J. Membrane Biol. 121, 133–140 (1991)
002226319100062J

The Journal of
Membrane Biology

Lithium-Selective Permeation through Lipid Bilayer Membranes Mediated by a Di-Imide Ionophore with Nonsymmetrical Imide Substituents (ETH1810)

Amira Zeevi and Rimona Margalit
Department of Biochemistry, George S. Wise Life Science Center, Tel-Aviv University, Tel-Aviv 69978, Israel

Summary. The neutral, noncyclic Li^+-selective ionophore ETH1810, which is a di-imide, differs structurally from previous similar ionophores by removal of the intramolecular symmetry of the N-imide substituents. Properties of this ionophore, as a potential carrier of lithium, were probed through studies of ionophore-induced changes in electrical properties of lipid bilayer membranes. ETH1810 was found capable of transporting lithium and other monovalent cations, across lipid bilayer membranes, forming 2:1 ionophore:ion membrane-permeating species. It was found to be 10-fold more potent than ETH1644, which was the previous best ionophore of this type. The selectivity sequence among alkali cations was found to be: Li^+ (1) > Na^+ (0.009) > K^+ (0.004) > Cs^+ (0.0035). Among the physiological alkali cations, it constitutes a 40 (*vs.* Na^+) to 160% (*vs.* K^+) improvement over ETH1644. ETH1810 was also found to be capable of acting as a carrier of biogenic amines and related molecules, with the following selectivity sequence: tryptamine (20) > phenylethylamine (7.8) > tyramine (4.3) > serotonin (2.5) > Li^+ (1) > NH_4^+ (0.013) > dopamine (0.012). It was found that protons, at physiological concentrations, do not interfere with the lithium transport mediated by ETH1810. The relationship between the improvements in ionic selectivity and potency *vs.* the differences in structural features is discussed.

Key Words lithium · ionophore · ion transport · lipid bilayers · biogenic amines · ionic selectivity

Introduction

The lithium ion has been found to engage in numerous activities in biological systems, interacting with transport systems, with components of neurological systems and with enzymes [2, 4, 25, 27, 28, 34]. Some of these activities have found therapeutic applications, most notable among them being the treatment and control of manic-depressive illness [5, 14, 26, 31, 32]. Other therapeutic applications, still under investigation, are alcoholism, neutropenia, drug addiction, bulemia and anorexia [7, 8, 13, 29, 37].

Lithium is not a "physiological inorganic ion." A consequence of this situation is its poor permeability through biological membranes, whereas the physiological inorganic alkali cations, Na^+ and K^+, permeate through selective transport systems nature has designed for them. The poor permeability of lithium could be one of the factors contributing to the undesirable side effects accompanying lithium therapy and is also an obstacle and limitation in research into the biological activities of this ion [1, 3, 14–16, 21, 30, 31, 36, 39].

Conceivably, lithium permeation through biological membranes could be improved by the application of lithium-selective transport systems. Such systems could serve, in the short run, as useful research tools and, in the long run, as aids in lithium-mediated therapy.

In the course of the last 15 years, a variety of noncyclic synthetic lithium-selective ionophores have become available [6, 9, 11, 12, 23, 24, 33, 35, 41]. Among them, in the molecules developed by Simon et al. [9, 23, 24, 41] and by Shanzer et al. [33], imide oxygens, capable of acting as ligands for Li^+, are found to be a conserved structural feature. In contrast, both the backbone and the substituents on the imide nitrogens have undergone considerable structural changes throughout the years.

Currently, on a structural basis, these ionophores can be divided into two groups (to be denoted groups I and II), as shown by the examples illustrated in Fig. 1. Besides differences in the backbone, the major structural differences between the two groups are most pronounced in the state of oxygens, available for ion liganding. Members of group I have considerably more oxygens per molecule than members of group II. Within group I, besides offering additional oxygens, the various N-imide substituents can produce inductive effects of the backbone oxygens. Inductive effects of a similar type can also be produced by the N-imide substituents in group II. However, in the latter group these substituents

