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Permeability of Lipid Bilayer Membranes to Biogenic Amines and Cations: Changes Induced by Ionophores and Correlations with Biological Activities

M. Schadt and G. Haeusler

Research Department, F. Hoffmann-La Roche & Co., Ltd., 4002 Basel, Switzerland

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Summary. The permeation of various cations and biogenic amines across artificial lipid membranes (bilayer membranes) was investigated by means of electrical conductivity measurements and fluorescence spectroscopy. Their permeability properties were modified by doping them with five different carboxylic ionophores. The induced permeability changes were correlated with some biological activities of the ionophores.

Four out of five ionophores increased the permeability of doped membranes for Li^+, Na^+, K^+, Mg^{2+} and Ca^{2+}. Two of them showed a preference for K^+ whereas one (X-537A) increased the membrane permeability for K^+ as well as for Ca^{2+}. It was also found that the ionophores increased the permeability for serotonin, dopamine, norepinephrine and epinephrine. No direct coupling was found between the facilitated ion permeation and the permeation of biogenic amines induced by the ionophores. The measurements can be qualitatively explained by assuming that the permeation of biogenic amines is competitively inhibited by cations. It appears that one biogenic amine molecule forms a complex with one ionophore molecule, the complex acting as a carrier for biogenic amines. All ionophores investigated increased the bilayer permeability considerably for some biogenic amines. (A preference up to 420:1 for serotonin over epinephrine was measured for one specific ionophore.)

There was no correlation between the *in vitro* antibacterial activity (against bacillus E and bacillus TA) of the ionophores and their potency to change the ion permeability of doped membranes. The correlation found between the ionophore-induced permeation of biogenic amines through membranes and their antibacterial activity is probably without biological meaning. However, a rather good correlation was found between cardiac sympathetic effects of the ionophores and their ability to facilitate permeation of norepinephrine through artificial membranes.

Antibiotics acting as ion-transporting carriers in biological [8, 10, 15–21] and artificial lipid bilayer membranes [9, 13] as well as in bulk organic phases [14] are known as ionophores [19]. They increase the electrical conductivity of lipid barriers due to their ion complexing ability. Whether the

conductivity increase is ion selective or not depends on the complexing properties of the ionophore [9]. The first antibiotic recognized to be an ionophore [11, 12] was valinomycin, forming lipid-soluble complexes with monovalent cations.

The coccidiostatic Ro 2-2985 (X-537A) is a carboxylic ionophore [4]. It was isolated [2] in 1951 and its structure was analyzed [6] in 1970. Various interesting biological activities of Ro 2-2985 were reported [8, 10, 17, 20, 21, 24] within the last few years especially in cardiotonic actions [3, 10, 16, 17, 21]. Recently, it was found that Ro 2-2985 has not only ionophoric properties but also enhances the permeation of catecholamines in biological membranes [10] as well as in bulk organic phases [17].

In the present experiments a two-chamber set-up [22] was used for the measurements of the ion permeability and the penetration of biogenic amines (BA) through artificial lipid bilayer membranes [13]. The cation selectivity of doped bilayers and their selectivity for various BA was determined. The main object was to find correlations between some biological activites of structurally similar antibiotics and their ability to change the permeability induced by them in artificial lipid bilayer membranes to cations and BA. A correlation was found to exist between the effect of ionophores on the permeation of BA across doped bilayers and their influence on the heart. This finding suggests that artificial lipid bilayer membranes can be useful as a tool to investigate and to correlate – in a comparatively simple and well-defined model system – complex physiological activities induced by some drugs.

Materials and Methods

Lipid Bilayer Membranes

Fig. 1 shows a schematic drawing of the experimental system. A Teflon cell was used consisting of two compartments with a 2-mm diameter hole in the separating wall across which the lipid bilayer is spread [13]. Both compartments were filled with electrolyte solutions. The formation of the bilayer was observed through the glass window in the outer wall of compartment 1. The purpose of the window was to monitor visually the flatness of the membrane to keep its surface tension at a minimum. This was done by adjusting the hydrostatic pressure across the bilayer to zero when electrolyte samples were taken from the cell. The pressure was adjusted with syringes dipping into the electrolyte solution (not shown in Fig. 1).

The temperature of the cell was set at 22 °C. A four-point probe set-up (four reversible Ag-AgCl electrodes) was used to measure membrane potential and membrane current independently of each other. The polarity of the variable voltage source V_0 could be chosen. The membrane potential V (Fig. 1) was measured with a Keithley vibrating reed electrometer, model 640, with an input impedance $R > 10^{16}$ ohms. A Keithley picoammeter, model 417, was used to measure the membrane current.

