cell membrane, it is most active when associated with a lipid bilayer. The activity of PKC has been shown to be affected by compounds which alter the bulk biophysical properties of a membrane (Epand & Lester, 1990). If only uncharged and zwitterionic compounds are considered, then those which activate PKC are hexagonal-phase promoters (Epand, 1987). In contrast, uncharged and zwitterionic compounds which are bilayer stabilizers (hexagonal-phase inhibitors) inhibit PKC (Epand, 1987; Epand et al., 1988, 1989, 1991). In addition, other factors such as charge play an important role in modulating PKC activity (Epand, 1987; Epand et al., 1988, 1991; Bottega et al., 1990). A number of positively charged compounds have been shown to inhibit polycationic substrate phosphorylation by PKC regardless of a compound's ability to modulate membrane biophysical properties (Epand, 1987).

Since both bilayer stabilizers and cationic drugs inhibit PKC (Epand, 1992), we attempted to combine both of these characteristics to enhance inhibitor potency. Our design strategy focuses on the use of a steroid backbone which possesses a compact fused ring arrangement. This allows for facile transfer of the drug from the aqueous phase to the membrane phase, where PKC is most active. Furthermore, the fused ring system of the steroid is planar; this avoids introducing hydrocarbon splay and therefore prevents expansion of the center of the lipid bilayer. Consequently, hexagonal phase formation is less likey to occur. Combining this feature with a large polar head group possessing amino functionalities generates a cationic amphiphile with a large ratio of head group to hydrocarbon cross-sectional area. This gives the molecule the property of being a good bilayer stabilizer. We compare the efficacy of cationic amphiphiles possessing a steroid with those containing a straight alkyl chain group as the hydrocarbon backbone on the activity of PKC. We also demonstrate that combining both bilayer-stabilizing ability and positive charge provides a basis for the rational design of novel PKC inhibitors.

EXPERIMENTAL PROCEDURES

Materials

Bovine brain phosphatidylserine was obtained from Avanti Polar Lipids, Alabaster, AL. *sn*-1,2-diolein was purchased from Nu Chek Prep, Inc., Elysian, MN. Phenylmethanesulfonyl fluoride (PMSF), histone III-S, *trans*-tamoxifen, and polylysine–agarose were obtained from Sigma Chemical Co., St. Louis, MO. DEAE-Sepharose fast flow, phenyl-Sepharose

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[1] Abbreviations: ACS, aqueous counting scintillant; CPD, cholesterylphosphoryldimethylethanolamine; DAG, *sn*-1,2-diacylglycerol; DEPE, 1,2-dielaidoyl-*sn*-glycero-3-phosphoethanolamine; DSC, differential scanning calorimetry; H_{II}, inverted hexagonal phase; T_h, bilayer–hexagonal phase transition temperature; HIV, human immunodeficiency virus; L_α, liquid crystalline lamellar phase; MDR, multidrug resistance; PMSF, phenylmethanesulfonyl fluoride; PS, phosphatidylserine.

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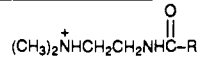
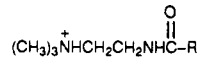
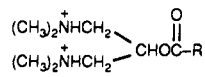
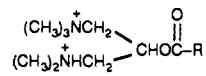
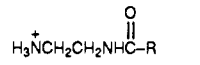
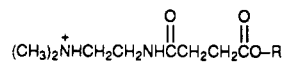
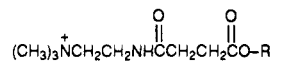
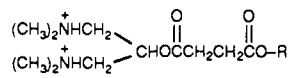
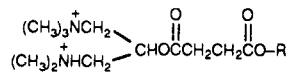
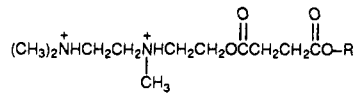
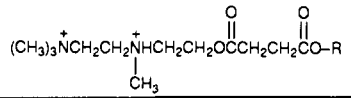
Table I: Effect of Cationic Steroids on the $L_\alpha \rightarrow H_{II}$ Transition Temperature of DEPE and Their Potency in the Inhibition of PKC in a Triton Mixed Micelle Assay Containing Calcium, Phosphatidylserine, and Diolein