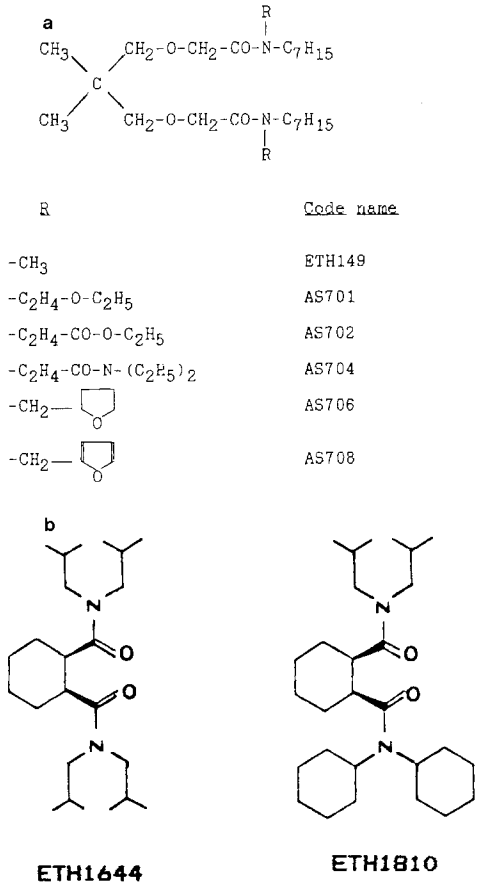

Fig. 1. Structure formulas of lithium-selective ionophores. (*A*) Molecules with a di-imide di-ether backbone. (*B*) Molecules with a di-imide backbone

do not offer additional oxygens for ion liganding, nor do they differ from one another to the extent observed among the members of group I.

We have previously investigated lithium-selective permeation of lipid bilayer membranes, mediated by ionophores listed in group I and by ETH1644 (of group II) [17–21, 40]. All molecules were found to act as lithium carriers in these membrane systems [18–20, 40]. The major difference among members of group I are in the magnitudes of ionic selectivity. While all select lithium over sodium, even the best of them (AS701) does not show high selectivity, giving a lithium/sodium permeability ratio of 13 [19, 20].

ETH1644 (of group II) did not show any significant improvement in potency (compared to AS701, the best of group I) but has been found to induce better selectivity, giving a lithium/sodium permeability ratio of 60 in lipid bilayer membranes [40]. Yet, considering the levels of sodium concentration in biological fluids [5], even this fivefold increase in selectivity was not considered sufficient with respect to expectations for in vivo performance. Therefore, the search for Li^+-selective ionophores which would induce a better (relative to the available ionophores) lithium selectivity in biological membranes is still on. It should also be noted that, for in vivo applications, the search for improved lithium selectivity has to be extended beyond sodium, to other ions also normally present in a living system.

The present communication is another step in that search. It is a report of molecular-level studies conducted in planar lipid bilayer membranes with ETH1810, which is another representative of group II. The first part of this report is focused at the activity of this ionophore as a lithium carrier. The second part is focused at the selectivity profile with respect to ions and molecules regularly present in biological systems such as Na^+, K^+, protons, and biogenic amines.

Materials and Methods

The ionophore used in this study [ETH1810] was a kind gift from W. Simon. Glyceril monooleate (GMO)[1] and soybean phosphatidyl choline type IV-S (asolectin) were purchased from Sigma Chemical. All other reagents were of analytical grade.

Membranes were formed on the aperture (usually 1-mm diameter) of a Teflon cell from lipid/decane (25 mg/ml for GMO and 50 mg/ml for asolectin) solutions. Steady-state electrical properties of the membranes and the pH of the bathing solutions were measured using previously described methods [17, 19, 20, 40].

Results and Discussion

ETH1810 as a Lithium Carrier in Lipid Bilayer Membranes

The combination of the following two types of experiments can provide an assessment of the ability of ETH1810 to act as a lithium carrier: (*i*) Membrane conductance: The presence of the ionophore in a membrane bathed by lithium salts should induce an increase in membrane conductance, with quantitative dependences on ionophore and salt concentrations. (*ii*) Membrane potential: The dependence of the electrical potential drop across the membrane, on the gradient of lithium chloride as the single salt in the system, should reflect the fact that the transported species is an ion with a net charge of +1.

[1] *Abbreviations:* DA, dopamine; GMO, glyceril monooleate; PEA, phenylethyl amine; Ser, serotonin; Trp, tryptamine; Tyr, tyramine.

Typical results for such experiments are illustrated in the three sections of Fig. 2. The increase of membrane zero-current conductance, for GMO membranes, with the increase in ionophore concentration is illustrated in Fig. 2*a* for four sets of data, differing from one another in the magnitude of the constant lithium chloride concentration in the system. The data illustrate quite clearly that there is a substantial increase in the membrane conductance induced by the ionophore. Moreover, the increase is regular, showing a second power dependence on ionophore concentration.

The increase in membrane zero-current conductance with the increase in lithium activity, at a constant ionophore concentration, (replotted from data of the type given in Fig. 2*a*) is illustrated in Fig. 2*b*. These data also show a regular increase of conductance with concentration, in this case to the first power. Taking the ion and ionophore concentration dependences together indicates a 2 : 1 ionophore : ion stoichiometry for the membrane-permeating species. This is similar to the stoichiometries found for previously studied ionophores [20, 40].

