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Adsorption of aminopyridines to phosphatidylserine membranes

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Aminopyridines belong to the class of compounds which facilitate synaptic transmission at low calcium concentration, an effect associated with the block of K^+ channels, enhanced entry of calcium into presynaptic terminals and greater release of transmitter. We have measured the zeta-potential of phosphatidylserine vesicles in the presence of aminopyridines and some related compounds in order to relate the strength of association of the aminopyridines with their biological effectiveness. The dependence of zeta-potential on the concentration of aminopyridines was analyzed in terms of the Langmuir-Stern-Grahame adsorption model. The rank order of the association constants (in M^{-1}) obtained in the study was as follows: 3,4-diaminopyridine (6.5), 4,5-diaminopyrimidine (3.8), 4-aminopyridine (2.6), 3-aminopyridine (1.8), 2-aminopyridine (1.6), 4-dimethylaminopyridine (0.5), 4-aminopyridine methiodide (0.2), and, as control, calcium (12.1). The comparison of association constants with published results of the electric potential maps obtained by the CNDO/2 method suggests that binding to phosphatidylserine membrane increases with the density of excess charge on the protonated aminopyridine ring. We find that the sequence of potencies of aminopyridines in blocking K^+ channels, in releasing transmitter, and in the shifts of calcium concentration dependence of synaptic transmission are about the same as the sequence of association constants with the phosphatidylserine membrane. Assuming that the binding domain for aminopyridines in the presynaptic terminal has similar adsorption properties as the phosphatidylserine membrane, we estimate the electric potential difference between the domain and the external solution to be between −300 and −340 mV.

Introduction

Aminopyridines (APs) are biologically active N-heterocyclic compounds whose primary sites of action are at nerve synapses. These compounds are known as anticurare agents [1] for their ability to reverse the neuromuscular blockade produced by d-tubocurarine [2]. APs are antagonists of the paralysis produced by botulinum toxin [3] and can counteract the muscle fatigability in myasthenia gravis [4]. At lower concentrations APs enhance synaptic transmission [5,6] and induce repetitive postsynaptic responses to a single presynaptic stimulus [7–9]. However, at elevated concentrations they block synaptic transmission [10].

In studies of synaptic transmission in bullfrog sympathetic ganglia it was found that the amplitude of the postganglionic compound action potential (CAP) decreased as a function of decreasing $[Ca^{2+}]$ in the external medium. The sigmoidal curve describing the Ca^{2+} concentration dependence of synaptic transmission was shifted markedly to the left (lower $[Ca^{2+}]$) by 3,4-diaminopyridine [10]. In a subsequent report Matsumoto and Riker [11] extended their analysis of Ca^{2+}-dependent shifts to include other APs and obtained similar results.

The effects of APs on synaptic transmission are believed to be related to the AP-induced block of potassium channels in presynaptic membranes [12–14]. This block of potassium channels prolongs the falling phase of the action potential, enhancing calcium influx, and thereby increasing transmitter release [15]. Studies of Kirsch and Narahashi [14] with squid axon and Molgo et al. [16] with frog neuromuscular junction suggest that the active site of APs is at the internal surface of nerve membrane and that the biological activity is associated with the cationic form of APs. The neutral form is deemed important for membrane permeation when APs are applied externally.

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Structure-activity studies [13,14,16,17] have revealed that amino or hydroxy groups on the pyridine ring are essential for pharmacological activity in nerve membrane, thereby suggesting some possible properties of an AP receptor site. It has been concluded that the site does not discriminate between the molecular structures of APs, specifically the position of the amino group on the pyridine ring. Peradejordi et al. [17] calculated maps of electrostatic potentials for a series of neutral and protonated APs. Common electrostatic potential distribution patterns were found only for the biologically active protonated species in which the cloud of positive charge resides on the ring and there is a potential minimum next to the amino group. It was proposed that association with a membrane receptor may be a two-step process: first, coulomb attraction involving the N-heterocyclic ring and a negatively charged receptor group, followed by hydrogen bonding [17]. In the context of this proposed mechanism of action of APs, we found it important to study adsorption of these compounds on a biologically relevant model membrane system: the phosphatidylserine bilayer.

Recent studies of interactions of calcium channel antagonists [18–20] and beta-adrenergic blocking drugs [21] with biomembranes indicate the existence of multistep processes: (a) nonspecific interactions of ligand/drug with the membrane coupled with specific interactions with the receptors, and (b) active involvement of the lipid matrix in the orientation of ligands at the membrane/water interface, favoring binding to the receptor and enhancing the kinetics of membrane response [22].

