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A model for lasalocid-mediated electrical transport of biogenic amines through lipid bilayer membranes

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Summary

The ionophore lasalocid (X-537A) has been shown to form charged complexes with some biogenic amines in lipid bilayer membranes; however, the bulk of amine transport occurs by neutral complex formation with lasalocid. A model for membrane transport is proposed which takes into account both types of interaction. The fate of the charged amine–ionophore complexes upon the addition of *p*-tyramine is also discussed.

Introduction

Lasalocid is a carboxylic acid antibiotic (Berger et al., 1951) that acts as an ionophore by forming complexes with various cations rendering them hydrophobic and thus enhancing their transport through lipid membranes (Pressman, 1973). In solution, lasalocid complexes with monovalent and divalent cations as well as with aliphatic amines (Lindenbaum et al., 1979), and has been shown to transport such ions across artificial lipid bilayer membranes (Celis et al., 1974; Schadt and Haeusler, 1974; Kafka and Holz, 1976; Holz, 1977; Degani, 1978; Kinsel et al., 1982a and b). Its physiological role resides with its ability to transport Ca^{2+} and catecholamines across such membranes and hence affect the cardiovascular system (Pressman, 1973; Holz, 1975). The general scheme proposed for ion transport mediated by ionophores involves: (1) formation of a carrier–cation complex at the membrane–solution interface; (2) transport of the complex through the membrane;

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(3) release of the cation at the opposite interface; and (4) return of the ionophore to complete the cycle (McLaughlin and Eisenberg, 1975).

Recently we have determined the affinity of various biogenic amines for complexation with lasalocid (from monolayer, partitioning and flux experiments) and defined the stoichiometry of the complexes responsible for membrane transport and conductance (Kinsel et al., 1982 a, b). A correlation between amine structure and binding characteristics was also developed. These data have now been expanded and diversified to permit the proposal of a model describing the electrical conductance associated with transport of biogenic amines through lipid bilayer membranes modified with lasalocid. While the preponderance of amine flux occurs as the electrically-silent 1 : 1 complex, this model emphasizes the transport of the conducting species. Such ionophores may eventually serve as carriers to improve drug delivery.

Materials and methods

All chemicals were as described earlier (Kinsel et al., 1982a). Lipid bilayer membranes were formed as reported previously, according to the method of Mueller (Mueller et al., 1963). The aqueous solution bathing the membrane (3 ml on each side) was buffered with 10 mM Hepes (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonate) (using concentrated HCl or NH_3 to adjust pH) and contained ascorbic acid (5×10^{-4} M) as anti-oxidant. Measurements of membrane conductance vs ionophore concentration were carried out at pH = 7.0 with amine added to both sides of the membrane and held constant at 50 mM. Membrane conductance vs amine concentration was also determined at pH 7.0 maintaining ionophore concentration at 3.5 mM in the lipid solution. The pH dependency of membrane conductance was determined holding amine (50 mM) and ionophore (3.5 mM) concentrations constant and varying the pH in the two aqueous compartments.

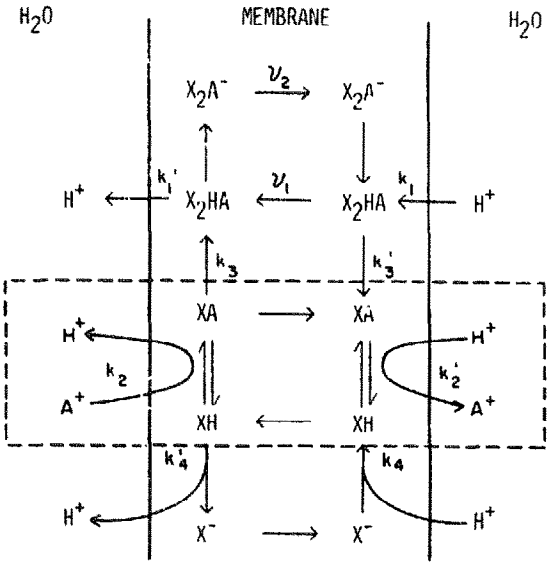
Inhibition studies with *p*-tyramine were carried out in pH 7.0 (10 mM) Hepes buffer, maintaining ionophore concentration (3.5 mM) and amine concentration (50 mM) constant as described above. *p*-Tyramine was introduced incrementally to both sides of the membrane as a methanolic solution (0.7 M).