The dependence of the membrane zero-current potential of GMO membranes, on the gradient of lithium activity across the membrane, in the presence of the ionophore, is illustrated in Fig. 2*c*. If there are only one salt and one type of ion/ionophore permeant species in the system, the dependence of V_o (the potential drop across the membrane) on the ion activity gradient can take the following form:

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

where a_i' and a_i'' denote ion activities in the aqueous phases on both sides of the membrane, z is the net charge of the permeant ion and n is the ion stoichiometry in the membrane-permeating complex. For the present case, $n = 1$ has already been determined (recall Fig. 2*b*). Therefore, in the presence of LiCl as the single salt in the system, the slope of the potential (in mV units) *vs.* the logarithm of the ion activity gradient could take the values of either -58.5 or $+58.5$, for Cl^- or Li^+, respectively. The slope expected for a monovalent cation is illustrated, along the experimental data, in Fig. 2*c*. The agreement between the expectation and the experimental data is clear support for Li^+ being the transported ion.

In conclusion, the experimental data show quite clearly that ETH1810 is capable of transporting the lithium ion across lipid bilayer membranes, forming a permeating species with a 2 : 1 ionophore : Li^+ stoichiometry.

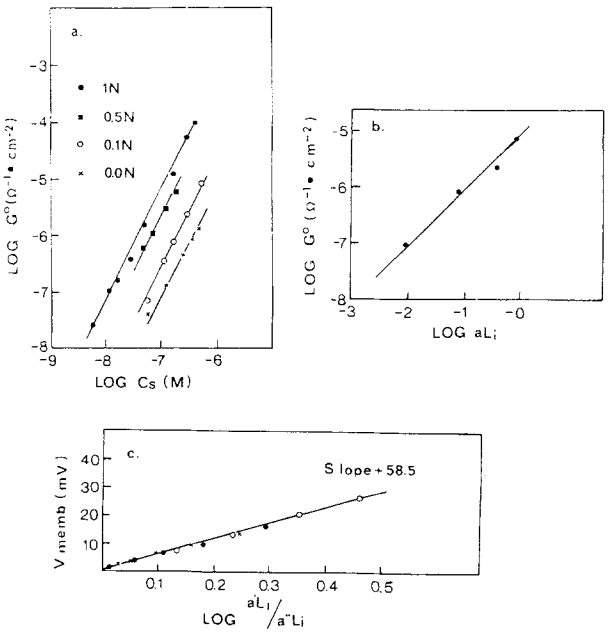

Fig. 2. Ionophore-induced zero-current conductances and potentials of GMO membranes for assessment of the ability of ETH1810 to act as a lithium carrier. (*a*) The increase in membrane zero-current conductance with the increase in ionophore concentration, in the presence of LiCl in the membrane-bathing solutions, at the concentrations listed on the figure. Ordinate: logarithm of membrane conductance. Abscissa: logarithm of ionophore concentration in the aqueous phase. Points are experimental: lines are drawn to a slope = 2. (*b*) The increase in membrane zero-current conductance with the increase in activity of the lithium ion, at a constant ionophore concentration of 0.1 μM, in the presence of LiCl in the membrane-bathing solutions. Ordinate: logarithm of membrane conductance. Abscissa: logarithm of lithium activity in the aqueous phase. Points are experimental; line is drawn to a slope = 1. (*c*) The increase in the zero-current potential drop across the membrane with the increase in the gradient of lithium activities across the membrane ("dilution potentials"), at a constant ionophore concentration of 0.5 μM. Ordinate: membrane potentials. Abscissa: logarithm of the ratio of the activities of lithium on both sides of the membrane. Points are experimental; data are pooled from three separate experiments each represented by a different type of symbol; line is drawn to the slope indicated on the figure

Selectivity Profile: Lithium *vs.* Alkali Cations

Membrane Zero-Current Conductances in the Presence of Alkali-Cation Salts

A measure of the ionic selectivity induced in a membrane by an ionophore, acting as a carrier, can be obtained by comparison of the membrane zero-current conductances determined in a series of experiments in which the salt entity is varied. A typical

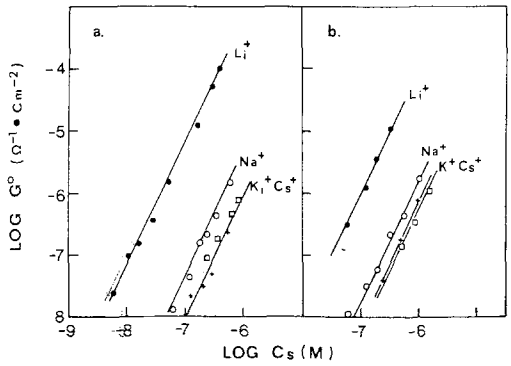

Fig. 3. The increase in zero-current membrane conductance with the increase in ionophore concentration, in the presence of 1 N chloride salts of the alkali cations listed on the figure. Ordinate: logarithm of membrane conductance. Abscissa: logarithm of ionophore concentration in the aqueous phase. Points are experimental; lines are drawn to a slope = 2. (*a*) GMO membranes. (*b*) Asolectin membranes

