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Selective Transport of Li^+ across Lipid Bilayer Membranes Mediated by an Ionophore of Novel Design (ETH1644)

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Summary. The neutral noncyclic, lithium-selective ionophore ETH1644, which is structurally different from previously available ionophores of this type, is a selective carrier of Li^+ in lipid bilayer membranes of various lipid composition. The ionophore forms a 2 : 1 carrier/cation complex, and the rate-limiting step in the overall transport process is the diffusion of the carrier/ion complex across the membrane.

The selectivity sequence for lithium *vs.* other ions normally found in biological systems is: Li^+ (1) > Na^+ (0.017) ≥ K^+ (0.017) > Cl^- (0.001), Ca^{2+} and Mg^{2+} are impermeant. At neutral pH protons do not interfere with the Li^+-carrying ability of this ionophore. On the basis of structural differences and supported by conductance data, it is argued that the improved selectivity of Li^+ over the other alkali cations is due more to a decrease in the affinities of the ionophore for the latter cations that to an increase of its affinity to Li^+. This ionophore can also act as a carrier of biogenic amines (catecholes, indoles and derivatives), with the structure of the permeant species and mechanism of permeation similar to that observed with the alkali cations. The selectivity sequence is: tryptamine (18.1) > phenylethylamine (11.6) > tyramine (2.4) > Li^+(1) > serotonin (0.34) > epinephrine (0.09) > dopamine (0.05) > norepinephrine (0.02), showing the ionophore to be more selective to Li^+ than to any of the neurotransmitters studied.

Key Words lithium · ionophore · ion-transport · lipid bilayers · biogenic amines

Introduction

Synthetic noncyclic ionophores for Li^+ have been available for the last decade. Until recently all molecules [6, 16, 24] had a diether diimide backbone, differing among them only in the residues located at one of the N-imide positions (Fig. 1). These molecules act as Li^+-selective carriers in lipid bilayer membranes, and (all) have a lyotropic selectivity sequence, but with differences in the magnitudes of selectivity [14–16]. The best of them, AS701, favors Li^+ over Na^+ by a factor of 13.

Since the lithium ion has poor permeability through biological membranes, a selective Li^+ carrier could, conceivably, be of use in any such system where an improved permeability of this ion is desired. In the short run, this would make the ionophore a useful research tool. In the long run, there are potential therapeutic applications, for example in the treatment of manic-depressive illness [8, 20, 21, 23] and in certain hematological disorders [5, 11, 22, 25]. To perform as such, an ionophore would have to select Li^+ over other physiological ions by a margin better than has been achieved so far. This rationale, and the availability of a new ionophore, ETH1644 [26], have lead us to study it as a potential carrier of lithium in a system modeling biological membranes: planar lipid bilayers.

Our strategy was the following: first, test whether this ionophore can transport Li^+ (at all) across the bilayer and, if affirmative, to assess the type of mechanism. Next, provided the first step is satisfactory, determine the selectivity between lithium and sodium, which is the most-likely inorganic ion competitor in biological systems. Proceeding beyond this point would depend on whether this ionophore should prove to be as good as, or better than, the best of the former series. The third stage would include determination of the selectivity of lithium *vs.* various ions which are normally present in a biological system, some of which we found to be carried by ionophores of the former design [12, 17]. These ions fall, roughly, into three groups: monoatomic cations and anions—Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-; protons; polyatomic quaternary amines—NH_4^+ and biogenic monoamines (catecholes and indoles). We have also added Rb^+ and Cs^+ to the list of ions studied.

Materials and Methods

The ionophore used in this study (ETH1644) was a kind gift from W. Simon. Glyceril monooleate (GMO)[1] and soybean phosphati-

[1] *Abbreviations used:* DA—dopamine. EP—epinephrine. GMO—glyceril monooleate. NE—norepinephrine. PE—phosphatidylethanolamine. PEA—phenylethylamine. Ser—serotonin. Trp—tryptamine. Tyr—tyramine