	amphiphile[a]	slope (K/mol fraction)	IC_{50} (μM)
III		147 ± 7	258
IV		159 ± 24	12
V		52 ± 5	643
VI		239 ± 7	11
VII		44 ± 6	246
VIII		57 ± 5	191
IX		203 ± 10	59
X		126 ± 4	408
XI		244 ± 9	15
XII		136 ± 4	164
XIII		190 ± 7	20

[a] R is the cholesteryl backbone without its OH group.

CL-4B, and Sephacryl S-200HR were obtained from Pharmacia-LKB. Leupeptin was from IAF Biochem. Int., Montreal, Quebec, and [γ-^{32}P]adenosine 5′-triphosphate was from NEN, Quebec. Cationic amphiphiles were synthesized as described in the supplementary material (see paragraph at end of paper). The structures of these amphiphiles are given in Table I. The amphiphiles are prepared as chloride or iodide salts. Using ion exchange or testing the effects of sodium halides, we could demonstrate that the halide anion did not effect lipid polymorphism or PKC activity at the low concentrations used for the amphiphiles.

Methods

Isolation of Protein Kinase C. Protein kinase C was purified from rat brain to near homogeneity by the procedure previously described by Huang et al. (1986) using chromatography on DEAE-Sepharose, phenyl-Sepharose, Sephacryl-S200 HR, and polylysine–agarose. After eluting from polylysine–agarose, the uncontaminated fractions containing PKC were pooled. The high salt concentration was removed using Amicon YM-10 ultrafiltration, concentrated, frozen in liquid nitrogen, and stored at −80 °C. Full activity was regained after rapid thawing. Trace impurities (116K, 66K, and 50KM_r) could still be detected when the gel was heavily silver stained. The enzyme gave a specific activity of 200 nmol of phosphate incorporated per minute per milligram of protein when assayed for histone phosphorylation using the Triton mixed micelle assay with 6.5 mol % phosphatidylserine, 2.5 mol % DAG, and 100 μM calcium present. Specific activities ranging from 30 nmol/(min·mg) (Hannun et al., 1985) to 600 nmol/(min·mg) (Hannun & Bell, 1988; Loomis & Bell, 1988) have been observed for PKC using the Triton mixed micelle assay under the same conditions.

Mixed Micelle Assay of Protein Kinase C. The Triton X-100 assay previously described by Bell and co-workers was used to measure enzyme activity (Hannun et al., 1985). Phosphatidylserine and 1,2-diolein with and without additive were dissolved in a solution of chloroform/methanol (2:1 v/v). Solvent was evaporated with a stream of nitrogen, and last traces were removed using a vacuum desiccator at 40 °C. The lipid films were then solubilized by the addition of 3% Triton X-100, vortexed vigorously for 30 s, and then incubated at 30 °C for 10 min to allow for equilibration. A 25-μL aliquot of this solution was used in a final assay volume of 250 μL, containing 20 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 200 μg/mL histone III-S, 100 μM $CaCl_2$, 10 μM [γ-^{32}P]adenosine 5′-triphosphate, and 2.75 mM Triton X-100, with 300 μM (6.5 mol %) phosphatidylserine and 107 μM (2.5 mol %) 1,2-diolein. For controls, 25 μL of 20 mM EGTA replaced the $CaCl_2$. To initiate the reaction, 150 ng of protein was added. After briefly mixing, the tubes were incubated for 10 min at 30 °C. The reaction was terminated by adding 1 mL of cold 0.5 mg/mL BSA and 1 mL of cold 25% trichloroacetic acid. This mixture was passed through a GF/C Whatman filter and washed five times with 2 mL of 25% trichloroacetic acid. After drying, the filters were counted with 6 mL of ACS scintillation fluid.