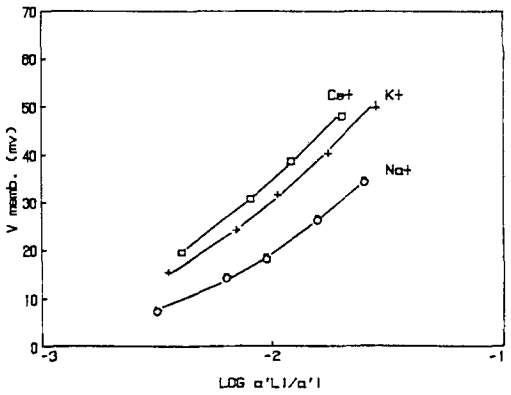

Fig. 4. Zero-current potentials of asolectin membranes in the presence of ETH1810 and salt mixtures. Both membrane-bathing solutions are symmetrical with respect to the concentrations of the ionophore (at 0.5 μM) and a single chloride salt of a desired alkali cation, at 1 N. Constant ionophore concentration potential drops across the membrane generated by additions of lithium chloride to one membrane-bathing solution only. Ordinate: membrane potentials. Abscissa: logarithm of the ratio of activities of lithium to the other alkali cation at the lithium-containing side. Points are experimental; solid curves are theoretical expectations drawn according to Eq. (2) in the text for the magnitudes of permeability ratios listed in Table 1

case is illustrated in Fig. 3, where the increase in zero-current conductances with the increase in ionophore concentration are illustrated for the chloride salts of Li^+, Na^+, K^+ and Cs^+ in GMO and in asolectin membranes. These data indicate a selectivity sequence of $Li^+ > Na^+ > K^+ > Cs^+$. Similar to the finding with lithium (recall Fig. 2*a*), the data for the other ions also indicate an ionophore stoichiometry of 2.

The magnitudes of membrane conductances induced in the presence of ETH1810, shown here, were found to be 10-fold higher (under similar lithium and ionophore concentrations, for the same membrane composition) than those induced in the presence of ETH1644 or AS701 [20, 40]. For example, in GMO membranes, in the presence of 1 N LiCl, 0.1 μM ETH1810 induces a membrane conductance of $1 \times 10^{-5}\ \Omega^{-1}\ cm^{-2}$ (Fig. 3) while 0.1 μM ETH1644 induces membrane conductance of $1 \times 10^{-6}\ \Omega^{-1}\ cm^{-2}$. This is a clear indication of an improvement in ionophore potency. The differences between the asolectin and GMO data are attributed to the membrane lipid composition, which lead to differences in membrane fluidity and in membrane surface potential [22].

Membrane Zero-Current Potentials in the Presence of Alkali-Cation Salts

Typical results for the increase in the zero-current potential drop across asolectin membranes, with the increase in the activity ratio of lithium to another alkali cation in the system, are illustrated in Fig. 4. Similar results were obtained for GMO membranes. Lithium-to-alkali cation permeability ratios were obtained from data of this type according to the following equation:

$$V_o = \frac{RT}{F} \ln \frac{a'_{Li} + (P_i/P_{Li})a'_i}{(P_i/P_{Li})a''_i} \qquad (2)$$

where V_o is the membrane potential drop, a_{Li} and a_i are the activities of lithium and an alkali cation (denoted i) and P_i/P_{Li} is the corresponding permeability ratio. The superscripts $'$ and $''$ differentiate (recall Eq. (1)) between the two aqueous phases on both sides of the membrane.

The permeability ratios obtained are listed in Table 1, for both GMO and asolectin membranes, and are compared there to the corresponding ratios determined in the presence of ETH1644. The data in Table 1 make it quite clear that ETH1810 induces membrane ionic selectivity which, for the present objectives, is improved compared to ETH1644. The increase in selectivity is across the board, i.e., not only lithium *vs.* sodium but also lithium *vs.* the two other, larger, alkali ions tested. The trend observed is independent of the lipid composition of the membrane. The implications of the observed selectivities, with respect to structure-activity relationships will be discussed in a later section.