By analogy, we have adopted the concept that the interaction between APs and the potassium/calcium channel system triggering the release of transmitter involves both specific and nonspecific steps. We quantify the nonspecific interactions in terms of the adsorption constants of APs with negatively charged lipid membranes obtained from the measurements of the ζ-potential of lipid vesicles. We have found not only that the sequence of association constants of the series of APs with the membrane is similar to those found in various pharmacological studies on nerve axons and synapses, but that there is a proportionality between the association constants of APs with phosphatidylserine membranes (as determined from the ζ-potential studies with lipid vesicles) and the value of the AP concentration corresponding to the half-maximal shift of the calcium concentration dependence curve for synaptic transmission [10,11].

Materials and Methods

Bovine brain phosphatidylserine (PS) (20 mg/ml in chloroform) was purchased from Avanti Polar Lipids (Birmingham, AL) and used without further treatment. A 0.5–1.0 ml aliquot of the original chloroform solution was mixed with about 50 ml of analytical grade chloroform. A thin lipid film was formed on the lower half of a round-bottom flask using a rotary evaporator. After pumping for an hour to ensure removal of chloroform, the flask was shaken with 15–30 ml of a buffered solution of 0.1 M tetramethylammonium chloride (TMACl) and 1 mM EDTA to form a suspension of lipid vesicles. TMACl was selected because the cation does not appreciably adsorb to PS membranes [23].

The source of 3,4-diaminopyridine (3,4-DAP), 4-aminopyridine (4-AP), and 3-aminopyridine (3-AP) was Aldrich Chemical Co. (Milwaukee, WI); 4-dimethylaminopyridine (4-DMAP) and 4,5-diaminopyridine (4,5-DAPM) were obtained from Sigma Chemical Co. (St. Louis, MO), while 2-aminopyridine (2-AP) was from Reilly Tar and Chemical Corp. (Indianapolis, IN).

A 1 M stock solution of each aminopyridine in buffer was prepared, using Mops (3-*N*-morpholinopropanesulfonic acid) for electrophoretic mobility measurements at pH 7.2, and citrate/phosphate/borate buffer for other pH values. 4,5-DAPM, 3-AP and 2-AP are only partially ionized at physiological pH (respective pK_a values are 6.03, 5.98 and 6.86). Adsorption characteristics of these species were obtained at pH values corresponding to 80% and 20% ionization, and averaged values of K are reported. For each of twelve AP concentrations, 1 ml of vesicle stock suspension was mixed with an appropriate volume of aminopyridine solution and buffered TMACl solution to a total volume of 10 ml. These vesicle suspensions were subsequently used in electrophoretic mobility determinations.

The measurements were done with a Mark 1 electrophoretic instrument (Rank Brothers, Bottisham, Cambridge, U.K.) using a cylindrical cell with a 2 mm internal capillary. The cell voltage and current were monitored with Data Precision 245 and Dana 4200 digital multimeters. The polarity of the applied voltage was alternately reversed to minimize electrode polarization and the drift velocities for both polarities, which usually agreed within a few percent, were averaged. The velocity of vesicles was measured as a function of depth of the microscope's focus and the velocity at the stationary layer was obtained by the linear regression method. Generally at least twenty measurements for each polarity were made for each concentration point. The mobilities were found to be independent of vesicle size. The Helmholtz equation was used to determine the value of ζ-potential from the electrophoretic mobility μ,

$$\mu = \epsilon\epsilon_0\zeta/\eta \qquad (1)$$

where ϵ is the relative dielectric constant, ϵ_0 the permittivity, η the viscosity and ζ is the ζ-potential value.

Adsorption model

We have used a set of assumptions known as the Gouy-Chapman-Stern adsorption model which were introduced by McLaughlin and Harary [24]. The model combines the Langmuir adsorption isotherm with the Boltzmann factor correction for the local concentration of adsorbing species at the membrane/water interface and Grahame's equation relating the membrane surface potential to the membrane surface charge density. It has been shown that this model can be used to describe adsorption of inorganic and organic ions on phospholipid membranes [23–29].

We assume that three ionic species may simultaneously adsorb to the negatively charged membrane surface. These are singly charged cations of the "background" electrolyte (simple salt and buffer components) suspending the PS vesicles, aminopyridine, and calcium ions. The adsorption model consists of three major components: (a) distribution of ions between water and the membrane surface, (b) determination of the membrane surface charge density, and (c) the relationship between ζ-potential, membrane surface potential and the surface charge density.

(a) Distribution of ions between water and membrane surface. We assume that all ions, including calcium, form 1:1 complexes on adsorption to phosphatidylserine. We denote as L_t and L_f the total and free (not populated) surface densities of lipids in the membrane.