Estimation of the Intrinsic pK of Cationic Amphiphiles in Triton Micelles. The intrinsic pK of sphingosine, tamoxifen, and compounds III and XV in a Triton micelle were estimated using ^{1}H NMR. NMR spectra were recorded on a Bruker-AM500 spectrometer. A sample for NMR was prepared by taking a 10% Triton solution made up in 5% D_2O and 95% deionized distilled water and vortexing vigorously with the appropriate quantity of drug to give a 10 mg/mL solution. The resulting suspensions required brief sonication. 1,3-Bis-(diethylamino)-2-propyl behenate (XIV) gave a clear, homogeneous solution after sonication, while tamoxifen and [2-[(3β-cholesterylcarbonyl)amino]ethyl]dimethylamine (III) remained cloudy until the pH was brought below 5.5. Drug-containing micelles were titrated with 10 or 100 mM NaOH or 10 or 100 mM HCl, taking a pH reading before and after the NMR spectra were recorded. The ionization behavior of the cationic amphiphiles under these conditions may not be identical to their behavior in the PKC assay mixture. The lower concentration of cationic amphiphile and the presence of PS and histone will all modify the surface charge and hence the surface pH.

Differential Scanning Calorimetry. DEPE (10 mg) and different concentrations of additive were dissolved together in a solution of chloroform/methanol (2:1 v/v). The solvent was evaporated under a stream of nitrogen, leaving a lipid film deposited on the walls of a glass test tube. Traces of remaining solvent were removed into a liquid nitrogen trap under high vacuum for 2 h at 40 °C. The lipid film was hydrated with 2 mL of 20 mM PIPES, 1 mM EDTA, 150 mM NaCl, and 0.02 mg/mL NaN_3, pH 7.4, warmed to about 45 °C, and vortexed vigorously for 30 s to suspend the lipid. Both buffer and suspended lipid solution were degassed for 20 min prior to loading into the reference and sample cell, respectively, of an MC-2 high-sensitivity differential scanning calorimeter. A scan rate of 45 K/h was employed. The en-

Table II: Effect of Cationic Nonsteroid Amphiphiles on the $L_\alpha \rightarrow H_{II}$ Phase Transition Temperature of DEPE and Their Effect on the Inhibition of PKC in a Triton Mixed Micelle Assay Containing Calcium, Phosphatidylserine, and Diolein[a]

	amphiphile	slope (K/mol fraction)	IC_{50} (μM)
XIV[a]	$(CH_3)_2NCH_2$ / $(CH_3)_2NCH_2$ — CHOC$(CH_2)_{20}CH_3$	−104 ± 1	DNI
XV[a]	$(CH_3)_2NCH_2$ / $(CH_3)_2NCH_2$ — CHOC$(CH_2)_{22}CH_3$	−110 ± 5	DNI
XVI	$(CH_3)_3\overset{+}{N}CH_2$ / $(CH_3)_2\overset{+}{N}HCH_2$ — CHOC$(CH_2)_{20}CH_3$	277 ± 10	26
XVII	$(CH_3)_3\overset{+}{N}CH_2$ / $(CH_3)_2\overset{+}{N}HCH_2$ — CHOC$(CH_2)_{22}CH_3$	246 ± 9	40
XVIII	tamoxifen trimethylammonium iodide	83 ± 14	19
	tamoxifen	−146 ± 8	194

[a] The tertiary amine groups in these amphiphiles are not protonated at pH 7.4 (DNI, does not inhibit).

thalpy of the bilayer to hexagonal transition temperature was determined by fitting the hexagonal phase transition trace to a single van't Hoff component using Microcal Co. software. The transition temperature of this fit curve was used as the value for the $L_\alpha \rightarrow H_{II}$ transition temperature (T_h). In cases where the transition was sharp and symmetrical, the fit T_h was the same temperature of the maximum excess heat capacity ($C_{p,max}$) of the experimental curve. For the broader, less cooperative transitions, the fit T_h deviated only slightly (<0.2 °C) or not at all from the observed temperature of $C_{p,max}$.