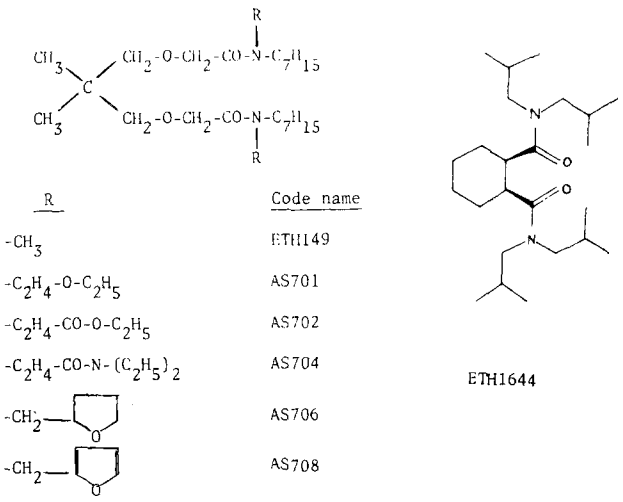

Fig. 1. Structure formulas of Li^+-selective ionophores

dyl choline type IV-S (asolectin) were purchased from Sigma Chemical Co.

Membranes were formed on the aperture (usually 1 mm diameter) of a Teflon cell from lipid/decane solutions (25 mg/ml for GMO, 50 mg/ml for asolectin). Steady-state electrical properties of the membrane were measured using previously-described methods [4, 14, 16, 17]. To avoid contamination of the aqueous phases, their pH was measured in aliquots of 250 μl, taken out at desired intervals (symmetrically, from each aqueous phase) in the course of an experiment.

Results and Discussion

Ionophore-Mediated Transport of Li^+

In the presence of 1 N LiCl, we have found this ionophore to induce considerable increase in the zero-current conductance of lipid bilayer membranes, independant of the lipid composition of the membrane. Typical examples are illustrated in Fig. 2*a*. Furthermore, for all lipid compositions tested, the increase of conductance with the increase in ionophore concentration is regular and second order, indicating an ionophore stoichiometry of 2. The observation that the membrane conductance, for any given ionophore concentration within the region studied, decreases from GMO to asolectin to PE, which is the direction of increase in membrane surface dipole potential as well as the magnitudes of the decrease, indicate that the permeant species is a carrier of a postively-charged ion [1, 7, 14]. Additional support for a carrier rather than a channel mechanism, comes from the combination of the small size of the ionophore (*recall* Fig. 1) and a stoichiometry of no more than two (Fig. 2*a*): A channel formed from two molecules would be too small to span the lipid bilayer [14].

To determine the stoichiometry of the charged species, we have followed the increase in membrane zero-current conductance with the increase in LiCl concentration, at a constant ionophore concentration. Typical results illustrated in Fig. 2*b* clearly show a first power dependence, giving an ion stoichiometry of 1.

In order to identify the ion carried (i.e., Li^+ or Cl^-) we have measured the increase in zero-current potential of GMO membranes, in the presence of a constant ionophore concentration, under a LiCl gradient. If only one type of ion/ionophore permeant complex is present in the system, the data should conform to the following equation:

$$V_o = \frac{RT}{F}\frac{n}{z}\ln\frac{a_i'}{a_i''} \qquad (1)$$

where $'$ and $''$ indicate parameters, in general, in the aqueous phases on both sides of the membrane, a_i is the activity of an ion in an aqueous phase, n in the ion stoichiometry (1 in this case), and z is the net-charge of the permeant species. We have plotted in Fig. 2*d* the theoretical expectations, according to Eq. (1), for both ions in the system, Cl^- and Li^+, and compared them to the experimental data, which fit unambiguously with the expectation for Li^+. Thus, the overall stoichiometry and composition of the permeant complex ETH1644 forms, in the presence of LiCl, is a 2:1 ionophore/Li^+ complex. In this respect, the present system is similar to all the ionophores of the former design [14–16]. Another similarity is in the type of mechanism. For the other Li^+-carriers we have found the rate-limiting step in the overall transport process to be the diffusion of the carrier/ion complex across the membrane [14–16]. For this type of mechanism the increase of membrane conductance with the increase of the potential drop applied across the membrane should be hyperbolic [10], as indeed found there [14–16] and in the present case, as exemplified by the data illustrated in Fig. 2*c*.