Membrane conductance values in each case represent an average of measurements for 3–5 membranes.

Results and discussion

A model for membrane transport

A model describing cation transport through bovine brain lipid bilayer membranes doped with cyclodepsipeptides of the valinomycin group (Melnik, 1973; Ovchinnikov et al., 1974) has been adapted and altered to render it applicable to the transport of biogenic amines by the carboxylic acid ionophore, lasalocid (Scheme I). Previous studies (Kinsel et al., 1982b) have demonstrated that amine flux across lipid



Scheme I.

bilayers modified with lasalocid involves transport primarily (>99%) as 1:1 neutral complexes. Thus, the amine (A^+) which is protonated in the aqueous phase (at the pH of those studies (Tuckerman et al., 1959)) apparently interacts with the ionized form of ionophore (X^-) at one membrane interface to form an 'electrically silent' complex (XA) which is transported through the membrane and released at the opposite interface. The independence of amine permeability on applied potential across the membrane further suggests that the primary transported species is not charged (Kafka and Holz, 1976; Kinsel et al., 1982b).

In addition, higher order complexes can be formed by association of ionophore (XH) with the 1:1 neutral complex (XA) to yield aggregates X_2HA and X_2A involving one amine and two ionophore molecules. X_2HA and X_2A^- are presumed to have similar permeability coefficients (ν_1 and ν_2, respectively, which, therefore, can be expressed as ν) in accord with the Stokes-Einstein equation (i.e. they have similar molecular size). Such 2:1 charged complexes have been shown (Kinsel et al., 1982a) to form between lasalocid and various biogenic amines (e.g. phenylephrine, metanephrine and amphetamine) and are responsible for the conductance observed in lipid bilayer membranes in the presence of such amines. These higher order complexes, however, only represent a small fraction of the total concentration of ionophore species, since charged species contribute < 1% to total observed amine flux. The dissociated form of the weakly acidic ionophore (X^-) is itself membrane-soluble and contributes a background conductance (Kinsel et al., 1982a).

The model can be defined in terms of membrane conductance which is inversely proportional to the sum of membrane and surface resistance (Eqn. 1) (Melnik, 1973)

$$g_0 = \frac{F^2}{RT}(R_{\text{membrane}} + 2R_{\text{surface}})^{-1} \tag{1}$$

where F is Faraday's constant, g_0 is conductance and R and T have their usual meaning. These resistances can be expressed in terms of the charge-carrying species

defined by the model (Eqns. 1a and 1b)

$$R_{membrane} = \frac{1}{\nu_2[X_2A^-]} + \frac{1}{\nu_1[X_2HA]} \tag{1a}$$

$$R_{surface} = \{(k'_1 + k'_3)[X_2HA]\}^{-1} \tag{1b}$$

and introduced into Eqn. 1.

To express $[X_2A^-]$ and $[X_2HA]$ in terms of measurable quantities, substitutions are made in Eqns. 1a and 1b from equilibrium constants 2–5 obtained from the model,

$$K_1 = \frac{k'_1}{k_1} = \frac{[X_2A^-][H^+]}{[X_2HA]} \tag{2}$$

$$K_2 = \frac{k'_2}{k_2} = \frac{[XH][A^+]}{[XA][H^+]} \tag{3}$$

$$K_3 = \frac{k'_3}{k_3} = \frac{[XH][XA]}{[X_2HA]} \tag{4}$$

$$K_4 = \frac{k'_4}{k_4} = \frac{[X^-][H^+]}{[XH]} \tag{5}$$

where K_1 describes the acid dissociation of the 2:1 ionophore–amine complex, K_2 describes dissociation of XA by proton exchange, K_3 describes the breakdown of the 2:1 complex (X_2HA) to give the 1:1 aggregate (XA) and free ionophore, and K_4 is the acid dissociation constant of the ionophore. A mass balance on ionophore in the membrane is given by Eqn. 6

$$X_T = [XH] + [X^-] + [XA] + [X_2HA] + [X_2A^-] \tag{6}$$

where $[X_2HA]$ and $[X_2A^-]$ are presumed to contribute negligibly (based on flux measurements) to X_T and with Eqns. 2–5 transform Eqn. 1 into an expression describing membrane conductance in terms of pH and total ionophore (X_T) and protonated amine (A^+) concentration, according to the proposed model (Eqn. 7):

$$g_0 = \frac{F^2}{RT} \cdot \frac{\nu \frac{K_1}{K_2K_3}[A^+][X_T]^2[H^+]}{\left\{[H^+] + K_1\left(1 + \frac{2\nu}{k'_1 + k'_3}\right)\right\}\left\{\frac{[A^+]}{K_2} + [H^+] + K_4\right\}^2} \tag{7}$$

Recall, however, that the transport of amine as charged species (as determined from conductance measurements) accounts for $<0.1\%$ of total amine transport.