We consider the adsorption of three kinds of ions: singly charged cations of background electrolyte, aminopyridine cations, and divalent cations. Their respective bulk and interfacial concentrations are $[C_0]_{aq}$, $[C_0]_s$, $[C_1]_{aq}$, $[C_1]_s$, $[C_2]_{aq}$ and $[C_2]_s$. K_0, K_1 and K_2 denote the respective association constants and L_{0m}, L_{1m} and L_{2m} are the surface densities of lipid-ion complexes. The adsorption isotherms for individual ionic species are:

$$L_{0m} = K_0 \cdot [C_0]_s \cdot L_f \tag{2a}$$

$$L_{1m} = K_1 \cdot [C_1]_s \cdot L_f \tag{2b}$$

$$L_{2m} = K_2 \cdot [C_2]_s \cdot L_f \tag{2c}$$

The interfacial ionic concentrations at charged membrane surfaces are:

$$[C_0]_s = [C_0]_{aq} \cdot \exp(-eV_s/kT) \tag{3a}$$

$$[C_1]_s = [C_1]_{aq} \cdot \exp(-eV_s/kT) \tag{3b}$$

$$[C_2]_s = [C_2]_{aq} \cdot \exp(-2eV_s/kT) \tag{3c}$$

where V_s is the electric potential at the aqueous side of the membrane/water interface.

The balance of the number of sites, total, free and occupied is given by:

$$L_t = L_f + L_{0m} + L_{1m} + L_{2m} \tag{4}$$

In terms of respective membrane coverages:

$$\theta_0 = L_{0m}/L_t \tag{5a}$$

$$\theta_1 = L_{1m}/L_t \tag{5b}$$

$$\theta_2 = L_{2m}/L_t \tag{5c}$$

The adsorption model can be described by a set of three linear equations:

$$(1 + K_0[C_0]_s)\theta_0 + K_0[C_0]_s\theta_1 + K_0[C_0]_s\theta_2 = K_0[C_0]_s \tag{6a}$$

$$K_1[C_1]_s\theta_0 + (1 + K_1[C_1]_s)\theta_1 + K_1[C_1]_s\theta_2 = K_1[C_1]_s \tag{6b}$$

$$K_2[C_2]_s\theta_0 + K_2[C_2]_s\theta_1 + (1 + K_2[C_2]_s)\theta_2 = K_2[C_2]_s \tag{6c}$$

(b) Determination of membrane surface charge density. Since the complex between a phosphatidylserine binding site and monovalent cation is electrically neutral, the membrane surface charge density is determined by only two lipid species: negatively charged unpopulated phosphatidylserine and phosphatidylserine complexes with doubly charged cations, which are positively charged. The membrane surface charge density, σ_m, is equal to:

$$\sigma_m = -eL_f + eL_{2m} = -eL_t(1 - \theta_0 - \theta_1 - 2\theta_2) \tag{7}$$

(c) Relationship between ζ-potential, membrane surface potential and the surface charge density. The relationship between the surface potential and the membrane surface charge density is given by Grahame's equation:

$$\sigma_m = (V_s/|V_s|)\{2\epsilon\epsilon_0 kT\Sigma_j C_j[\exp(-z_j eV_s/kT) - 1]\}^{1/2} \tag{8}$$

The summation includes all ions, cations and anions, the bulk density of the corresponding species is denoted by C_j and valency by z_j. The relationship between the ζ-potential, defined as the electric potential between the shear plane and the bulk solution, was taken from work of Bentz and Nir [30].

Results and Discussion

Calcium

The dependence of the ζ-potential of phosphatidylserine (PS) vesicles on calcium concentration is shown in Fig. 1. The vesicles were suspended in 0.1 M sodium chloride at pH 7.2 buffered with 1 mM Mops. There are two notable features of the data: (i) At the lowest Ca^{2+} concentration (0.1 mM) the value of the ζ-potential was found to be -55.2 ± 3.1 mV, which is very close to that observed in the absence of Ca^{2+}, -60.1 ± 4.0 mV. The latter, the control value of ζ-potential in 0.1 M NaCl, is less negative than that found when tetramethylammonium chloride was used as the suspending electrolyte (-88.0 ± 1.3 mV). The difference between -55.2 mV and -88.0 mV is due to the specific adsorption of