Selectivity of Li^+ vs. Na^+

The first indication of a considerable Li^+ over Na^+ selectivity can be observed from the difference in the membrane zero-current conductances, in the presence of LiCl or NaCl, as illustrated in Fig. 3 (left-hand side) and from the conductance ratio of these ions listed in Table 1. To determine the ratio of the permeabilities of these two ions (P_{Na}/P_{Li}), we

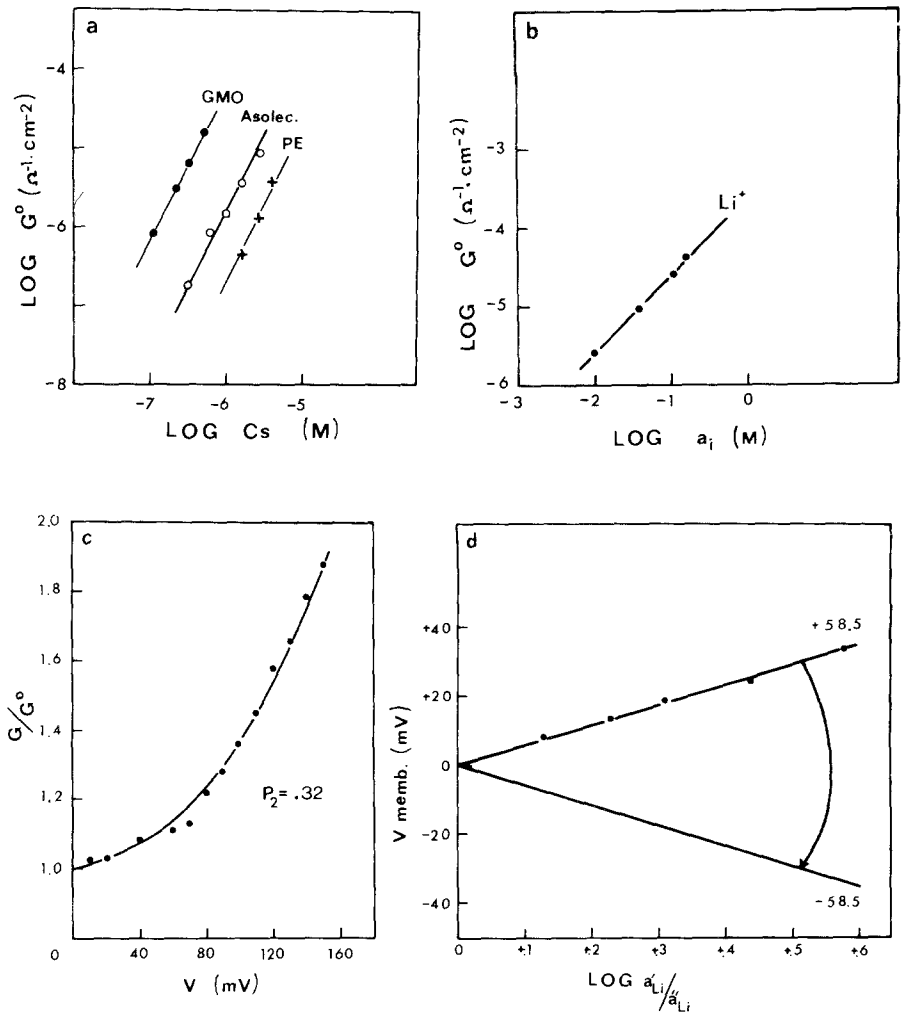

Fig. 2. (*a*) Dependence of zero-current conductances of GMO, asolectin and PE membranes on ionophore concentrations, in the presence of 1 N LiCl. *Ordinate:* Logarithm of membrane conductance. *Abscissa:* Logarithm of ionophore concentration in the aqueous phases. Points are experimental, lines drawn to a slope of 2. (*b*) Dependence of zero-current conductance of GMO membranes on LiCl concentration in the presence of 1×10^{-6} M ETH1644. *Ordinate:* Logarithm of membrane conductance. *Abscissa:* Logarithm of salt concentration in the aqueous phases. Points are experimental, line drawn to a slope of 1. (*c*) Conductance-voltage relationship of GMO membranes, in the presence of 1 N LiCl and 1×10^{-6} M ETH1644. *Ordinate:* The membrane conductance G, normalized to G^o—the membrane conductance at the limit of zero-current. *Abscissa:* The potential drop applied across the membrane. Points are experimental. The solid curve is the theoretical expectation for the type of mechanism discussed in the text, using a value of $P_2 = 0.32$ for the "barrier-shape" parameter (*see* reference 14 for details and for the quantitative expression). (*d*) Dependence of the zero-current potential drop across GMO membranes on the gradient of LiCl ("dilution potentials"). *Ordinate:* Membrane potentials. *Abscissa:* Logarithm of the ratio of the activities of lithium in the aqueous phases bathing the membrane. Points are experimental. The solid lines are the theoretical expectations according to Eq. (1), for Li^+ (positive slope) or for Cl (negative slope) being the transported ion