RESULTS

Inhibition of Protein Kinase C by Charged Amphiphiles. Drugs containing a steroid or straight-chain hydrocarbon backbone were synthesized to determine whether or not the nature of the hydrocarbon moiety alters the ability of a compound to inhibit PKC. The head group structure was also varied to ascertain whether or not the amount of charge or location of the charge (spatially close to or distant from the membrane surface) enhances the capability of an amphiphile to inhibit PKC activity. Drug potency was determined by measuring the concentration giving 50% inhibition of the enzyme. This is expressed as an IC_{50}. These IC_{50} values are used for comparative purposes only. It is recognized that these values are dependent on the assay system. With less Triton in the assay mixture, the IC_{50} will be lower and it will also depend on the amount of PS in the micelle or membrane. The IC_{50} is presented in Tables I and II for all the cationic amphiphiles studied.

Amphiphiles in DEPE Bilayers. Since neutral and zwitterionic compounds which raise T_h are inhibitors of PKC, we evaluated whether this property was a factor in modulating the PKC inhibitory potency in a series of homologous cationic amphiphiles. A convenient lipid to study the effects of additives on lamellar to hexagonal phase interconversion is DEPE (Epand, 1985). Generally, the concentration of additive was varied from 0 to 14 mol % of the lipid. An example of a series of DSC thermograms of DEPE with varying amounts of a cationic steroid is depicted in Figure 1. Pure DEPE undergoes a bilayer to hexagonal phase transition at approximately 65.6 °C. As the concentration of the drug is increased, the

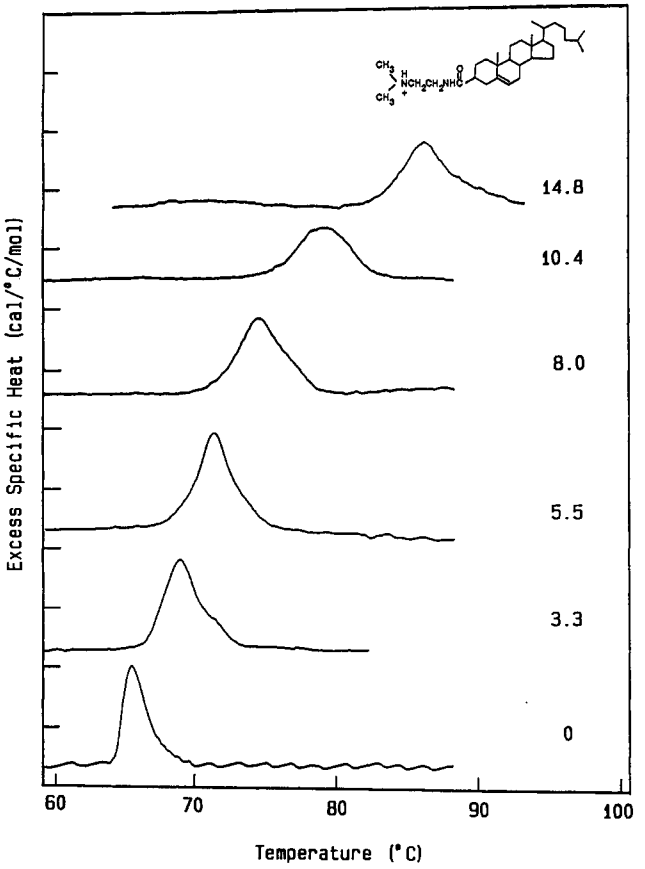


FIGURE 1: Differential scanning calorimetry (DSC) traces of the bilayer to hexagonal phase transition of DEPE in the presence of increasing concentrations of [2-[(3β-cholestrylcarbonyl)amino]ethyl]-dimethylamine (III), (inset, top right) hydrated with 20 mM PIPES, 1 mM EDTA, and 150 mM NaCl containing 0.02 mg/mL NaN_3, pH 7.4. The lipid concentration was 5 mg/mL. The concentration of additive as a mole percent of DEPE is given to the right of the DSC trace. The traces for various experiments were offset for clarity. Each division on the ordinate axis represents 100 cal/(K·mol).