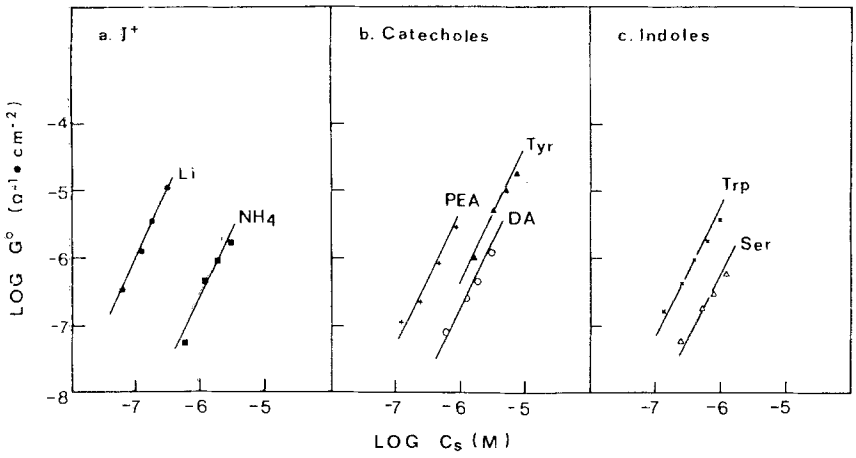

Fig. 5. The increase in zero-current conductances of asolectin membranes, with the increase in ionophore concentration, in the presence of chloride salts of the ions listed on the figure. (*a*) Li^+ and NH_4^+ at 1 N each. (*b*) Catechole amines at 10 mM each. (*c*) Indole amines at 10 mM each. In the two latter cases the ionic strength was held constant by 1 N CsCl. Ordinate: logarithm of membrane conductance. Abscissa: logarithm of ionophore concentration in the aqueous phase. Points are experimental; lines are drawn to a slope = 2

Table 1. Permeability ratios of the 2 : 1 carrier : ion complexes of ETH1810 and ETH1644, determined in asolectin and in GMO membranes

Ion	P_i/P_{Li}			
	Asolectin membranes		GMO membranes	
	ETH1810	ETH1644	ETH1810	ETH1644[a]
Li^+	1	1	1	1
Na^+	8.83×10^{-3}	1.25×10^{-2}	1.19×10^{-2}	1.68×10^{-2}
K^+	4.42×10^{-3}	1.14×10^{-2}	6.21×10^{-3}	1.68×10^{-3}
Cs^+	3.47×10^{-3}	7.98×10^{-3}	5.32×10^{-3}	8.55×10^{-3}

[a] Data from [40].

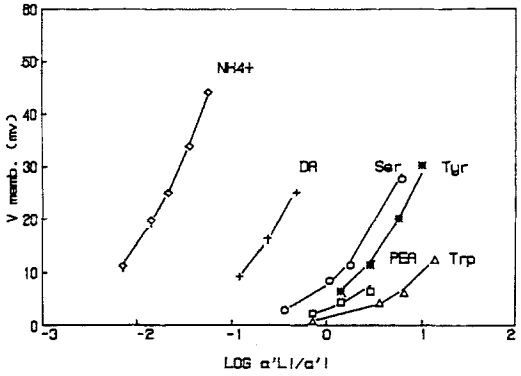

Fig. 6. Similar to Fig. 4, except the following: amine concentrations similar to those listed in Fig. 5. Ionic strength (barring NH_4^+) was held constant by 1 N CsCl. Constant ionophore concentration was held at 1 μM. Theoretical expectations were drawn according to Eq. (3) in the text for the magnitudes of permeability ratios listed in Table 2

Selectivity Profile: Lithium *vs.* Biogenic Amines

Typical results for the increase in zero-current conductance of asolectin membranes, in the presence of salts of ammonium and of several biogenic amines (or their models) are illustrated in Fig. 5. These data indicate clearly that, similar to previously studied ionophores [17, 20], ETH1810 is also capable of carrying protonated biogenic amines across lipid bilayer membranes, in an ionophore stoichiometry of 2. The data also indicate there is selectivity among the biogenic amines.

In order to obtain further quantitative data on the selectivity, membrane zero-current potentials were measured in the presence of a gradient of lithium and a desired amine salt, at constant ionophore concentrations and at constant ionic strength (held by 1 M CsCl). Typical results of this type of data are illustrated in Fig. 6. The desired permeability ratios of the amines with respect to Li^+ were obtained from data of the type illustrated in Fig. 6, according to the following equation:

$$V_o = \frac{RT}{F} \ln \frac{a'_{Li} + (P_{Cs}/P_{Li})a'_{Cs} + (P_{amine}/P_{Li})a'_{amine}}{(P_{Cs}/P_{Li})a''_{Cs} + (P_{amine}/P_{Li})a''_{amine}} \quad (3)$$

where a_{Li}, a_{Cs}, and a_{amine} are the activities of lithium, cesium and the specific amine in system, and P_{Cs}/P_{Li} and P_{amine}/P_{Li} are the corresponding permeability ratios. The latter are listed in Table 2 and are compared there to similar ratios determined in the presence of ETH1644.