have measured the zero-current potentials of GMO membranes in the presence of a constant ionophore concentration and LiCl/NaCl gradients (*see* references 10 and 14 for additional details of the experimental design and the data processing). Typical results are illustrated in Fig. 4. The points are the experimental data (each an average of 2–4 measurements) giving a ratio of 1.68×10^{-2}; the curve is the theoretical expectation, drawn according to the following equation for the magnitude of the ratio listed above:

$$V_o = \frac{RT}{F} \ln \frac{a'_{Na}(P_{Na}/P_{Li}) + a'_{Li}}{a''_{Na}(P_{Na}/P_{Li})}. \quad (2)$$

As can be seen in Fig. 4, there is good agreement between the experimental data and the theoretical

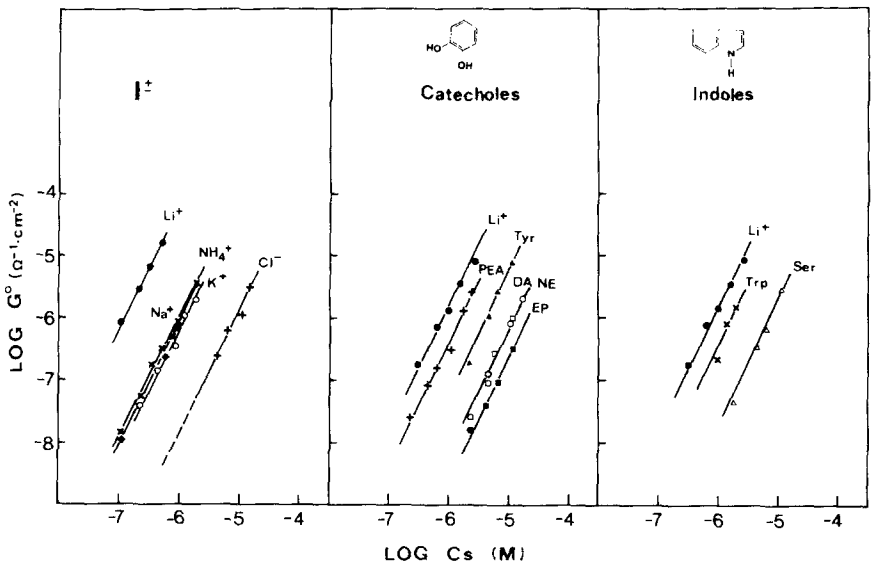

Fig. 3. Dependence of zero-current membrane conductance on ionophore concentration, for the following systems: *Left-hand section:* GMO membranes and 1 N chloride salts of the indicated cations, excepting $CaCl_2$ for the chloride ion itself. *Middle and right-hand sections:* asolectin membranes, in the presence of the indicated amine at the following concentrations: 10 mM for PEA, Trp, Tyr and Ser. 50 mM for DA, NE and EP. LiCl is 1 N. Ionic strength of biogenic amine systems kept constant by 1 N CsCl. *Ordinate:* Logarithm of membrane conductance. *Abscissa:* Logarithm of ionophore concentration in the aqueous phases. Points are experimental. Lines drawn to a slope of 2

Table 1. Permeability and conductance ratios of the 2 : 1 carrier/ion complexes of ETH1644 determined in GMO membranes

Ion	P_i/P_{Li}	G_i''/G_{Li}''
Li^+	1.00	1.00
Na^+	1.68×10^{-2}	2.04×10^{-2}
K^+	1.68×10^{-2}	1.12×10^{-2}
Rb^+	8.09×10^{-3}	1.05×10^{-2}
Cs^+	8.55×10^{-3}	7.94×10^{-3}
NH_4^+	2.01×10^{-2}	1.58×10^{-2}
Cl	9.94×10^{-4}	2.24×10^{-4}

expectation over the entire range of potentials measured and salt concentrations employed. This agreement indicates not only low experimental scatter but also that the permeabilities determined are true parameters, independent of membrane potential and of salt concentration, as expected for the mechanism deduced from the data of the conductance-voltage relationship (*recall* the previous section and Fig. 2*c*). The permeability ratio obtained is also in good agreement with the ratio of conductance for these two ions, listed in Table 1. The factor by which lithium is favored over sodium in this ionophore system, a fivefold increase from the best ionophore of the former lot, is encouraging in two respects: it justifies proceeding to the third step of this study (outlined in the strategy presented in the introduction); moreover, this ratio is of a magnitude realistic to consider studies in biological systems.