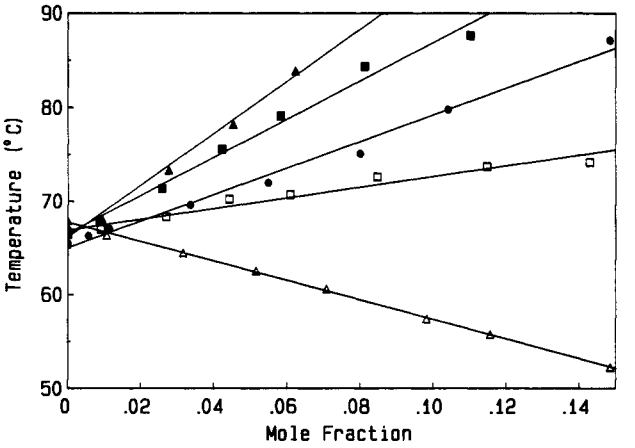


FIGURE 2: Shift in the bilayer to hexagonal phase transition temperature of DEPE as a function of different concentrations of additives. The lipid (5 mg/mL) and additive were hydrated with 20 mM PIPES, 1 mM EDTA, 150 mM NaCl, and 0.02 mg/mL NaN_3, pH 7.4. Points are for compounds III (●), VIII (□), IX (■), XIV (△), and XVI (▲).

temperature at which the lipid undergoes this transition is altered. The change in T_h is plotted as a function of mole fraction of additive in Figure 2. The slope of this linear plot is a useful parameter describing the effect of an additive on lipod polymorphism. Hexagonal phase promoters (bilayer destabilizers) give rise to negative slopes while bilayer stabilizers (inhibitors of the hexagonal phase) display positive

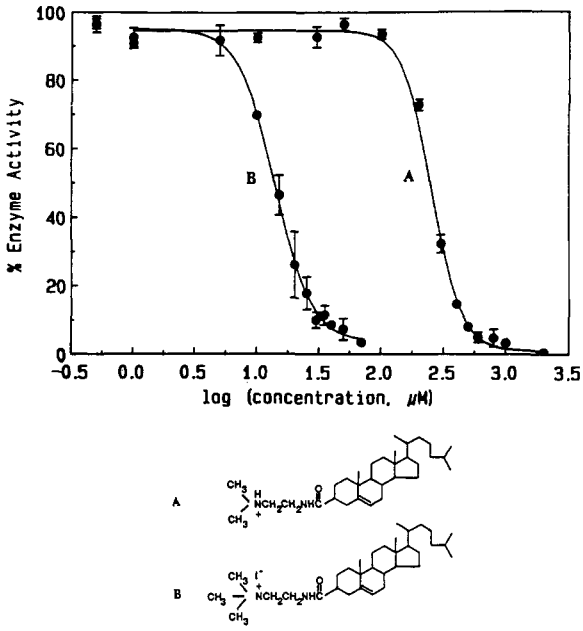


FIGURE 3: Effect of a cationic tertiary amine steroid (A), compound III, and its quaternary ammonium counterpart (B), compound IV, on the inhibition of calcium-, phosphatidylserine-, and dioleinstimulated PKC assayed using Triton X-100 mixed micelles as described under Methods.

slopes. The larger the magnitude of the slope, the more potent the additive is at either stabilizing or destabilizing the membrane. All cationic steroid derivatives are bilayer stabilizers (Table I). The bilayer-stabilizing effect of the cationic steroids is markedly increased when the positive charge is fixed on the molecule by quaternization. However, what is particularly interesting is the effect of the tertiary amine straight-chain amphiphiles on bilayer stability (Table II). These compounds are potent hexagonal-phase promoters despite their large head group. Tamoxifen, a commonly used antineoplastic drug, is also a potent hexagonal-phase promoter (Table II). However, after placing a permanent positive charge on these drugs, bilayer-stabilizing ability is markedly enhanced, particularly for the straight-chain amphiphiles (Figure 2, Table II).