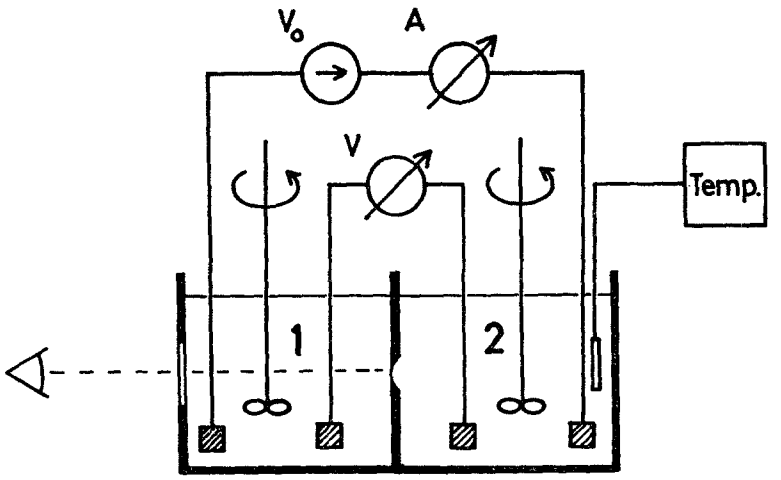
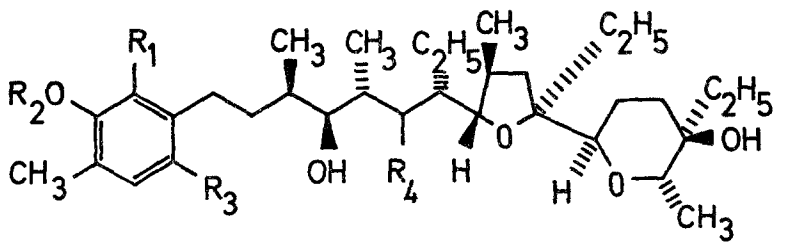


Fig. 1. Schematic drawing of the experimental system for penetration measurements of ions and biogenic amines through lipid bilayers. A four-point probe set-up is used to measure the electrical current A and the voltage V independently of each other. V_o = voltage source. Stirrers are mounted in each compartment

The electrical conductivity of doped bilayers could be measured for ionophore concentrations in the membranes of up to 0.4%. However, the bilayers became mechanically unstable for concentrations exceeding 0.2%, thus limiting the lifetime of a membrane to 5 to 10 min. Furthermore, the electrical stability decreased at high ionophore concentrations. The membrane current began to fluctuate versus time when a constant voltage was applied, thus reducing the reproducibility of the measurements. For practical reasons the ionophore concentration range was limited to 0.01 to 0.2%. (When using Ro 2-2985 as a dopand the "leak" conductivity of the undoped lipid bilayer became dominant at ionophore concentrations $\gtrsim$0.01% thus preventing the detection of conductivity changes induced by the ionophore.)

To determine the passive membrane permeability for BA the following procedure was used: Each cell compartment was filled with 10 ml electrolyte solution (0.1 M, pH 6). After the membrane became black [13], 200 μliters of a 10% BA stock solution were added to compartment 2. To achieve rapidly a homogenous electrolyte composition and to reduce the thickness of the unstirred layer at the membrane surface, Teflon stirrers were used in both compartments (Fig. 1). The time was monitored from the instant that the BA solution was added. After certain time intervals electrolyte samples were taken from compartment 1. The amount of BA penetrating the membrane from compartment 2 into compartment 1 was determined by measuring the fluorescence intensity of the samples. A Perkin-Elmer fluorescence spectrophotometer, model MPF-2A, was used with the excitation wavelength set at 280 nm. Except for serotonin, the fluorescence was measured at 320 nm. The respective wavelengths for serotonin were 300 nm and 335 nm. Large BA concentration gradients (0.2%) were used across the bilayers to get high enough BA fluxes and hence well-detectable fluorescence signals from the samples taken. All measurements were corrected for cumulative sampling dilution.

The lipid stock solution [22] was prepared by dissolving 25 mg of synthetic dioleoyl-L-α-lecithin (Supelco) in 2.5 ml of *n*-decane (Merck). The membrane solution was made prior to the experiments by mixing 100 μliters of lipid stock solution. The latter was



	R_1	R_2	R_3	R_4
Ro 2-2985	CO_2Na	H	H	O
Ro 20-0006	CO_2Na	H	Br	O
Ro 7-9431	CO_2Na	H	H	NOH
Ro 20-0083	NO_2	Na	NO_2	O
Ro 7-9409	CO_2Na	$COCH_3$	H	O

Fig. 2. Formulas of the ionophore Ro 2-2985 and the four derivatives used as dopands in the experiments

prepared by dissolving the antibiotics in *n*-decane containing less than 10% ethanol. The ionophores were supplied by J. W. Westley of Hoffman-La-Roche Inc., Nutley, New Jersey. The formulas of Ro 2-2985 [6] and of the derivatives used in the present experiments are shown in Fig. 2.

The biogenic amines epinephrine (E), norepinephrine (NE), dopamine (DA) and serotonin (5-HT) were supplied by Fluka. All BA stock solutions—with the exception of the 5-HT solution—were made up of 10% BA dissolved in the same electrolyte solution in which the membrane was suspended (Fig. 1).

We checked that the electrolytes did not influence the fluorescence spectra of the BA solutions. The 5-HT stock solution (pH 2.5%) was prepared by dissolving 0.5 g of serotonin-hydrogen-oxalate in 10 ml of bidistilled water. Then $CaCl_2$ was added in a molar ratio 5-HT/$CaCl_2$=1:1.2 to precipitate Ca-oxalate. All stock solutions could be kept in the dark in a refrigerator for several days without change in their fluorescence yield.