The data listed in Table 2 show quite clearly that some of the biogenic amines tested here are more permeant with this ionophore system than Li^+. The normal in vivo concentrations of these amines are

Table 2. Permeability ratios of the 2 : 1 carrier : ion complexes of ETH1810 and ETH1644 with biogenic amines and related molecules, determined in asolectin membranes

Amine	P_{amine}/P_{Li}	
	ETH1810	ETH1644
Li^+	1	1
NH_4^+	0.013	0.045
Catecholes and derivatives		
PEA	7.78	11.6
Tyr	4.29	2.39
DA	0.12	0.05
Indoles		
Trp	20.3	18.1
Ser	2.46	0.34

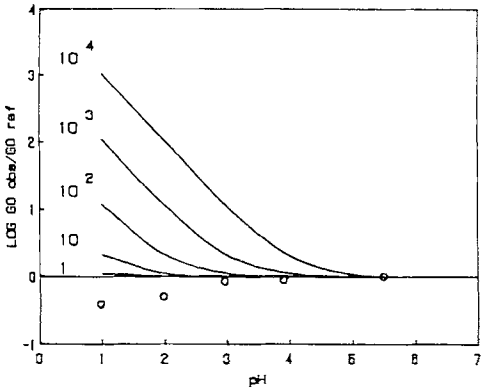

Fig. 7. The effect of pH on the zero-current conductance of asolectin membranes in the presence of 1 N LiCl and 0.5 μM ETH1810 in the membrane-bathing solutions. Ordinate: membrane conductance. Abscissa: pH of the membrane-bathing aqueous solutions. Points are experimental; solid curves are theoretical expectations drawn according to Eq. (4) in the text for the proton/lithium permeability ratios listed on the figure

sufficiently low, which suggests that their competition with lithium, on the ionophore, should not be significant. Obviously, this issue, as well as the effects of the ionophore on the biogenic amines themselves requires further studies at levels of organization higher than the molecular. On the other hand, similar to members of groups I and ETH1644, ETH1810 does have the ability to act as a carrier of biogenic amines. This ability could find applications as a research tool and in the construction of selective electrodes for the determination of such amines.

Protons

The effect of the pH of the membrane-bathing solutions, on membrane zero-current conductance, in the presence of constant lithium and ionophore concentrations was probed over the pH range of 1–6. Data of this type were then used to assess whether protons, under physiological concentration conditions, might interfere with lithium transport mediated by this ionophore.

If both protons and lithium are transported by this ionophore, the ratio of the zero-current conductances determined at a given pH, to that determined at another pH chosen as a reference point, can take the following form [40]:

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

where G^o_{pH} and G^o_{ref} are the zero-current membrane conductances at any given pH and at that of the reference (chosen to be 5.5), a_{Li} and a_H the lithium and proton concentrations and P_H/P_{Li} the corresponding permeability ratio.

The experimental conductance ratios obtained, for asolectin membranes, are illustrated in Fig. 7, together with the theoretical expectations, calculated for selected proton-to-lithium permeability ratios covering the range of $1–10^4$.

These data make it quite clear that the permeability ratio of protons to lithium does not exceed, for the present case, the magnitude of 1. This relatively low ratio gives an acceptable measure of confidence that there would be no significant interference of protons in ETH1810-mediated lithium transport under currently applied conditions. As already mentioned, in order to avoid toxicity and undesirable side effecs, plasma-lithium ratios should not exceed 1 mM [5]. This gives a lithium-to-proton concentration ratio of 10^4, for which a P_H/P_{Li} of 1 is quite safe. Moreover, this ratio of 1 is sufficiently low to prevent any significant interference, even if future developments (such as the application of an ionophore) would allow a reduction of one or two orders of magnitude in the administered lithium doses.

General Discussion: Structure-Activity Relationships

Two distinct findings indicate that with respect to activity as a lithium carrier, ETH1810 is an improvement over previously studied ionophores. Compared to ETH1644, ETH1810 is 10-fold more potent and induces a higher lithium *vs.* sodium selectivity, resulting in a 40% increase in the corresponding permeability ratio. Compared to the structural differences between ionophores of the group I and

ETH1644 (recall Fig. 1), those between the latter and ETH1810 seem less drastic: a departure from symmetry in the N-imide substituents, achieved through the replacement of isobutyls by cyclohexyls, on one of the N-imides. Yet, being the only difference between the two ionophores, this replacement has to account for the increases in selectivity and in potency.

The replacement of isobutyls by cyclohexyls could affect several nonmutually exclusive molecular properties: hydrophobicity—which is expected to affect ionophore solubility in the membrane; electron density at the oxygen atoms and cavity size—both of which are expected to affect selectivity.

The magnitudes of the Hansch Hydrophobic Substituent Constant (π), which can be used as a measure for relative hydrophobicities [10, 38], are 1.8 and 2.51 for an isobutyl and a cyclohexyl, respectively. This increase in the π value, from isobutyl to cyclohexyl, implies that ETH1810 is more hydrophobic than ETH1644. The increased hydrophobicity of ETH1810 should make it more soluble (*vs.* ETH1644) in the membrane, which should increase the partition coefficient of the ionophore : ion complex into the membrane. For similar conditions, this should result in a higher membrane conductance, and therefore a higher potency of ETH1810 *vs.* ETH1644, as was indeed observed.