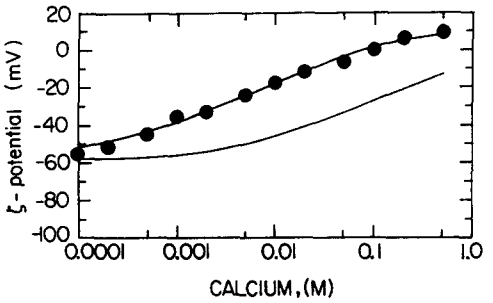


Fig. 1. The change of ζ-potential of phosphatidylserine (PS), vesicles as a function of concentration of calcium. The solution suspending the PS vesicles contained 0.1 M NaCl as a background electrolyte, and 0.001 M Mops (pH 7.2). The solid curves illustrate the predictions of the Langmuir-Stern-Grahame adsorption model assuming 0.65 nm^2 of membrane surface area per lipid molecule and the shear surface to be located 0.2 nm in front of the surface of the membrane. The upper curve is computed for an intrinsic association constant for calcium of 12.1 M^{-1} and 1 M^{-1} for sodium. The lower curve illustrates the change of the potential due to the adsorption and screening of sodium ions.

sodium on PS membranes. (ii) At the high end of the Ca^{2+} concentration range the values of zeta become positive and saturate. The potential reversal was observed at a Ca^{2+} concentration of 0.1 M ($\zeta = 0.0 \pm 1.0$ mV). These data confirm results obtained for PS membranes by other workers; Eisenberg et al. [23], in their studies of adsorption of monovalent cations to negatively charged membranes, reported ζ-potentials between -91.5 ± 2.0 and -90.0 ± 1.5 mV for 0.1 M tetramethylammonium chloride, and between -62.0 ± 1.0 and -61.5 ± 1.5 mV for 0.1 M sodium chloride. In later studies, McLaughlin et al. [26], obtained -85 mV for 0.1 M tetramethylammonium chloride and -58 mV for 0.1 M sodium chloride. The association constant for sodium from the adsorption model described above, assuming a surface area of 0.7 nm^2 per phosphatidylserine molecule and the shear surface to be located at a distance of 0.2 nm from the membrane surface, ranges between 0.6 M^{-1} and 1.0 M^{-1}. Our ζ-potential results for PS membranes are consistent with the assumption of 0.65 nm^2 per lipid molecule [31], shear surface distance of 0.2 nm [29], and a 1.0 M^{-1} association constant for sodium. The values of ζ-potential predicted from the model are -88.4 mV for 0.1 M tetramethylammonium chloride and -58.7 mV for 0.1 M sodium chloride.

The lower solid curve in Fig. 1 indicates the dependence of the ζ-potential of PS membranes if there were no specific adsorption of Ca^{2+}. The decrease of the magnitude of zeta potential at higher Ca^{2+} concentration is due to the screening effect of Ca^{2+}. The solid curve through the data is the model prediction based on the 'best' value of the Ca^{2+} association constant obtained from the minimum of χ^2 [32]. The value of the Ca^{2+}-PS membrane association constant obtained from the fit of the adsorption model to all data, 12.1 M^{-1}, is in close agreement with that obtained from the calcium concentration at the cross-over ζ-potential ($\zeta = 0$), which was found to be 10.0 M^{-1} [25]. This close agreement assures the validity of the aminopyridine association constant values obtained from the fit of the model to the aminopyridine concentration dependence of the ζ-potential.