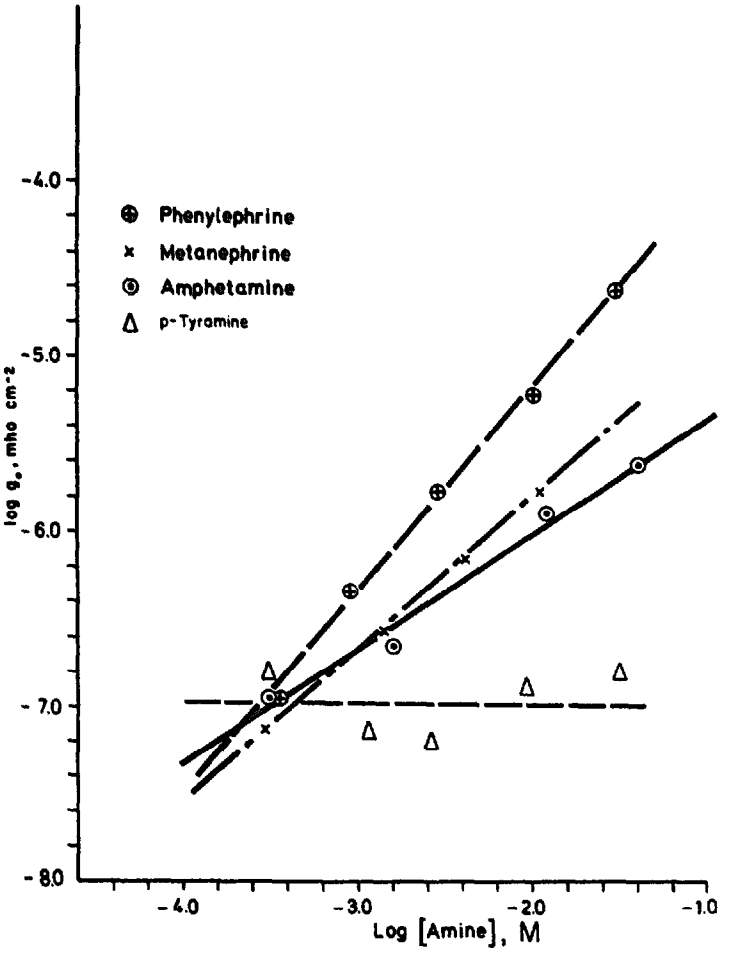


Fig. 1. Specific membrane conductance, g_0 (mho·cm^{-2}), plotted vs amine concentration (mol·l^{-1}) on both sides of the membrane. The aqueous phase was buffered at pH 7.0 (10.0 mM Hepes) and ionophore concentration in the membrane-forming lipid solution was 3.5 mM. Each data point represents an average value for 3–5 membranes.

According to the model, conductance should have a first-order dependency on $[A^+]$ (under the experimental conditions (pH = 7.0) chosen for the relatively weakly complexed amines used in this study, $K_4 \gg [H^+] + \frac{[A^+]}{K_2}$), a second-order dependence on $[X_T]$ and should show more complex behavior with pH change. In the absence of ionophore, no perceptable amine permeation of the bilayer membrane was observed. Membrane conductance is related to amine concentration (at constant ionophore concentration and pH) by the equation $g_0 = bC_{amine}^{\beta}$ (where β is the molecularity of the amine in the complex and 'b' is a constant). Conductance was determined as a function of amine concentration and plots of $\log g_0$ vs $\log C_{amine}$ (Fig. 1) were linear with slopes of 1 (within experimental error) indicating a first-order dependency on amine concentration in accord with Eqn. 7. If total amine permeability is monitored, however, flux reaches steady-state, apparently at a