Steroid-Based Cationic Amphiphiles and PKC Activity. The cationic steroids possessing a tertiary amine head group inhibited PKC with an IC_{50} in the range of 150–650 μM (Table I). However, there was a marked effect upon their quaternization. Introducing a fixed positive charge on the steroid resulted in inhibitors which were generally 10-fold more potent than their tertiary amine counterparts. The dose–response curves for a steroid containing a tertiary amine head group and its corresponding quaternary amine counterpart (Figure 3) show that upon quaternization the enzyme inhibition curve is markedly shifted to lower amphiphile concentrations. Apart from quaternization, the size and structure of the amine moiety did not appear to contribute significantly to the inhibition of the enzyme (Table I).

Cationic Straight-Chain Amphiphiles and PKC Activity. In order to assess whether or not the hydrocarbon backbone contributes to inhibitor potency, straight-chain hydrocarbon derivatives (compounds XIV and XV) were designed to be analogous to the cationic steroid amphiphiles V and X. When the steroid backbone is replaced with a straight-chain hydrocarbon, these amphiphiles do not inhibit PKC at concentrations up to 1 mM. Their lack of potency at inhibiting PKC, and their tendency to promote hexagonal-phase formation, suggests that they may be uncharged in a membrane or micelle. These compounds were not activators of PKC either in the absence or in the presence of DAG.

Tamoxifen Analogues and PKC Activity. As had been noted for sphingosine, not all cationic inhibitors of PKC are bilayer stabilizers (Bottega et al., 1990). Another cationic hexagonal-phase promoter is tamoxifen (Table II). Despite its effects on lipid-phase behavior, tamoxifen inhibited PKC (Table II) with a potency comparable to that of the tertiary amine steroids (Table I). Quaternizing tamoxifen however resulted in a bilayer-stabilizing inhibitor that is 10-fold more potent that tamoxifen itself (Table II). Its potency was comparable to the quaternized steroid derivatives.

Titration of Tertiary Amine Amphiphiles in Triton Micelles. In order to correlate the potencies of the inhibitors with the charge of the amphiphile in the Triton micelle, the protonation state of amphiphiles containing a tertiary amine head group was studied with ^1H NMR spectroscopy. Amphiphiles of interest included 1,3-bis(dimethylamino)-2-propyl behenate (XIV), tamoxifen, and [2-[(3β-cholesterylcarbonyl)-amino]ethyl]dimethylamine (III). The chemical shift of the methyl substituents present on a tertiary amine nitrogen is sensitive to the protonation state of the neighboring nitrogen atom. When the amino group is deprotonated, these protons give a characteristic chemical shift at about 2.2 ppm. Protonation at lower pH's leads to deshielding of the methyl protons and, therefore, a downfield shift to approximately 2.9–3.1 ppm. The deshielding effect is larger for XIV because of two neighboring amine groups, resulting in chemical shifts closer to 3.1 ppm. Conveniently, there is an absence of proton resonances in the region of 1.8–3.2 ppm of the Triton micelle spectrum. The shift of the methyl protons for XIV in Triton micelles is shown in Figure 4. The change in ionization state of the drug with respect to pH is depicted in Figure 5. What is striking is that, at the pH of the enzyme assay (pH 7.5), this compound is for the most part deprotonated. The apparent pK of XIV in Triton micelles is 5.5. Apart from electrostatic repulsion, the broad titration curve can be attributed to the presence of two tertiary amine sites on this molecule as opposed to one. If one amino functionality is protonated, it will be more difficult to protonate the second one. On the other hand, tamoxifen and III still bear a significant amount of positive charge at pH 7.5 (Figure 5). The apparent pK's of these drugs are 6.9 and 7.2, respectively.