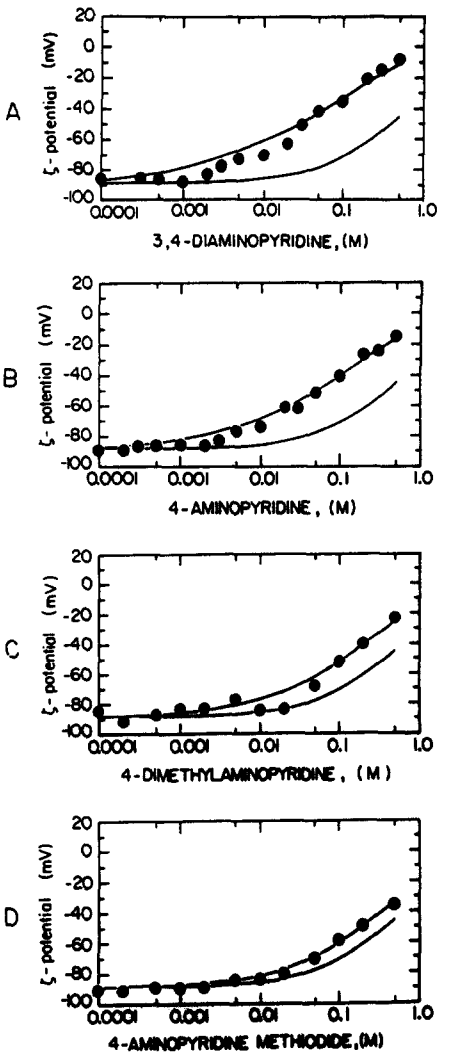


Fig. 2. Panels A–D show the effects of 3,4-diaminopyridine (A), 4-aminopyridine (B), 4-dimethylaminopyridine (C), and 4-aminopyridine methiodide (D) on the ζ-potential of phosphatidylserine vesicles in 0.1 M tetramethylammonium chloride as a background electrolyte with 1 mM Mops at pH 7.2. The solid curves in each panel illustrate the prediction of the Langmuir-Stern-Grahame adsorption model assuming 0.65 nm^2 of membrane surface area per lipid molecule with the shear surface located 0.2 nm in front of the membrane surface. The upper curves are computed for intrinsic association constants for the aminopyridines of 6.5 M^{-1} (A), 2.6 M^{-1} (B), 0.5 M^{-1} (C), and 0.2 M^{-1} (D), and zero for tetramethylammonium. The lower curves illustrate the screening affect of the aminopyridines on the ζ-potential in the absence of adsorption.

Aminopyridines

To avoid masking effects caused by the adsorption of alkali ions, 0.1 M tetramethylammonium chloride was used as the suspending medium. In the absence of aminopyridines the average ζ-potential was about -88 mV, a value consistent with the prediction of the diffuse double layer theory and the assumption of the absence of specific adsorption of tetramethylammonium cation.

In the plots of ζ-potential versus the aminopyridine concentrations shown in Figs. 2 and 3, the lower curve gives the variation in the magnitude of the ζ-potential due to screening of negative charge on the PS membrane by aminopyridine cations, as predicted from the diffuse double layer theory. Any further decrease in the magnitude of the ζ-potential is caused by the specific adsorption of aminopyridine cations partially neutralizing the negative surface charge of the membrane. The greater the gap between the data and the screening curve, the greater the specific adsorption of the cation. Thus, the weakest specific adsorption is exhibited by 4-aminopyridine methiodide, and the strongest by 3,4-diaminopyridine.

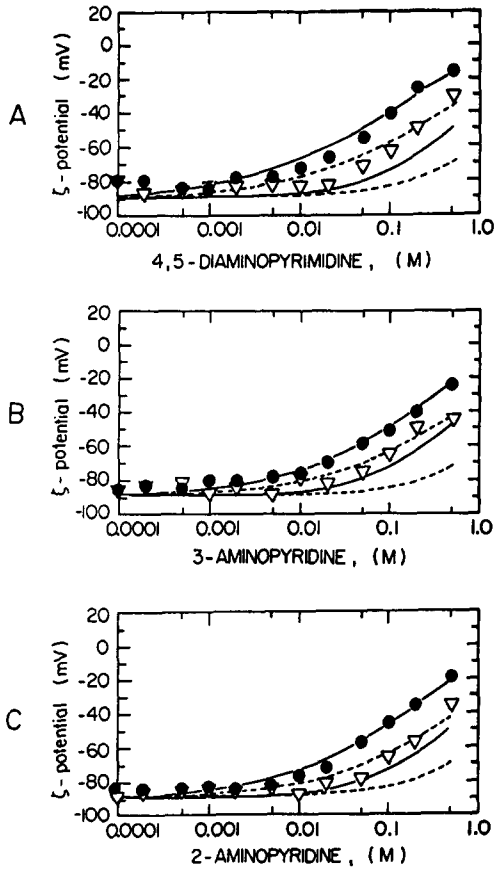
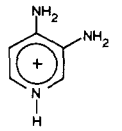
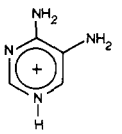
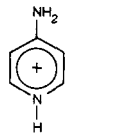
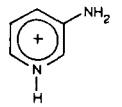
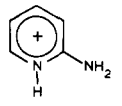
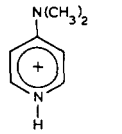
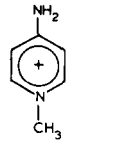


Fig. 3. Panels A–C show the effects of 4,5-diaminopyridine (A), 3-aminopyridine (B), and 2-aminopyridine (C) and their degree of ionization on the ζ-potential of phosphatidylserine vesicles in 0.1 M tetramethylammonium chloride as a background electrolyte with phosphate/citrate/borate buffer (2/2/0.5 mM, respectively). The filled symbols and solid curves correspond to the higher degree of AP ionization at pH 5.4 (A,B), 6.2 (C), while the open symbols and broken curves correspond to the lower ionization at pH 6.6 (A,B), and 7.4 (C). The upper solid and broken curves in each panel illustrate the prediction of the Langmuir-Stern-Grahame adsorption model assuming a 0.65 nm^2 membrane surface area per lipid molecule with the shear surface located 0.2 nm in front of the membrane. Intrinsic association constants for the cations are 3.8 M^{-1} (A), 1.8 M^{-1} (B), and 1.6 M^{-1} (C), and zero for tetramethylammonium. The lower solid and broken curves illustrate the screening effects of the respective cations on the ζ-potential in the absence of adsorption.