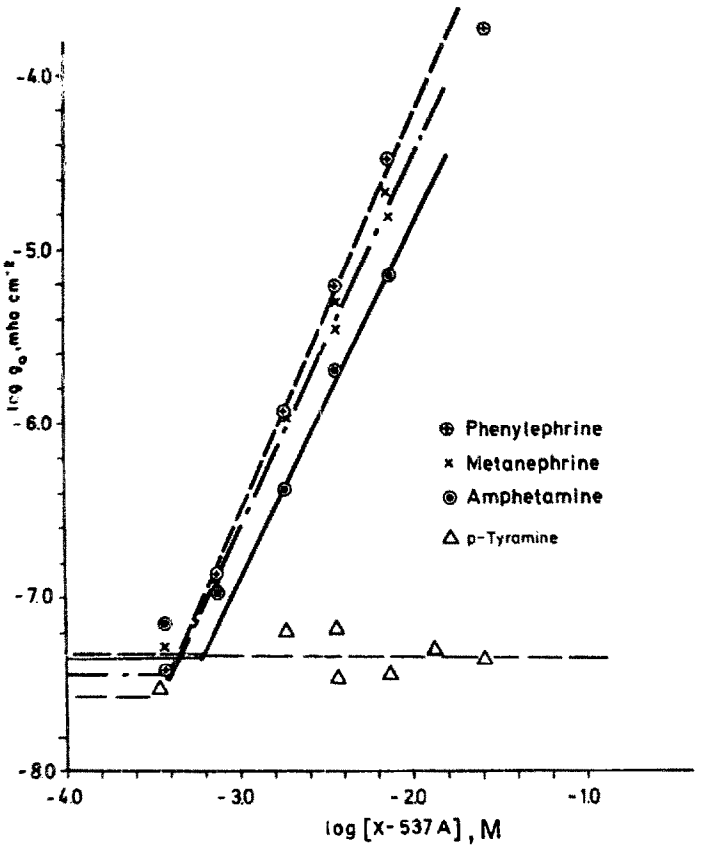


Fig. 2. Dependence of specific membrane conductance, g_0 (mho·cm^{-2}), on ionophore concentration (mol·l^{-1}) in the lipid solution used to form the membrane. The aqueous solution bathing the membrane was buffered at pH 7.0 (10.0 mM Hepes) and contained 50.0 mM amine (10.0 mM in the case of epinephrine). Each data point represents an average value for 3–5 membranes. The initial portion of each graph represents the membrane conductance in the absence of ionophore.

concentration of amine at which essentially all X is complexed (Kinsel et al., 1982b). Since the predominant species transported through the membrane is electrically silent (XA), it is not sensitive to conductance measurements. At constant amine concentration and pH, membrane conductance was determined as a function of ionophore concentration and can similarly be described by the equation $\log g_0 = aC^{\alpha}_{X\text{-}537A}$ (where α is the molecularity of ionophore in the complex and 'a' is a constant). Logarithmic plots of the data were linear (Fig. 2) with slopes approximately 2, indicating a second-order dependency of conductance on ionophore concentration, again in accord with Eqn. 7.

According to Eqn. 7, at constant amine and ionophore concentration, the variation of membrane conductance with change in pH should be of the form

$$g_0 = \frac{F^2}{RT} \frac{c_1[H^+]}{(c_2 + [H^+])(c_3 + [H^+])^2} \tag{8}$$

where c_1, c_2 and c_3 are constants. Membrane conductance, g_0, was measured and

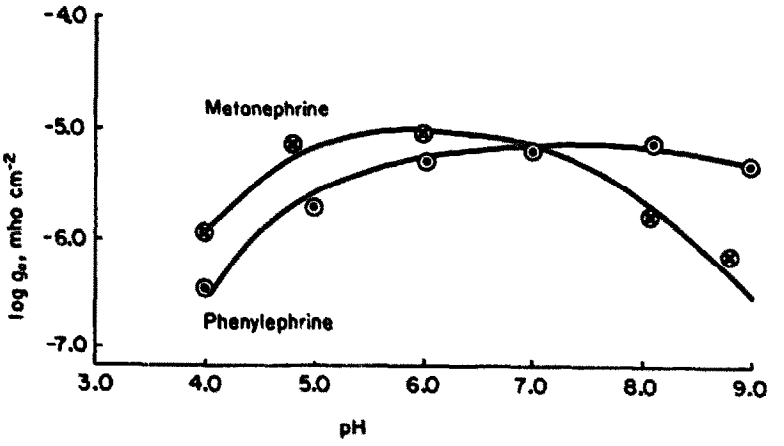


Fig. 3. Relation between specific membrane conductance, g_0 (mho·cm^{-2}) and pH of the aqueous phase containing 10.0 mM Hepes. Amine concentration on both sides of the membrane was 50.0 mM and ionophore concentration in the membrane-forming lipid solution was 3.5 mM. Each data point represents an average value for 3–5 membranes.