Selectivity of Li^+ vs. Monoatomic Cations and Anions

Two independent types of data clearly show the high selectivity of Li^+ over K^+, Rb^+, Cs^+ and Cl^- (in addition to the already-discussed case of Na^+): The increase of membrane zero-current conductance with the increase in ionophore concentration in the presence of any of these ions, for which typical data are illustrated in Fig. 3. The zero-current membrane potentials measured in a salt mixture of LiCl and a chloride salt of and any of these ions, for which typical data are illustrated in Fig. 4 (*see also* Table 1). It should be noted that, even though Cl^- has poor permeability in this ionophore system, where relevant its contribution to the measured potential was taken into account. The theoretical expectation for such systems can be expressed in the following form:

$$V_o = \frac{RT}{F} \ln \frac{a_i'(P_i/P_{Li}) + a_{Cl}''(P_{Cl}/P_{Li}) + a_{Li}'}{a_i''(P_i/P_{Li}) + a_{Cl}'(P_{Cl}/P_{Li})} \qquad (3)$$

where a_i and a_{Cl} are the activities of the i^{th} ion and Cl^-, and P_i/P_{Li} and P_{Cl}/P_{Li} are the corresponding permeability ratios.

As to Ca^{2+}, Mg^{2+} and determination of the permeability ratio of chloride to lithium, the results of dilution-potential experiments in the presence of $CaCl_2$ or $MgCl_2$ (data not shown) clearly identify the chloride ion and not the divalent cation to be the ion transported (however poorly) in these cases. Hence to the limit of the sensitivity of our instrumentation, these ions are impermeant in the present ionophore system (in lipid bilayer membranes). The permeability of chloride to lithium could be determined from membrane potentials measured in $LiCl/CaCl_2$ or $LiCl/MgCl_2$ gradients. Indeed, the data shown in Fig. 4 have been obtained from such experiments. The selectivity sequence we have obtained for the alkali cations is a permitted sequence in the Eisen-

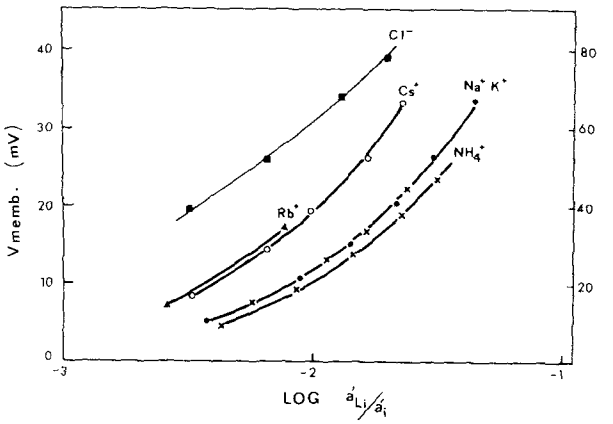

Fig. 4. Zero-current potentials of GMO membranes in the presence of 1×10^{-6} M ETH1644 and salt mixtures. One-sided additions of LiCl to a chloride salt of the indicated cations (the latter symmetrical in concentration with respect to both sides of the membrane). For Cl^-—a calcium salt. *Ordinate:* Observed membrane potential; right-hand scale is for Cl^- and lefthand scale is for all other ions. *Abscissa:* Logarithm of the ratio of activities of lithium to the other ion, on the membrane side of the lithium additions. Points are experimental. Solid curves are the theoretical expectations, drawn according to Eq. (2), for the permeability ratios listed in Table 1