TABLE I

Association constants of aminopyridines and calcium for phosphatidylserine membranes

Membrane active ion	Assoc. const. (M^{-1})
 3,4 Diaminopyridine	6.5
 4,5 Diaminopyrimidine[a]	3.8
 4 Aminopyridine	2.6
 3 Aminopyridine[a]	1.8
 2 Aminopyridine[a]	1.6
 4 Dimethylaminopyridine	0.5
 4 Aminopyridine methiodide	0.2
Calcium	12.1

[a] Average from measurements at two pH values.

It should be noted that in all studies pH was sufficiently high so that protonation of the carboxyl group of PS was insignificant. First, pK_a of the carboxyl group is 3.6 ± 0.1 [33], second, the value of ζ-potential at low AP concentrations (Fig. 3) was the same as at higher pH values (Fig. 2) indicating that protonation of PS was insignificant.

The values of association constant obtained from the

minimum χ^2 analysis, along with the molecular structures of individual cations, are given in Table I. The presence of a methyl group on the pyridine nitrogen or on the amino group causes a dramatic decrease of aminopyridine adsorption on the membrane; a 5–10-fold change of the association constant was observed between 4-aminopyridine and 4-dimethylaminopyridine or 4-aminopyridine methiodide. Finally, the location of the amino group on the pyridine ring also affects the strength of the aminopyridine–membrane interaction: the association constant of 4-aminopyridine is about 50% greater than that for 3-aminopyridine and 2-aminopyridine. On addition of an amino group to 4-aminopyridine, in position 3, the strength of pyridine–membrane interaction significantly increased, from 2.6 M^{-1} for 4-aminopyridine to 6.5 M^{-1} for 3,4-diaminopyridine. The presence of a second nitrogen in the ring (4,5-diaminopyrimidine versus 3,4-diaminopyridine) weakens adsorption to the membrane.

Interesting conclusions may be derived from the comparison of charge distributions in the APs and their association constant with PS membranes. Quantum-mechanical calculations of group charge [17] indicated that (a) the protonated pyridine ring carries a positive charge in excess of +1.0 unit charge, (b) there is a net positive charge on the pyridine proton of about 0.21, and (c) the NH_2 group carries a small net negative charge.

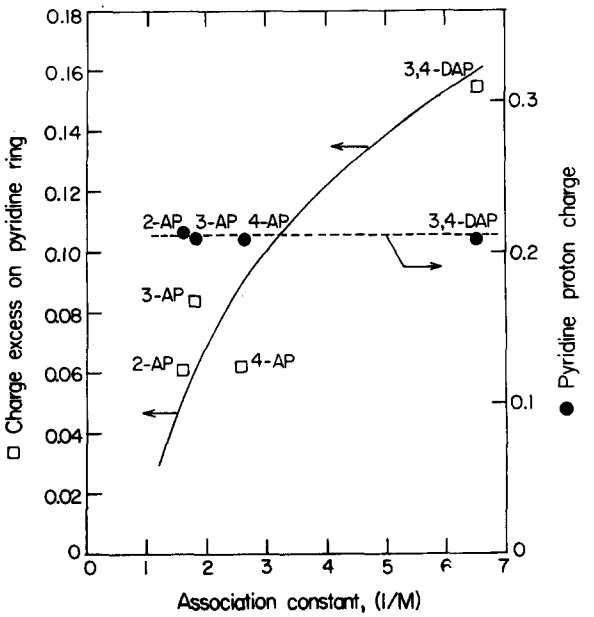


Fig. 4. Structure-activity relationship between the excess charge on the pyridine ring (squares), the pyridine proton charge (circles) and the association constant with the membrane. The quantities on the ordinates were obtained by quantum chemistry methods given in Ref. 17, the values of the association constant are given on the abscissa. The results indicate that the association constant is insensitive to the pyridine proton charge (dashed line) whereas it increases with the excess charge on the pyridine ring (solid curve). The solid curve corresponds to the best fit according to $K = K_0 \cdot \exp(12.9\,\Delta q)$, where Δq is the excess charge on the pyridine ring and $K_0 = 0.82\ M^{-1}$.

In Fig. 4 we have plotted the excess charge on the pyridine ring (data associated with the solid curve) and the charge on the pyridine proton, (both sets of data from Ref. 17) versus the association constant of APs with the PS membrane as obtained from the ζ-potential studies. The data suggest that AP binding to the membrane depends on the excess charge residing on the pyridine ring, or on the amino-group (charges which are equal in magnitude and opposite in polarity), rather than on the localized charge at the site of the proton on the pyridine nitrogen. This suggests that adsorption of aminopyridines on PS membranes is determined in part by electrostatic effects; that is, by the attractive force between the positively charged aminopyridine ring and a negatively charged oxygen on the phosphoryl ester segment of PS, and by dipolar interactions between the aminopyridine dipole and atomic charges within the glycerol region.