DISCUSSION

Structure–Activity Studies. Positively charged amphiphiles inhibit PKC. Quaternary ammonium compounds containing a permanent positive charge were found to be the most potent inhibitors of the kinase. This conclusion is also valid for vesicle-based assays of PKC activity (unpublished results). In the present study using the micelle assay, the tertiary amine amphiphiles gave half-maximal inhibition of the enzyme at concentrations approximately 10-fold higher (150–650 μM) than those of the corresponding quaternary amines (Tables I and II). The lack of inhibition of PKC by XIV correlates with its lack of charge in the conditions of Triton micelle assay. The fact that the C22 or C24 tertiary amines induce H_{II} phase formation suggest they partition deeply into the membrane; this partitioning is facilitated by their lack of charge. Thus the pK of an amphiphile in a membrane will be determined not only by the nature of the ionizable head group but also by the hydrocarbon moiety to which it is attached. The latter will determine the position of the head group near the bilayer surface and hence the polarity of its environment. Increased hydrophobicity of the amphiphile

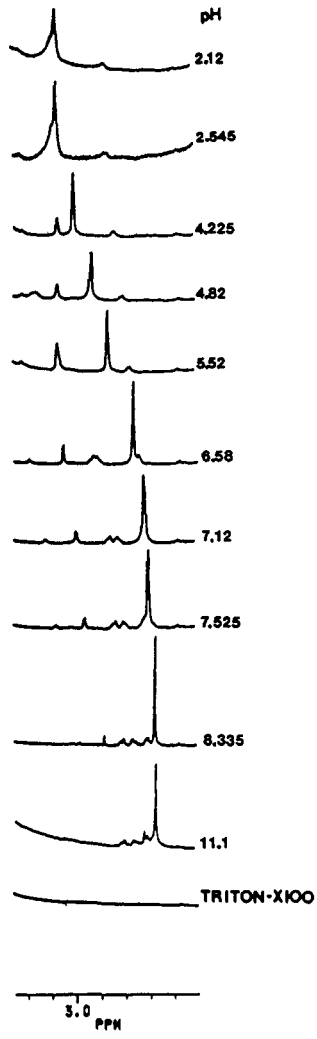


FIGURE 4: pH dependence of the chemical shift of the tertiary amine methyl protons of 1,3-bis(dimethylamino)-2-propyl behenate, compound XIV (10 mg/mL), in 10% Triton X-100 made up in 95% H_2O and 5% D_2O. D_2O served as a lock signal for the magnetic field throughout the experiment. The spectra were recorded at 500 MHz using presaturation of the water signal. The bottom spectrum depicts the absence of ^1H resonances of Triton X-100 between 2.0 and 3.4 ppm. The addition of drug accounts for the numerous resonances in the remaining spectra. The most intense peak was assigned to the tertiary amine methyl protons of XIV and was used to generate the titration curve.

will lower the pK of a basic head group and will be more likely to lower T_h. When a permanent positive charge is fixed on XIV to produce XVI, its potency is equivalent to that of quaternized tamoxifen (tamoxifen trimethylammonium iodide, Table II) and the quaternary ammonium steroids (Table I). Perhaps the most convincing evidence that the amount of positive charge on amphiphile determines how it will affect PKC comes from studies with sphingosine. We have previously shown that there is a decrease in inhibition of PKC by sphingosine with increasing pH concomitant with a change in the ionization state of sphingosine (Bottega et al., 1990). At pH 7.5, sphingosine is only 47% ionized and is consequently more charged than the tertiary amine steroids or tamoxifen. Its potency in inhibiting PKC (IC_{50} = 100 μM; Hannun et al., 1986), as well as its charge, is intermediate between the less charged and less potent tertiary amine amphiphiles and the more charged and more potent quaternary ammonium compounds. However, at pH 8.5 where about 78% of the positive charge is removed, sphingosine activated the enzyme to a small extent as predicted on the basis of its effect on lipid