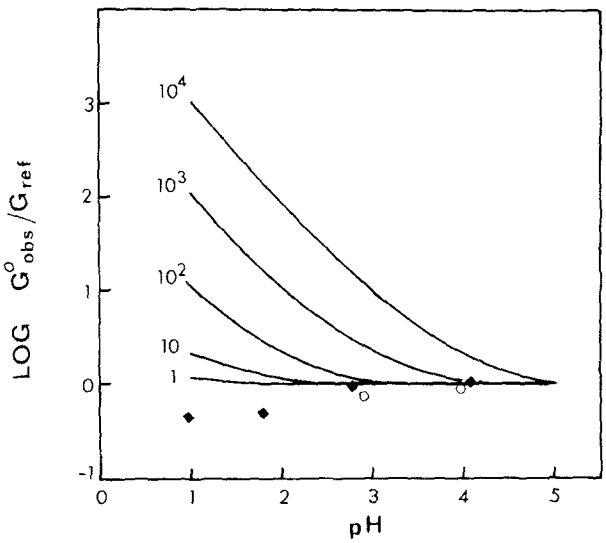

Fig. 5. The effect of pH on the zero-current conductance of GMO and asolectin membranes, in the presence of 1 N LiCl and 5×10^{-7} M ETH1644. *Ordinate:* Logarithm of the membrane conductance at a given pH normalized to the membrane conductance at the pH of 5.1. *Abscissa:* pH of the aqueous phases bathing the membrane. Points are experimental, solid curves are the theoretical expectations, drawn according to Eq. (3), for the permeability ratios of proton to lithium listed in the figure

man series [3] and similar to that obtained for the other lithium ionophores [14–16].

Protons

We tested whether protons could interfere with the performance of this ionophore as a selective carrier for Li^+ under physiological conditions. To that end we have measured the effect of decreasing the pH symmetrically on both sides of the membrane, by HCL additions, on the membrane conductance in the presence of IN LiCl and the ionophore. For GMO membranes the pH range was 1–5, for asolectin 3–5. Under these conditions ionophore-independent proton conductance in phospholipids is not promoted [9]. The magnitudes of membrane conductance measured at various pH levels (normalized to the conductance at pH 5.1) were compared to the theoretically-expected normalized conductances for various permeability ratios of protons to lithium, running over the range of $1–10^4$. The rationale for choosing the upper limit of this range was the following: *In vivo,* to avoid toxicity, plasma lithium concentrations should not exceed 1 mM [20]. This gives a concentration ratio of 10^4 for Li^+/H^+, for which a permeability of protons exceeding that of lithium by two orders of magnitude or more would indicate the possibility of proton interference. The theoretical expectation based on ref. [25] is:

$$\frac{G^o_{pH}}{G^o_{ref}} = \frac{1 + (P_H/P_{Li})(a_H)}{1 + (P_H/P_{Li})(a_H)_{ref}} \tag{4}$$

where G^o_{pH} is the membrane conductance at any given pH and G^o_{ref} is that conductance at a specific pH, chosen here to be 5.1.

The expected conductance ratios, for several proton to lithium permeability ratios, together with the experimental data, are plotted in Fig. 5 (in the logarithmic form) *vs.* the pH (the differences from one experimental point to the other are due mainly to membrane breakages, the trend having no theoretical significance). This comparison allows us to conclude that in lipid bilayer membranes the P_H/P_{Li} ratio induced by the investigated ionophore would not cause any interference with its activity in transporting Li^+ under physiological salt conditions.

NH_4^+ and Biogenic Amines

We have added NH_4^+, dopamine, epinephrine, norepinephrine and serotonin and several of their derivatives (under pH conditions where these amines are fully protonated, carrying a net charge of +1) to the ions studied in the course of this investigation. These species are present in biological systems, and several of the Li^+-selective ionophores we have previously studied carry them [12, 17].

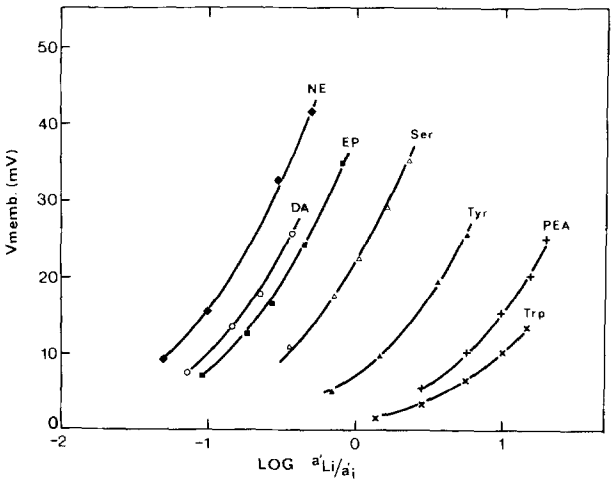

Fig. 6. Zero-current potentials of asolectin membranes, in the presence of ETH1644 and salt mixtures of LiCl and the biogenic amine indicated. One-sided additions of lithium. Ordinate and abscissa are as defined in Fig. 4. Points are experimental, solid curves are theoretical expectations, according to Eq. (2), for the permeability ratios listed in Table 2