Isolated Perfused Cat Heart

Cats of either sex weighing 1.9 to 3.1 kg were anaesthetized with sodium pentobarbital (Nembutal®; 45 mg/kg i.p.) and given i.v. 5,000 IU of heparin. The heart was prepared according to the method of Langendorff [7] and removed from the animal in connection with the vertebral column.

Perfusion was performed at a constant pressure of 60 cm H_2O and 37 °C with a modified Krebs-Henseleit solution (g/1,000 ml): NaCl, 6.7; KCl, 0.35; $CaCl_2$, 0.28; $MgSO_4$, 0.29; KH_2PO_4, 0.2; $NaHCO_3$, 2.1; glucose, 2.0; saturated with 95% O_2 and

5% CO_2, pH adjusted to 7.3. The cardiac contractile force was measured with a water-filled rubber balloon which was inserted through an incision of the left atrium into the left ventricle and connected to a Statham pressure transducer. The pressure changes triggered a Grass 7P4 tachograph. The cardiac contractile force and the heart rate were recorded on a Grass 7 polygraph.

For depletion of cardiac norepinephrine stores cats were pretreated with 5 mg/kg i.p. reserpine (Serpasil®, Ciba-Geigy) given 16 hr before the isolation of the heart. Cardiac sympathectomy was performed by injecting cats with two doses of 20 mg/kg i.v. of 6-hydroxydopamine hydrobromide on day 1 and two doses of 50 mg/kg i.v. on day 7. The experiments on the isolated heart were carried out 3 to 7 days after the last injection of 6-hydroxydopamine. The dose-schedule of the treatment with 6-hydroxydopamine was the same as that used previously and which was shown to produce a virtually complete cardiac sympathectomy [3].

Results

Ionic Transport

To determine the ionophore-induced preference of bilayers to transport cations, current measurements were made at constant membrane potential $V_M = 50$ mV where Ohm's law is still applicable. Various electrolyte solutions (NaCl, KCl, LiCl, $CaCl_2$, $MgCl_2$) and different ionophore concentrations were used (Fig. 3). The results in Fig. 3 are mean values taken from at least four different bilayers. The conductivities σ corresponding to a fixed membrane ionophore concentration of 0.07% were measured for all

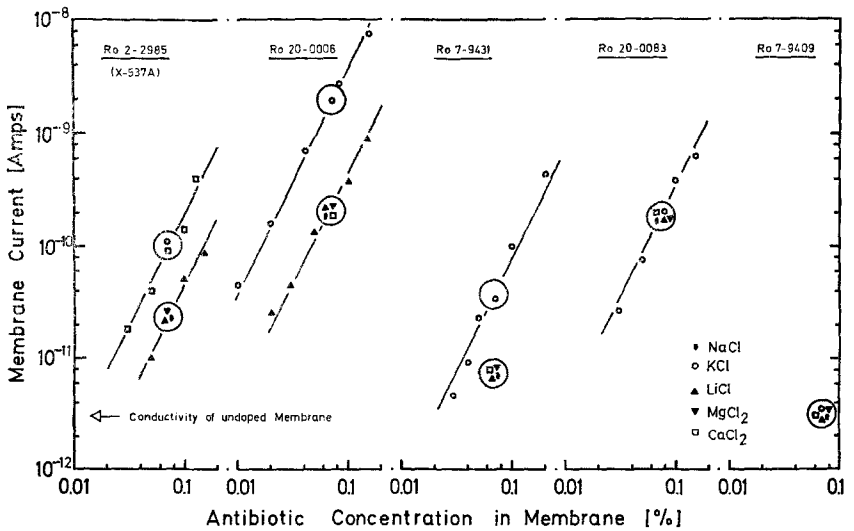


Fig. 3. Membrane current versus ionophore concentration measured at constant membrane potential $V_M = 50$ mV. Five different ionophores and five electrolyte solutions were used

Table 1. Ionic conductivities and biological activities induced by some ionophores

	LiCl	NaCl	KCl	$MgCl_2$	$CaCl_2$	σ/σ_o	σ_s/σ_o	C	BE	BTA
Ro 2-2985	45	45	200	45	200	7.6	34	++	100	100
Ro 20-0006	420	420	3800	420	420	71	640	+	67	124
Ro 20-0083	360	360	360	360	360	61	–	–	–	5
Ro 7-9409	5.9	5.9	5.9	5.9	5.9	1	–	+	2	1
Ro 7-9431	15	15	77	15	15	2.5	13	–	10	10
Pure lipid bilayer	5.9	5.9	5.9	5.9	5.9	–	–	–	–	–

Ion conductivity of doped lipid bilayer membranes suspended in various electrolytes (first five columns). The figures have to be multiplied by 10^{-11} to get the conductivities in ohm^{-1}. σ/σ_o = ratio of unselective conductivity increase, σ_s/σ = ratio of ion-selective conductivity increase (*see text*). Ionophore concentration in membrane = 0.07%. C = coccidiostatic activity. BE and BTA = relative *in vitro* antibacterial activity against bacillus E and bacillus TA, respectively (Ro 2-2985 = 100%).

electrolytes (encircled values in Fig. 3). The conductivities versus ionophore concentration σ(conc.) were measured for K^+, Li^+ and Ca^{2+} only. However, since the slope of σ(conc.) did not change for different ionophores (Fig. 3) and ions, it may be assumed that it is the same for Na^+ and Mg^{2+}.