Aminopyridines are probably located within the membrane/water interface rather than at the membrane surface, as shown by X-ray diffraction studies of locations of comparable molecules, such as propranolol [18,19] and dihydropyridine calcium channel antagonists [20]. X-ray and neutron diffraction studies will be necessary to address experimentally the issue of aminopyridine location within the membrane.

Structure-activity relationships of aminopyridines and their analogs in biological systems

Several groups of investigators have examined series of aminopyridines and analogs to determine their rank order of potency in modifying neural membrane function. For example, Kirsch and Narahashi [14] measured K^+ channel block by APs and hydroxypyridines in squid axon and observed the potency order 3,4-DAP > 4-AP > 2,3-DAP > 4-HP > 2,6-DAP > 3-HP > 2-HP. Molgo et al. [16] measured AP-induced increases in quantal content at Mg^{2+}-blocked frog neuromuscular junctions and obtained the potency order 3,4-DAP > 4-AP > 4-AQ > 3-AP > 2-AP.

Previous reports from the laboratory of one of the authors (W.K.R.) have shown [10] that the well-known Ca^{2+} dependence of synaptic transmission in sympathetic ganglion is shifted markedly to the left (to lower $[Ca^{2+}]_o$) by APs. In other words, concentrations of $[Ca^{2+}]_o$ otherwise insufficient to maintain synaptic transmission become fully effective for normal synaptic transmission in the presence of APs [10,11]. In these studies the potency order for shifting the Ca^{2+} dependence of synaptic transmission was 3,4-DAP > 4-AP > 3-AP > 4,5-DAPM > 4-APMI. Thus, the AP potency order determined by three groups of investigators is in reasonable accord with the rank order of magnitudes of association constants of APs and PS membranes found

in the present work (Table I), with the exception of 4,5-DAPM which has two ring nitrogens and is not an aminopyridine.

The pronounced leftward shift in the $[Ca^{2+}]_o$ dependence of synaptic transmission produced by APs [10,11] suggests enhanced entry or binding of Ca^{2+} at presynaptic nerve terminals. In the following discussion, therefore, we will adopt as a working hypothesis that the enhanced presynaptic entry or binding of Ca^{2+} is due to the blockage of K^+ channels by the APs. The greater the number of K^+ channels blocked, the greater the Ca^{2+} entry or binding, and the greater the release of transmitter. With this hypothesis the primary AP receptor site would be the K^+ channel. It is possible, according to the data of Molgo et al. [16], that the APs act at the intracellular face of the K^+ channel, and that this binding domain has characteristics similar to that of the PS headgroup. The consequences of the assumption of similarity of the binding domain at the AP receptor site in the K^+ channel in biomembranes to that in PS membranes will now be explored in some detail.

The density of membrane-bound aminopyridine, $(AP)_m$, is equal to:

$$(AP)_m = L_t K(\text{app})[AP]_{aq}/(1 + K(\text{app})[AP]_{aq}) \quad (9)$$

where $[AP]_{aq}$ is the bulk aqueous concentration. In terms of the general adsorption model given earlier, $[AP]_{aq} = [C_1]_{aq}$ and $(AP)_m = L_{1m}$. The apparent association constant of aminopyridine with the binding domain at the presynaptic terminal, K(app), can be related to the intrinsic association constant, K(int), according to:

$$K(\text{app}) = K(\text{int}) \cdot \exp(-eV_s/kT) \quad (10)$$

V_s is the electric potential difference between the binding domain and the external solution. K(int) corresponds to K_1 in the general model.

If the magnitude of a physiological response, B, is determined by the number of potassium channels blocked by APs, then the response should be proportional to the maximum amplitude of the response, B_{max}, and to the fraction of channels associated with APs.

$$B = B_{max} \cdot K(\text{app})[AP]_{aq}/(1 + K(\text{app})[AP]_{aq}) \quad (11)$$

At half-maximal physiological response, $B^\star = B_{max}/2$, the aqueous concentration of aminopyridine is $[AP^\star]_{aq}$ and it follows that:

$$K(\text{app})[AP^\star]_{aq} = 1 \quad (12)$$

Similar conclusions would follow if we assume that the effect of APs on synaptic transmission is a sequential process, similar to that proposed by Herbette and collaborators [18–20] for some cardiovascular drugs in which the lipid bilayer matrix plays an active role in positioning the drug for a favorable interaction with the receptor. In this case the adsorption of drug is followed by its lateral diffusion within the membrane toward the receptor. In the case of APs it would be the inner lipid monolayer that would play the active role since APs are known to be effective when applied from the inside of squid axon membrane [13,14].