We have found this ionophore to form 2 : 1 complexes with ammonium and with the biogenic amines studied, similar to its complexes with alkali cations and similar to the complexes of the other lithium ionophores with biogenic amines [12, 17]. This is exemplified for the ionophore stoichiometry by the data illustrated in Fig. 3. To determine the ionophore-induced selectivity of each of these amines relative to lithium, thus estimating their potential for interference in biological systems, zero-current potentials of asolectin membranes were measured under LiCl/Biogenic-amine salt gradients. Typical results are illustrated in Fig. 6, where the points are the experimental data and the solid curves are the theoretical expectations according to Eq. (2), for the magnitudes of the permeability ratios listed in Table 2. Since the experimental conditions required maintaining a constant ionic strength (1 N CsCl was used) where relevant the contributions of Cs^+ and Cl^- to the observed membrane potential were taken into account. The data clearly show that this ionophore induces a considerable span of selectivities among the biogenic amines.

Since the biogenic amines studied are either carried better than NH_4^+ or comparable to it, it seems obvious that more than the favorable electrostatic interaction of the quaternary amine head with the imide oxygens of the ionophore is involved here. We suggest hydrophobic interactions of the lipophylic moieties of a biogenic amine with similar-natured regions of the ionophore, as discussed in detail elsewhere [12, 17].

As to the possible interference of these biogenic amines in the transport of Li^+ this ionophore could mediate in a biological system: The low permeability of the neurotransmitters among the biogenic amines studied, together with their low *in vivo* levels [18, 19] indicate that a serious interference is unlikely. Also, in this respect the present ionophore is better than AS701 (which is the best for lithium, among the former), since ETH1644 is less selective to biogenic amines than AS701 [17].

Table 2. Permeability and conductance ratios of the 2 : 1 carrier/biogenic-amine complexes of ETH1644 to the lithium complex, determined in asolectin membranes

Amine	P_i/P_{Li}	G_i''/G_{Li}''
Tryptamine	18.10	25.12
Phenylethylamine	11.56	25.12
Tyramine	2.39	3.98
Serotonin	0.34	0.60
Epinephrine	9.10×10^{-2}	5.10×10^{-2}
Dopamine	5.09×10^{-2}	8.53×10^{-2}
Norepinephrine	1.65×10^{-2}	8.53×10^{-2}

Molecular Factors Involved in the Improved Selectivities Induced by ETH1644

The data presented in this report show that ETH1644 is presently the best Li^+-selective carrier among the noncyclic, neutral, synthetic ionophores available. It selects Li^+ over Na^+ by a factor of 60 and has the least possibility of interference from other ions normally present in a biological system. These findings and the structural differences between this ionophore and the others (*recall* Fig. 1) raise the following question: Can those structural differences account for the improvement in selectivity?

Owing to the replacement of the ether oxygens by carbon units in the "backbone" of ETH1644, a molecule of this ionophore has only two oxygenic ligands, whereas the other ionophores have four or more per molecule. Among the alkali cations lithium can form stable complexes with a coordination of four [2], which can be supplied in any of the available ionophores (of both designs) by the four "backbone" imide oxygens present in a 2 : 1 carrier/ion complex. In fact, analysis of thermodynamic data of hydration and solvation of alkali cations [13, 14] has lead to the conclusion that for Li^+ these imide oxygens are the major ligands. Yet ionophores such as ETH149 and the AS-R series have additional oxygens which can accommodate cations larger than lithium, requiring a coordination of six or more. Thus, the abundance of ligands in these ionophores, together with their noncyclic

structures which enables the formation of cavities to fit the size of any given ion, makes it possible for these ionophores to bind several species of alkali cations with similar affinities, at the expense of the span in selectivities [14–16]. In this respect, ETH1644 is quite different. It has no excess of oxygen ligands—only two per ionophore molecule, sufficient to satisfy the liganding needs of Li^+, but not of larger cations. An experimental observation that bears on this issue comes from membrane conductance. If one compares the conductances observed in the present case with data of similar type and experimental conditions for the other ionophores [14–16], it can be seen that the lithium conductances are of similar level, but those of the other alkali cations in this system are much lower in the present system.

Taking the issues discussed above into consideration, we propose that the improved Li^+ selectivity of the present ionophore is not due to an increase in the binding of lithium to the ionophore, but rather to a decrease in the binding of the other alkali cations to it.

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