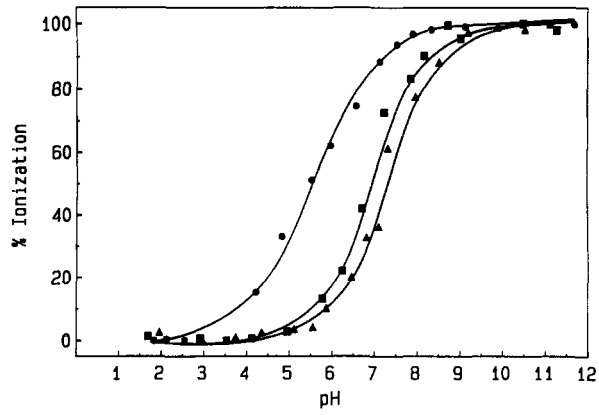


FIGURE 5: pH titration curves of 1,3-bis(dimethylamino)-2-propyl behenate, XIV (●), tamoxifen (■), [2-[(3β-cholesterylcarbonyl)-amino]ethyl]dimethylamine, III (▲), in Triton X-100 measured with ^1H NMR spectroscopy. The drug concentration (10 mg/mL) in a solution of Triton X-100 made up in 95% H_2O and 5% D_2O. D_2O served as a lock signal for the magnetic field throughout the experiment.

polymorphism (Bottega et al., 1990). In addition, *N*-acetylsphingosine, an analogue of sphingosine without a positive charge, does not alter the activity of isolated PKC nor the effect of this enzyme in human platelets (Hannun et al., 1987). In fact, *N*-methyl-*N*-isopropylsphingosine, an analogue with an increased head group hydrophobicity, activated PKC (Merrill et al., 1989).

Through an inductive effect from the attached methyl substitutions, a tertiary amine nitrogen would be expected to protonate more easily than a primary or secondary amine. However, the reverse is the case. The steric crowding around the nitrogen atom makes the protonation of tertiary amines more difficult (or deprotonation easier). Consequently, tertiary amines have a lower pK than primary or secondary amines. This effect would be even greater in the membrane. Since tertiary amines are more hydrophobic, they would partition deeper into the membrane and this would make deprotonation in a more hydrophobic environment more favorable. Tamoxifen, a tertiary amine, is 10-fold less potent than *N*-desmethyltamoxifen, a drug with one less methyl substituent on the nitrogen atom (O'Brian et al., 1988), probably because tamoxifen is less charged in the conditions of the PKC assay. Moreover, amino acridines possessing primary amine substitutions are more potent than those possessing tertiary amine substitutions at the same site (Hannun & Bell, 1988), again consistent with the expected pK values. In addition, Merrill et al. (1989) reported that *N*-methylsphingosine (IC_{50} = 4 mol %) is more potent than *N*,*N*-dimethylsphingosine (IC_{50} = 10 mol %). It is likely that *N*,*N*-dimethylsphingosine is largely deprotonated in the Triton micelle as was observed for 1,3-bis(dimethylamino)-2-propyl behenate (XIV). However, more recently the difference in inhibitory potency between sphingosine and *N*,*N*-dimethylsphingosine appeared somewhat less in a vesicle assay than in the Triton micelle assay (Khan et al., 1990). We find little difference in the Triton micelle assay in inhibitory potency between the tertiary amine steroid amphiphile (III) and its primary amine counterpart (VII) (Table I). It is possible that the basic groups of both of these amphiphiles are largely deprotonated under conditions of the PKC assay, leading to their low inhibitory potency.

In summary, a number of cationic amphiphiles are inhibitors of PKC. The potency of inhibition is not sensitive to changes in the structure of the amphiphile, but it does require the maintenance of a positive charge in a membrane environment.

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SUPPLEMENTARY MATERIAL AVAILABLE

A description of the synthesis and characterization of the new cationic amphiphiles used in this work (17 pages). Ordering information is given on any current masthead page.

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