The double logarithmic plots of conductivity against ionophore concentration in Fig. 3 show that there exists a quadratic dependence on the ionophore concentration, $\sigma \propto (\text{conc.})^2$, for K^+, Li^+ and Ca^{2+}. The ion preference and the relative increase of σ varied considerably for different ionophores. No increase in conductivity could be measured with respect to undoped lipid bilayers when Ro 7-9409 was used (Fig. 3), whereas Ro 20-0083 increased σ unselectively with respect to undoped lipid bilayers by a factor of 100 (the factors refer to an antibiotic concentration of 0.07%). Ro 7-9431 caused σ to increase by a small degree with no preference for Na^+, Li^+, Mg^{2+} and Ca^{2+} whereas $\sigma(K^+)$ increased by a factor of 4. Ro 20-0006 increased σ with no preference for the same ions by a factor of 100 whereas $\sigma(K^+)$ increased by a factor of 1,000. Therefore, both derivatives, Ro 20-0006 and Ro 7-9431, were found to be ion-selective with respect to K^+. The measurements shown in Fig. 3 confirm the ion-preference of Ro 2-2985 for both K^+ and Ca^{2+} with respect to Na^+, Li^+ and Mg^+ as reported from ion-complexation studies in a two-phase distribution system (bulk) [14, 17].

Table 1 summarizes the conductivity measurements of Fig. 3. It shows σ (ohm^{-1}) of bilayers doped with various ionophores in the presence of different electrolyte solutions. σ/σ_o in Table 1 is the ratio of the unselective increase in conductivity of a doped bilayer compared with the "leak"

conductivity σ_o of an undoped lipid bilayer. σ_s/σ_o signifies the selective increase in conductivity of a doped bilayer ("selective" refers to the most permeable ion(s), whereas "unselective" refers to the less permeable ones). Columns BE and BTA show the relative *in vitro* activity (growth inhibition) of the antibiotics *vs.* bacillus E and bacillus TA as reported by J. W. Westley *et al.* [24]. Column C shows the coccidiostatic activity of the ionophores (J. W. Westley, *personal communication*).

Transport of BA

Fig. 4 shows measurements of the penetration of NE across membranes doped with various concentrations of two different ionophores, Ro 2-2985 and Ro 20-0006. Each measurement was made 60 min after the BA gradient was established. The amount of NE penetrated depended linearly on the antibiotic concentration in the bilayer (Fig. 4). The extrapolated straight

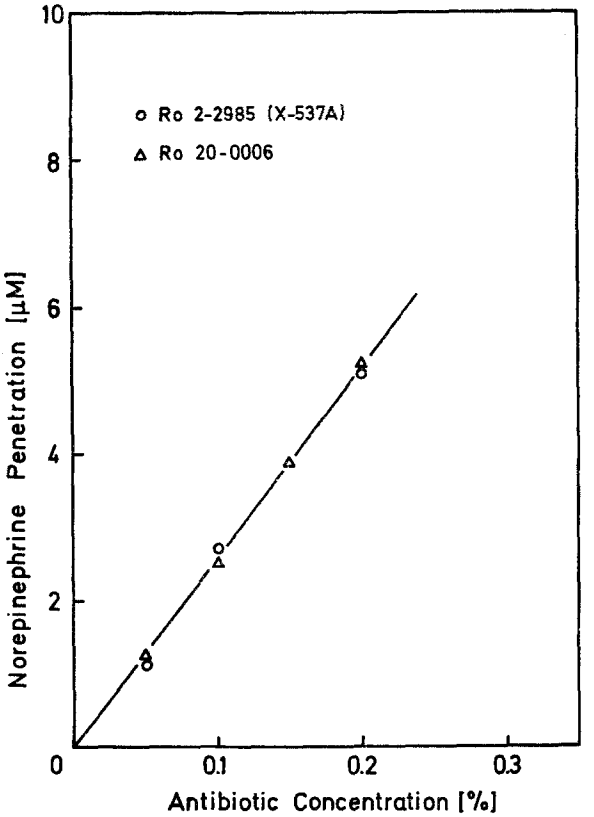


Fig. 4. Penetration of NE through doped lipid bilayer membranes plotted against the concentration of Ro 2-2985 and Ro 20-0006, respectively. 0.1 M $CaCl_2$ was used as an electrolyte solution