The magnitudes of the Taft Electronic Substituent Constant (σ^*), which reflects an inductive effect of a substituent are -0.13 and -0.15 for isobutyl and cyclohexyl, respectively [10, 38]. This slight increase in electron density at the oxygen ligands does not seem sufficient to account alone for the improvement in selectivity.

The Taft Steric Parameter (Es^c), corrected according to Hancock, is proportional to the diameter of the substituent [10, 38]. The magnitudes of this parameter, -2.48 and -2.64 for isobutyl and cyclohexyl, respectively, imply a modest increase in diameter for the cyclohexyl substituent. If replacement of the isobutyls by the larger cyclohexyls would decrease the cavity accommodating the complexed ion, this could account for improvement in ionic selectivity (with minor support of the inductive effect).

The observed improvement in lithium selectivity could be due, for the most, not because ETH1810 binds lithium better but because it binds other ions poorer, than ETH1644 (recall Table 1, with respect to larger ions, such as potassium and cesium). Moreover, for performance in vivo as a carrier, it is preferable to strive for improvement in selectivity by decreasing, rather than by increasing, binding constants. Increase in binding might result in the introduction of (or increase in) kinetic limitations [20].

In conclusion, the novel structural features of ETH1810 result in a carrier which is better, in terms of potency and lithium selectivity, than former ones. It is proposed that this ionophore can well serve, in terms of its performance as a lithium-selective carrier, as a research tool. It is also proposed that its favoring of lithium over sodium is of sufficient magnitude to merit serious consideration of extending molecular-level studies with ETH1810 to investigations at higher levels of organization.

References

1. Amidsen, A., Schou, M. 1978. Lithium. *In:* Side Effects of Drugs. Annual 2. M.N.G. Dukes, editor. pp. 17–29. Excerpta Medica, Amsterdam
2. Avissar, S., Schreiber, G., Danon, A., Belmaker, R.H. 1988. Lithium inhibits adrenergic and cholinergic increases in GTP binding in rat cortex. *Nature* **331:**440–442
3. Burnham, C., Braw, R., Karlish, S.J.D. 1986. A Ca-dependent K channel in "luminal" membranes from the renal outer medulla. *J.Membrane Biol.* **93:**177–186
4. Dilsaver, S.C. 1986. Cholinergic mechanisms in affective disorders. Future directions for investigation. *Acta Psychiatr. Scand.* **74:**312–334
5. Ehrlich, B.E., Diamond, J.M. 1980. Lithium, membranes and manic depressive illness. *J. Membrane Biol.* **52:**187–200
6. Gadzekpo, V.P.Y., Hungerford, J.M., Kadry, A.M., Ibrahim, Y.A., Xie, R.Y., Christian, G.D. 1986. Comparative study of neutral carriers in polymeric lithium ion selective electrodes. *Anal. Chem.* **58:**1948–1953
7. Gallichio, V.S. 1988. Lithium and hematopoietic toxicity. *Acta Haematol.* **79:**192–197
8. Gallichio, V.S., Chen, M.G. 1981. Influence of lithium on proliferation of hematopoietic stem cells. *Exp. Hematol.* **91:**804–810
9. Guggi, M., Fiedler, V., Pretsch, E., Simon, W. 1975. A lithium ion-selective electrode based on a neutral carrier. *Anal. Lett.* **8:**857–866
10. Hansch, C., Leo, A.J. 1979. Substitution Constant for Correlation Analysis in Chemistry and Biology. J. Wiley, New York
11. Hiratani, K. 1982. Synthesis and properties of noncyclic polyether compounds: IV. A new synthetic ionophore exhibiting selective lithium ion transport. *Chem. Lett.* 1021–1024
12. Hiratani, K., Taguchi, K. 1987. Synthetic polyether dicarboxylic acids exhibiting lithium ion-selective transport. *Bull. Chem. Soc. Jpn.* 3827–3833
13. Hsu, G.L.K. 1984. Treatment of bulimia with lithium. *Am. J. Psychiatry* **141:**1260–1262
14. Johnson, F.N. (editor). 1987. Depression and Mania Modern Lithium Therapy. IRL, Oxford—Washington, DC
15. Johnson, G. 1984. Lithium. *Medical J. Aust.*, Oct. 27, pp. 595–601
16. Kersten, L. 1987. The gastrointestinal system. *In:* Depression and Mania Modern Lithium Therapy. F.N. Johnson, editor. pp. 196–202. IRL, Oxford—Washington, DC
17. Margalit, R., Azuri-Yam, R., Zeevi, A. 1985. Serotonin, do-

pamine and related molecules are transported selectively by noncyclic neutral Li^+-selective ionophores: Implications for electrodes and for neurological studies. *In:* Ion Measurements in Physiology and Medicine. M. Kessler, D.K. Harrison, and Y. Hoper, editors. pp. 48–53. Springer-Verlag, Berlin—Heildelberg