plotted (after subtracting the conductance of the membrane without ionophore) as a function of pH for phenylephrine and metanephrine (Fig. 3). The form of the curves generated by computer fit of Eqn. 7 (X_T and A^+ held constant) was consistent with that expected from the general polynomial expression (Eqn. 8) in which the c_1, c_2 and c_3 terms all contribute to the observed variation in conductance. Calculations using Eqn. 8 reveal that the value of g_0 is quite sensitive to the numerical values of the individual c's; a 5% variation in K_1, K_2, K_3 and K_4 caused approximately a 40% variation in g_0. Attempts to mathematically describe g_0 with fewer than 3 parameters were unsuccessful. Thus, Eqn. 7 adapted from the general conductance equation (Eqn. 1) in terms of the model proposed in Scheme I, appears to adequately explain the conductance of amines through a bilayer membrane modified with ionophore lasalocid. It is difficult to ascribe physical significance to the shapes of these curves which are governed by the relative magnitude of c_1, c_2 and c_3, since these constants are heterogeneous, including contributions from acid dissociation and partition constants that cannot be determined independently.

The shape of the right-hand portion of the curve is governed by c_3 (i.e. $\frac{[A^+]}{K_2}$) and K_4. Since phenylephrine and metanephrine have similar pK_as (Tuckerman, 1959), K_4, the acid dissociation constant at the interface, and $[A^+]$, the concentration of protonated amine, are not likely to differ substantially and, therefore, it is anticipated that K_2 plays a dominant role in determining the magnitude of c_3. A higher affinity of lasalocid for an amine is reflected as a lower K_2 (higher c_3) value which when squared could account for the decrease in membrane conductance evidenced for metanephrine relative to phenylephrine.

Inhibition of membrane conductance

Amines that complex with and are transported by lasalocid *exclusively* as 1:1

neutral complexes (e.g. *p*-tyramine) appear to have the greatest affinity for the ionophore, i.e. K_2 is small. Higher order complexes do not appear to form (K_3 is small) either because generation of the 1 : 1 complex (XA) is followed by immediate membrane transport or the structure of the amine may not lend itself to aggregation with ionophore to form X_2HA or X_2A^-. Membrane conductance in the presence of *p*-tyramine, for example, is independent of amine concentration, ionophore concentration and pH as expected if no charged species are formed (Figs. 1 and 2). By virtue of its high affinity for the ionophore coupled with its ability to inhibit background membrane conductance attributed to ionophore (X^-) *p*-tyramine may also be capable of inhibiting the conductance across bilayer membranes of amines that form charged complexes with the ionophore. The addition of *p*-tyramine to membranes modified by lasalocid and containing either epinephrine, norepinephrine, phenylephrine, metanephrine or amphetamine (amines known to form charged complexes with lasalocid) caused a dramatic and complete reduction in membrane conductance to the limiting background conductance of the membrane (a factor of at least two orders of magnitude) (Fig. 4). No explanation is offered to explain the apparent increase in g_0 observed at lower *p*-tyramine concentrations. The amount of *p*-tyramine needed to inhibit membrane conductance was consistent with affinity constants of amines for ionophore as calculated from monolayer, partition-