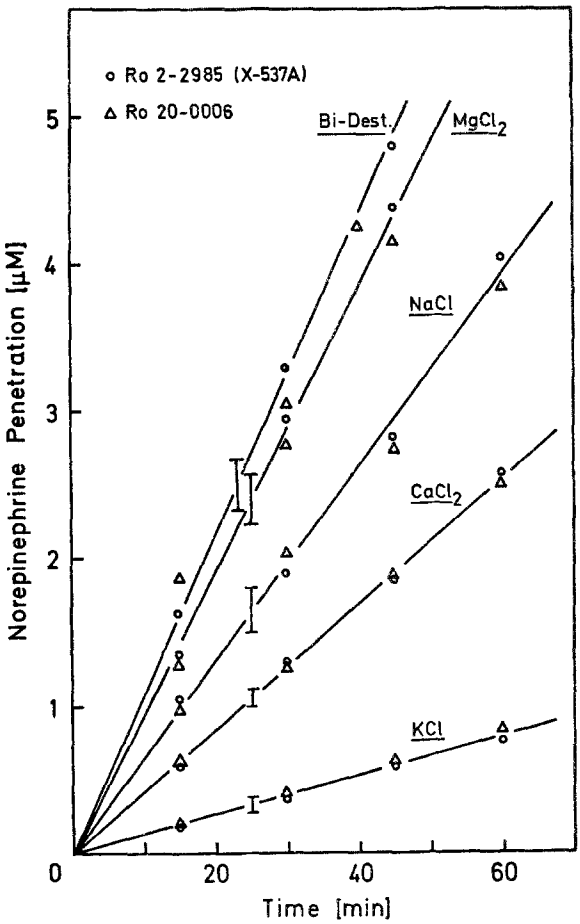


Fig. 5. Penetration of NE through doped bilayer membranes plotted against time. The membranes were suspended in various electrolyte solutions (0.1 M). Ro 2-2985 and Ro 20-0006 were used as dopands in concentrations of 0.1 %

line in Fig. 4 goes through zero, suggesting that the linear relationship is also valid at low ionophore concentrations (<0.01 %).

The time course of NE transport across doped bilayers versus time is depicted in Fig. 5. 0.1 % of Ro 2-2985 and Ro 20-0006 were used as dopands. The measurements were made to determine the influence of different electrolyte solutions (0.1 M, pH 6) on the penetration of NE across bilayers. Both ionophores gave identical results within experimental accuracy. With bidistilled and deionized water the penetration of NE was most pronounced. It increased by a factor of 10 compared with KCl (Fig. 5).

With A = membrane area (3.14×10^{-2} cm^2), Δc = NE concentration gradient across membrane, f = NE-flux density and [NE] = penetrated

Table 2. Permeability coefficients p for norepinephrine of lipid bilayer membranes

	Bi-Dist.	NaCl	KCl	$MgCl_2$	$CaCl_2$
p(NE) [cm sec^{-1}]	$5.0 \cdot 10^{-5}$	$3.0 \cdot 10^{-5}$	$6.0 \cdot 10^{-6}$	$4.3 \cdot 10^{-5}$	$1.9 \cdot 10^{-5}$

Membranes were doped with 0.1% of Ro 2-2985 and Ro 20-0006 (identical p-values for both ionophores) in the presence of various electrolytes (0.1 M).

Table 3. Permeability coefficients p measured for 5-HT, DA, NE and E in doped (0.1%) lipid bilayer membranes

	5-HT	DA	NE	E	C	BE	BTA	HA
Ro 2-2985	1300	750	190	18	++	100	100	$1.05 \cdot 10^{-7}$
Ro 20-0006	1300	750	190	18	+	67	124	$1.11 \cdot 10^{-7}$
Ro 20-0083	3.8	<0.5	<0.5	<0.5	–	–	5	$>10^{-5}$
Ro 7-9409	14	4.8	2.2	<0.5	+	2	1	$3.92 \cdot 10^{-6}$
Ro 7-9431	210	160	100	<0.5	–	10	10	$2.43 \cdot 10^{-6}$
Pure lipid bilayer	1.4	<0.5	<0.5	<0.5	–	–	–	–

The figures in the first four columns have to be multiplied by 10^{-3} to get p (μsec^{-1}). 0.1 M $CaCl_2$ was used as an electrolyte solution. C = coccidiostatic activity. HA = molar concentrations of ionophores required to increase the contractile force of cat hearts by 50%. BE and BTA = relative to *in vitro* activity against bacillus E and bacillus TA, respectively.

amount of NE after time t, one gets [1, 23]

$$f = \frac{[NE]}{t \cdot A} = p \cdot \Delta c \tag{1}$$

where p = permeability coefficient of NE. With a NE concentration of 0.2% in compartment 2 (compartment volume 10 ml) the concentration gradient becomes $\Delta c = 1.18 \times 10^{-5}$ M cm^{-3}. The permeability coefficients p(cm sec^{-1}) for NE were determined from the measurements in Fig. 5 using Eq. (1). The results are summarized in Table 2.

Fig. 6 depicts the penetration of different BA's against time through bilayers doped with 0.1% of Ro 2-2985 and Ro 20-0006, respectively. 0.1 M $CaCl_2$ was used as an electrolyte. The corresponding permeability coefficients p are shown in the two top columns of Table 3. The permeability of doped bilayers proved to vary considerably for different BA's: it was highest for 5-HT and 72 times smaller for E.

Table 3 shows the permeability coefficients p ($\mu sec^{-1} \times 10^{-3}$) measured for 5-HT, DA, NE and E in bilayers doped (0.1%) with different ionophores.