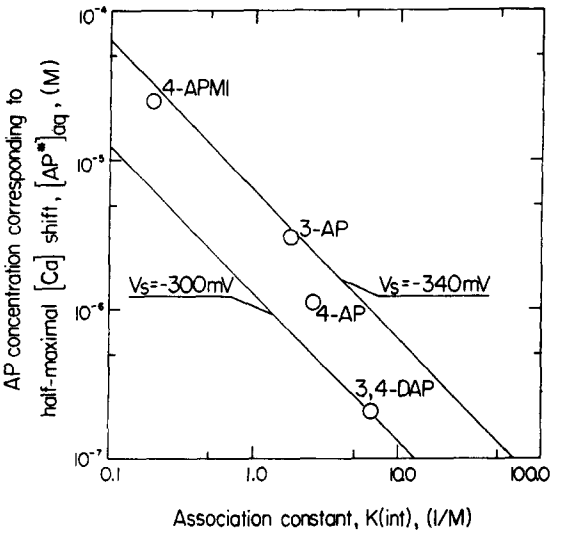


Fig. 5. Structure-activity relationship between the aminopyridine concentrations corresponding to half-maximal calcium concentration shift obtained from studies of synaptic transmission at low calcium concentration levels and the association constants of aminopyridines with PS membranes. The data indicate that the greater the association constant of aminopyridine with the membrane, the lower the concentration of aminopyridine needed to produce a half-maximal leftward shift in the calcium concentration required for normal synaptic transmission in the frog sympathetic ganglion (Ref. 10, datum for 3-AP is an unpublished observation). The straight lines correspond to the electric potential difference between the binding domain for protonated aminopyridines at the presynaptic membrane and the external aqueous solution.

In Fig. 5 we plot the ionized aminopyridine concentration $[AP^\star]$ corresponding to the half-maximal shift in the $[Ca^{2+}]_o$-dependence curve for synaptic transmission [11] versus the intrinsic association constant, K(int). It follows that, if the aminopyridine binding domain on or in the potassium channel has adsorption characteristics similar to those of the PS membrane, then the characteristic aminopyridine concentration $[AP^\star]_{aq}$, the intrinsic association constant, and the electric potential difference between the binding domain and the external aqueous solution are related according to:

$$[AP^\star]_{aq} = \exp(eV_s/(kT)/K(\text{int}) \quad (13)$$

The plot shown in Fig. 5 indicates that the results of synaptic transmission studies and adsorption characteristics obtained on the model membrane system indeed conform to eqns. 12 and 13. The data fall within the limits determined by an electric potential of the binding domain between −300 and −340 mV. Electric poten-

tial differences of such magnitude are quite common for phospholipid membranes. For example, the electric potential difference between the interior of electrically neutral egg-PC and PE membranes is 400–500 mV (positive inside) [34–36] and the potential jump occurs within the hydrophobic-hydrophilic boundary region. The −300 to −340 mV electric potential difference at the AP binding domain of the K^+ channel can be achieved by several ester groups with oxygens defining the AP binding domain.

In conclusion, the present data on association constants of APs with PS vesicle membranes are in good agreement with extensive neuropharmacological studies insofar as the rank order of AP potencies. With regard to the AP binding domain our analysis has adopted the conventional assumption that it is on or near a K^+ channel which, when blocked by APs, results in synaptic facilitatory effects. However, the data presented and cited in this report also allows speculation that the specific binding site for the APs in the vicinity of the K^+ channel has a negative potential of 300–340 mV and may be one normally occupied by a divalent cation, e.g. Ca^{2+}. For example, the magnitudes of association constants of the more potent APs, notably 3,4-DAP with an association constant of 6.5, are in the magnitude range of the association constant for Ca^{2+}, i.e., 12.1. This fact, together with the pronounced (5-fold) shift induced by 3,4-DAP in the Ca^{2+}-dependence curve for synaptic transmission [10,11], could be consistent with AP modulation at a Ca^{2+}-binding site. Indeed, one could interpret the results of Molgo et al [16] on Mg^{2+}-blocked frog neuromuscular junction as simply another manifestation of the AP-induced Ca^{2+} dependence shift; one in which the AP modulation of Ca^{2+} binding causes displacement of the bound Mg^{2+}, hence relieving the Mg^{2+} block and consequently increasing end plate potential amplitude, exactly as observed [16]. In this speculative alternative, therefore, the binding of APs to a membrane site might modulate Ca^{2+} binding, or possibly act directly at such a site as Ca^{2+}-like agonists. Future investigations will be needed to test this proposition.

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