18. Margalit, R., Eisenman, G. 1981. Ionic permeation of lipid bilayer membranes mediated by a neutral noncyclic Li^+-selective carrier having imide and ether ligands: I. Selectivity among monovalent cations. *J. Membrane Biol.* **61:**209–219
19. Margalit, R., Shanzer, A. 1981. A study of Li^+-selective permeation through lipid bilayer membranes mediated by a new ionophore (AS701). *Biochim. Biophys. Acta* **649:**441–448
20. Margalit, R., Shanzer, A. 1982. New Li^+-selective ionophores with the potential ability to mediate Li^+ transport in vivo: Ionic selectivity and relative potencies, studies in model membranes. *Pfluegers Arch.* **395:**87–92
21. Margalit, R., Zeevi, A., Shanzer, A. 1984. Electrogenic and selective transport of biogenic amiens across lipid bilayer membranes mediated by a neutral ionophore. *Biochim. Biophys. Acta* **774:**193–199
22. Mclaughlin, S. 1977. Electrostatic potential at membrane solution interfaces. *In:* Current Topics in Membrane and Transport. Vol. 9. F. Bronner and A. Kleinzeller, editors. pp. 71–144. Academic, New York
23. Metzger, E., Ammann, D., Asper, R., Simon, W. 1986. Ion selective liquid membrane electrode for the assay of lithium in blood serum. *Anal. Chem.* **58:**132–135
24. Metzger, E., Ammann, D., Schefer, U., Pretsch, E., Simon, W. 1984. Lipophilic neutral carriers for lithium selective liquid membrane electrodes. *Chimia* **38:**440–442
25. Muller-Oerlinghausen, B. 1985. Lithium long-term treatment: Does it act via serotonin? *Pharmacopsychiatria* **18:**214–217
26. Myers, D.H., West, T.E.T. 1987. Hormone system. *In:* Depression and Mania Modern Lithium Therapy. F.N. Johnson, editor. pp. 220–226. IRL, Oxford—Washington, DC
27. Pandy, G.N., Dorus, E., Davis, J.M., Tosteson, D.C. 1979. Li^+ transport in human red blood cells. Genetic and clinical aspects. *Arch. Gen. Psychiatry* **36:**902–908
28. Pleul, O., Oerlinghausen, M. 1986. Lithium therapy and the turnover of phosphatidylcholine in human erythrocytes. *Eur. J. Clin. Pharmacol.* **31:**457–462
29. Preskorn, S.H., Goodwin, D.W. 1987. Medical management of the depressed alcoholic patient. *Int. J. Psychiatry Med.* **17:**117–131
30. Ramsey, T.A., Cox, M. 1982. Lithium and the kidney: A review. *Am. J. Psychiatry* **139:**443–449
31. Schou, M. 1986. Lithium treatment: A refresher course. *Brit. J. Psychiatry* **49:**541–545
32. Schou, M. 1987. Use in other psychiatric conditions. *In:* Depression and Mania Modern Lithium Therapy. F.N. Johnson, editor. pp. 44–46. IRL, Oxford—Washington, DC
33. Shanzer, A., Samuel, D., Korenstein, R. 1983. Lipophilic lithium ion carriers. *J. Am. Chem. Soc.* **105:**3815–3818
34. Sherman, W.R., Gish, B.G., Hondar, M.P., Munsell, L.Y. 1986. Effects of lithium on phosphoinositide metabolism in vivo. *Fed. Proc.* **45:**2639–2646
35. Shinar, H., Navon, G., Klaui, W. 1986. Novel organometallic ionophore with specificity towards Li^+. *J. Am. Chem. Soc.* **108:**5005–5006
36. Stern, T.A., Lydiard, R.B. 1987. Lithium therapy revisited. *Psychiatr. Med.* **4:**39–68
37. Tisman, G., Herbert, V., Rosenblatt, S. 1973. Evidence that lithium induces human granulocyte formation, elevated serum vitamin B12 binding capacity in vivo and granulocyte colony production in vitro. *Br. J. Haematol.* **24:**767–771
38. Topliss, J.G. 1972. Utilization of operational schemes for analog synthesis in drug design. *J. Med. Chem.* **15:**1006–1011
39. Walker, R.G., Smith, P.K. 1987. Kidneys and the fluid regulatory system. *In:* Depression and Mania Modern Lithium Therapy. F.N. Johnsoon, editor. pp. 206–213. IRL, Oxford—Washington, DC
40. Zeevi, A., Margalit, R. 1985. Selective transport of Li^+ across lipid bilayer membranes mediated by an ionophore of novel design (ETH1644). *J. Membrane Biol.* **86:**61–67
41. Zhukov, A.F., Erne, D., Ammann, D., Guggi, M., Pretsch, E., Simon, W. 1981. Improved lithium ion-selective electrode based on a lipophilic diamide as neutral carrier. *Anal. Chim. Acta* **131:**117–122

Received 15 June 1990; revised 13 November 1990