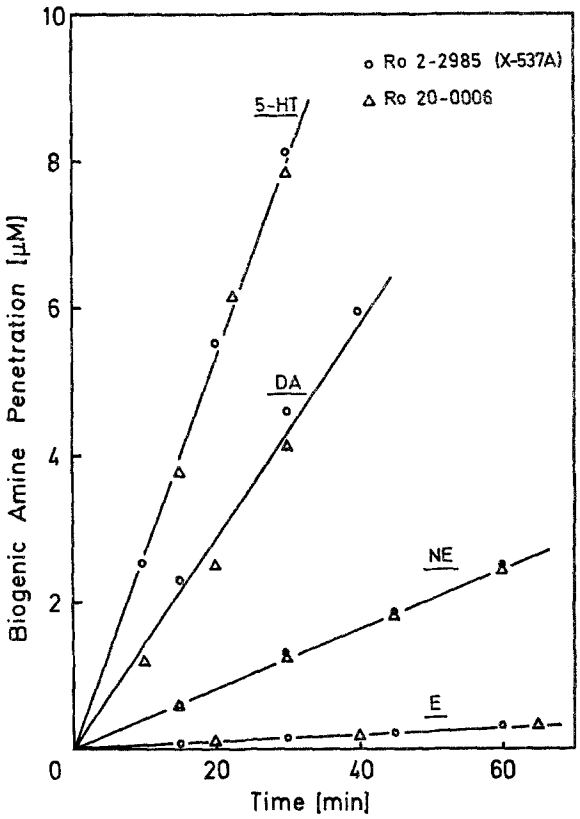


Fig. 6. Penetration of various biogenic amines through doped lipid bilayer membranes plotted against time. Serotonin (5-HT), dopamine (DA), norepinephrine (NE) and epinephrine (E) were used. 0.1 M $CaCl_2$ was used as an electrolyte solution. Ionophore concentration in membranes = 0.1 %

All measurements were carried out in 0.1 M $CaCl_2$. The amount of biogenic amines penetrating undoped lipid bilayers (Table 3) was found to be very small. p could only be measured for 5-HT (high fluorescence yield) in membranes lasting longer than 2 hr. The low lipid penetration guaranteed that the p-values in the doped bilayers were not artifacts due to "leaky" membranes but were actually induced by the ionophores. Columns BE and BTA show the relative *in vitro* antibacterial activities [24] against bacillus E and bacillus TA; column C refers to the coccidiostatic activity (as in Table 1).

Ionophore-Induced Release of NE from the Isolated Cat Heart

Apart from Ro 20-0083 all ionophores caused an increase in heart rate and in cardiac contractile force when added to the perfusion solution of the

isolated cat heart. These effects reached their maximum within 2 to 4 min after exposing the heart to the ionophores. The maximum, however, was not maintained and the positive chronotropic and inotropic effects faded within the next 10 to 15 min using perfusion solutions of constant concentrations. A second exposure of the hearts to the ionophores resulted in a smaller effect. Therefore, only one concentration of the ionophores was tested in a given heart.

Within a limited concentration range the ionophore-induced increases in rate and contractibility were concentration-dependent. Concentration-response curves were constructed by exposing the hearts to three different ionophore concentrations. Three hearts were used to obtain the mean value for each concentration of an ionophore. From the dose-response curves the ionophore concentrations that increased the cardiac contractile force by 50% were determined graphically. The numbers for these concentrations are given in Table 3, column HA. Ro 20-0083 was without an effect up to 10^{-5} M; higher concentrations were not tested.

The ionophore-induced increase in heart rate and contractibility was absent after depletion of the cardiac NE stores with reserpine or after destruction of adrenergic nerve endings with 6-hydroxydopamine.

In a concentration range 10 to 100-fold higher than necessary to elevate the cardiac contractile force by 50%, the ionophores produced cardiac arrest in diastole followed by a slowly developing contracture. These effects were observed irrespective of whether the hearts were from normal cats or from cats pretreated with reserpine or 6-hydroxydopamine.

Discussion

Ion Selectivity

X-ray crystallography analysis [6] of the barium salt of Ro 2-2985 revealed a complex consisting of two ionophore molecules binding one Ba^{2+} with their polar groups. The lipid-soluble molecules are thought to be wrapped around the complexed ion by hydrogen bonding [6] acting thus as an ion carrier [9, 19] as in nigericin [14]. The carrier properties of the Ca^{2+}-ionophore complex are thought to be responsible for some of the reported biological activities [10, 17, 21] of the ionophore.

If complexation of two Ro 2-2985 molecules with one ion – as observed in the crystalline state with Ba^{2+} – occurs also in the lipid part of biological membranes, it should occur in artificial lipid bilayers, too. Indeed, the square-law dependence $\sigma \propto (\text{conc.})^2$ of the ion conductivity of doped bilayers

found in the present experiments (Fig. 3) suggest that two carboxylic molecules are necessary for the transport of one ion across the membrane. This seems to be true not only for divalent but also for monovalent cations. Due to the inherent "leak" conductivity of undoped bilayers, it was not possible to verify whether $\sigma \propto (\text{conc.})^2$ can be extrapolated to low ionophore concentrations ($\lesssim 0.01\,\%$).

Comparing the results presented here with the conventional ion complexation measurements made by Pressman [14, 17] who used the same ionophore (Ro 2-2985), shows good agreement between the ion-complexing affinities found by Pressman and the ion selectivities for Ca^{2+} and Mg^{2+} reported here (Fig. 3). The complexing affinities (relative values) for Mg^{2+} and Ca^{2+} differ by a factor of 2.6 [14, 17], which compares well with the conductivity ratio $\sigma(Ca^{2+})/\sigma(Mg^{2+}) = 2.9$ of our results (Fig. 3). Pressman used a two-phase (bulk) water-lipid distribution system [14] to measure the complexation of cations with ionophores. Unfortunately, it is not possible to compare his results on monovalent cations with those he made with bivalent cations [17]. He found, however, in agreement with the conductivity measurements reported here, a higher affinity of Ro 2-2985 for K^+ than for Na^+ and Li^+.