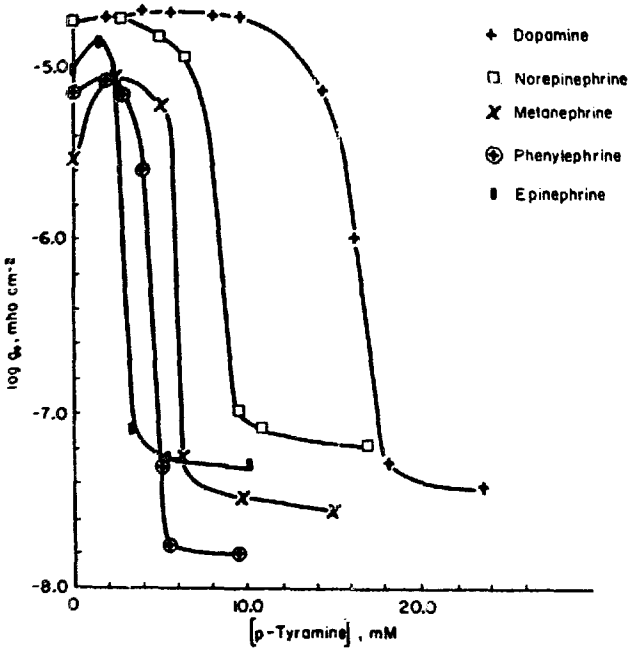


Fig. 4. Influence on specific membrane conductance, g_0 (mho·cm^{-2}), of increasing the concentration of *p*-tyramine (mol·l^{-1}) on both sides of the lasalocid-doped lipid bilayer membrane. The aqueous phase, containing 50.0 mM amine, was buffered at pH 7.0 (10.0 mM Hepes) and ionophore concentration in the lipid solution used to form the bilayer membrane was 3.5 mM. Each data point represents an average value for 3–5 membranes.

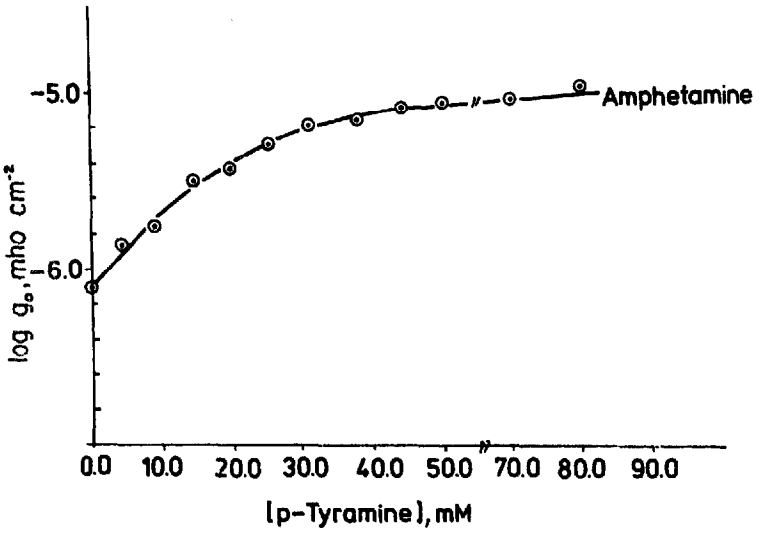


Fig. 5. Influence on specific membrane conductance, g_0 (mho·cm^{-2}), of increasing the concentration of *p*-tyramine (mol·l^{-1}) on both sides of the lasalocid-doped lipid bilayer membrane. The aqueous phase, containing 50.0 mM amine, was buffered at pH 7.0 (10.0 mM Hepes) and ionophore concentration in the lipid solution used to form the bilayer membrane was 3.5 mM. Each data point represents an average value for 3–5 membranes.

ing and flux data (Kinsel et al., 1982b), i.e. the amine exhibiting the strongest complexation with the ionophore requires the greatest amount of *p*-tyramine to suppress conductance. Thus, the required amount of *p*-tyramine needed to inhibit conductance increased (as determined by the inflection points in Fig. 4) in the order epinephrine < phenylephrine < metanephrine < norepinephrine < dopamine. The inhibitory effect of *p*-tyramine on conductance was not observed with amphetamine (Fig. 5) (which has a complexation constant similar to *p*-tyramine) within the range of tyramine concentration used, but would be expected to suppress conductance at higher concentrations. According to the proposed model, tyramine appears to act by either forming a tight complex with ionophore, depleting its availability (for the process governed by k'_2) for interaction with amines capable of forming charged complexes or by blocking k_3 (i.e. formation of higher order complexes from 1:1 (XA) complexes). The significance of such inhibition of membrane transport in physiological systems and the use of ionophores as novel drug delivery systems for amine-containing drugs is the basis for future studies.

Acknowledgements

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