Ion Selectivity, Coccidiostatic Activity and Antibacterial Activity

Several authors [5, 15, 21, 24] have suggested that carboxylic ionophores owe their biological activity to changes in the ion transport properties induced in biological membranes. It appears that so far no correlation has been attempted between the two aspects, i.e. using structurally similar molecules that possess different biological activities and comparing them with changes of ion transport properties induced by the ionophores in doped bilayer membranes.

A comparison of the two columns σ/σ_o and σ_s/σ in Table 1 with the coccidiostatic activity in column C shows no correlation between the ion conductivity changes in bilayers induced by the ionophores and their coccidiostatic activity. σ/σ_o of Ro 2-2985, for instance, is relatively small in comparison with the less potent ionophores Ro 20-0006 and Ro 20-0083 (Table 1) which induce large conductivity increases. (The latter ionophore even lacks coccidiostatic activity.) In contrast, Ro 7-9409 does not influence the bilayer conductivity ($\sigma/\sigma_o = 1$) while still exhibiting coccidiostatic activity. The same lack of correlation is found when the ion selectivity (σ_s/σ) is compared with the coccidiostatic activity of Ro 2-2985, Ro 20-006 and Ro 7-9431. These compounds are all selective for K^+ (Table 1), yet only the first two are

coccidiostatic. Ro 7-9409 does not seem to be ion-selective (no increase of conductivity was found) but it is a coccidiostatic agent.

Comparing the numbers of the two columns BE and BTA (Table 1) with those of columns σ/σ_o and σ_s/σ shows that there is no correlation between the relative *in vitro* activity of the ionophores *vs.* bacillus E and bacillus TA and their effects on ion permeability through artificial membranes. (It was suggested by one reviewer of this article that part of the poor correlation between biological and *in vitro* activity of the ionophores may be due to the fact that animals hydrolyze the acetylated derivative to the more active parent compound but its *in vitro* conductivity induction may be further reduced by its less favorable partition into the membrane.)

Penetration of Biogenic Amines

Several workers [3, 10, 17, 21] have reported studies on the influence of Ro 2-2985 on muscle contraction. They found that the ionophore not only has the ability to increase the permeability of membranes of the sarcoplasmatic reticulum to Ca^{2+} [10, 21] but also to increase the membrane permeability for catecholamines [10, 17]. The latter is most probably due to its ability to complex organic amines and to transport them across membranes. Concerning the transport to BA through doped lipid bilayers, several questions arise.

(*i*) To what extent do the investigated ionophores increase the BA-permeability of membranes and how selective are they for different biogenic amines ? The results in Table 3 answer this question. The permeability coefficients p of the four biogenic amines studied are identical for the ionophores Ro 2-2985 and Ro 20-0006. However, considerable differences exist between these two and the others. Ro 20-0083, for instance, induces a 340 times smaller increase in membrane permeability for 5-HT than the most active ionophore (Ro 2-2985). All ionophores in Table 3 are selective for biogenic amines. Their permeability decreases in the order: 5-HT, DA, NE and E.

The permeability coefficient ratios p_n/p_m for various biogenic amines (the indices refer to the various biogenic amines considered) reflect the relative preference of the ionophores for BA. Table 4 summarizes the ratios. The values are taken from Table 3. The results in Tables 3 and 4 show that the ionophores virtually do not distinguish between 5-HT and DA. Their preference increases for 5-HT and NE and is best when comparing E with the other BA's.

Table 4. Permeability coefficient ratios p_n/p_m of various biogenic amines penetrating doped (0.1%) lipid bilayer membranes

	p_S/p_{DA}	p_S/p_{NE}	p_S/p_E
Ro 2-2985	1.7	6.8	72
Ro 20-0006	1.7	6.8	72
Ro 20-0083	>7.6	>7.6	>7.6
Ro 7-9409	2.9	6.4	>28
Ro 7-9431	1.4	2.1	>420

The indices refer to the biogenic amines considered (S = 5-HT, DA = dopamine, NE = norepinephrine, E = epinephrine). 0.1 M $CaCl_2$ was used as an electrolyte solution.

The preference for BA of doped membranes induced by the ionophores (Fig. 5; Table 4) exceed by far the preference for cations (Fig. 3; Table 1) and no correlation seems to exist between the two properties. Ionophores that distinguish between BA do not necessarily distinguish between cations and vice versa. In contrast to Pressman, who found a rough correlation between the complexing affinities of different types of carboxylic ionophores for E and divalent cations [17], the results reported here do not suggest such a correlation. Ro 20-0083, for instance, induces a relatively high Ca^{2+} conductivity (Fig. 3) which is comparable to that induced by Ro 2-2985 and Ro 20-0006. However, the induced permeability for E (Table 3) does not parallel the increase in conductivity for Ca^{2+}.

The order of the relative complexing affinities of Ro 2-2985 for BA reported by Pressman [17] agrees with the permeabilities $p_{NE} > p_E$ of the measurements in Table 3. The complexing ratio $K_{NE}/K_E = 28$ determined in his two-phase water-lipid distribution system [14, 17] is in agreement within a factor of 4 with the penetration ratio $p_{NE}/p_E = 8.3$ of the measurements in Table 3.

(*ii*) The question whether the penetration of BA is linearly related (directly coupled) to the penetration of cations across doped lipid membranes may be important for the understanding of the biological activity of carboxylic ionophores. For instance, a consequence of direct coupling would be an enhanced ionophore-induced release of catecholamines from adrenergic nerve endings or chromaffin cells with increasing ion flux across membranes. Besides direct coupling one might think of another mechanism; i.e., the competitive inhibition of BA penetration by cations. In this case it is conceivable to assume that an ionophore molecule is unable to transport BA when its polar groups are saturated. Consequently, the penetration of

BA would be most heavily influenced by those cations that are transported across the membrane. In this case ionic gradients and membrane potentials would influence BA penetration less than in the case of direct coupling.

The linear relationship found between the concentration of ionophores and the transport of NE across bilayer membranes (Fig. 4) is contradictory to the square law dependence $\sigma \propto (\text{conc.})^2$ of the membrane conductivity against ionophore concentration (Fig. 3) if it is assumed that the same complex is responsible for the transport of both, ions and BA. The linear relationship in Fig. 4 suggests that only one ionophore molecule is necessary to complex one BA molecule, which is in contrast to the complexation of cations where two ionophore molecules are required. It seems, therefore, that different complexes are responsible for the transport of ions and the penetration of BA, thus excluding direct coupling.

The measurements in Fig. 5 show that the penetration of NE varies greatly when different electrolyte solutions are used. It is interesting to see that the ionophore-induced penetration of NE across the bilayers is most pronounced for bi-distilled and deionized water. The smallest penetration coefficients (Table 2) for NE were measured in $CaCl_2$ and KCl; i.e., in those electrolytes whose cations are preferentially transported across doped membranes (Fig. 3; Table 1). This finding is further evidence for the assumption that BA transport is not directly coupled to the ion transport: the more cations that are transported, the more cation-complexes containing two ionophore molecules are formed, thus reducing the number of uncomplexed ionophore molecules in the membrane. The concept of competitive inhibition agrees quantitatively well with the measurements of Figs. 3, 4 and 5. The mechanism between the transport of BA and ions across doped membranes will be further investigated.

(*iii*) The answer to the question whether there exists a correlation between the *in vitro* antibacterial activity *vs.* bacillus E and bacillus TA and the penetration of BA through doped lipid bilayers seems to be positive. The measured penetration coefficients (Table 3) correlate well with the relative *in vitro* activities. This correlation, however, is most likely accidental. As biogenic amines hardly influence the metabolism of bacteria, it seems more likely that the ionophores increase in an unspecific way the permeability of bacterial membranes to even other classes of molecules, e.g. bacterial metabolites. Further experiments are necessary to verify this aspect.

The increase in rate and contractility of the isolated cat hearts caused by the ionophores in low concentrations is undoubtedly due to a release of NE from the adrenergic nerve endings, since these effects were absent after depletion of NE stores with reserpine or after destruction of cardiac ad-

renergic nerve endings with 6-hydroxydopamine. Both Ro 2-2985 and Ro 20-0006 caused a positive inotropic effect at similarly low concentrations (Table 3). This is in agreement with the high permeability of NE induced by these antibiotics in artificial lipid membranes. Furthermore, Ro 20-0083 did not influence the cardiac contractile force up to a concentration of 10^{-5} M which agrees with the undetectably small penetration coefficient measured for NE in doped lipid bilayers (Table 3). The correlation between the cardiac effects and the ionophore-induced penetration of NE through doped membranes is not as good for Ro 7-9409 and Ro 7-9431 (Table 3). As a whole, however, the results obtained with the very simple model membranes correspond surprisingly well to the findings in the isolated cat heart.

Levy *et al.* [10] demonstrated recently that Ro 2-2985 increases the rate of isolated atria of rabbits at low concentrations, an effect that was attributed to a release of endogenous catecholamines. With increasing ionophore concentrations the resting tension was found to be elevated. This is in agreement with the development of the cardiac contracture observed in our experiments at high ionophore concentrations. It seems highly probable that this effect is due to an ionophore-induced change of the Ca^{2+} permeability of the myocardiac membranes of the cells. (Catecholamines are not involved since the ionophore-induced contracture was observed after the depletion of NE with reserpine or after the destruction of the cardiac adrenergic nerve endings with 6-hydroxydopamine.) This finding is in agreement with the square law dependence $\sigma \propto (\text{conc.})^2$ and the linear relationship $[\text{NE}] \propto (\text{conc.})^1$ measured for the ion conductivity and the penetration of NE in bilayer membranes, respectively (Figs. 3 and 4). Due to the different power dependences, the ion conductivity must become dominant over the penetration of NE at high ionophore concentrations which is in agreement with the finding that cardiac concentration effects are governed by the ion transportation properties of the ionophores at high concentrations; i.e. their NE transportation properties are overruled.

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