Biochemical Pharmacology, Vol. 38, No. 11, pp. 1755–1762, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

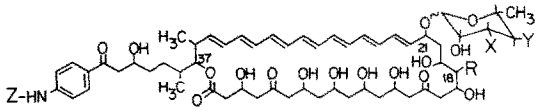
RELATIONSHIP BETWEEN IONOPHORIC AND HAEMOLYTIC ACTIVITIES OF PERIMYCIN A AND VACIDIN A, TWO POLYENE MACROLIDE ANTIFUNGAL ANTIBIOTICS

B. CYBULSKA,*† E. BOROWSKI* and C. M. GARY-BOBO‡

* Department of Pharmaceutical Technology and Biochemistry, Technical University of Gdansk, 80–952 Gdansk, Poland and ‡ Service de Biophysique, Département de Biologie, CEN Saclay, 91191 Gif-Sur-Yvette Cedex, France

(*Received* 24 *May* 1988; *accepted* 29 *November* 1988)

Abstract—The ionophoric and hemolytic activities of two antifungal aromatic heptaenes: vacidin A and perimycin A, were studied on human red blood cells. Measurements of hemolysis, K^+ influx and efflux, H^+ movement and potential difference across the cell membrane, show that the hemolytic activity, being related to the K^+ permeability induced by the polyene, is strongly dependent on the ability of this polyene to induce H^+ movement. It was shown that: (1) both antibiotics have approximately the same efficiency in inducing K^+ permeability, but a 100-fold difference in their hemolytic activity; (2) their hemolytic activity is related to their ability to induce H^+ movement; (3) the protonophoric activity requires the existence of a free carboxyl group in the macrolide ring, as in vacidin A. The hemolytic activity is determined by the intrinsic efficiency of a K^+/H^+ exchange induced by this polyene. With perimycin A, which lacks the free carboxyl group, the hemolytic activity is dependent on the Cl^- conductive flux which slows down the K^+ flux.



	R	X	Y	Z
Perimycin A	CH_3	OH	NH_2	CH_3
Vacidin A	COOH	NH_2	OH	H

Fig. 1. Structure of perimycin A and vacidin A.

Perimycin A and vacidin A (Fig. 1) are antifungal antiobiotics belonging to the group of large macrolide ring polyenes, subgroup of aromatic heptaenes [1]. These antibiotics are biologically much more active than the non-aromatic polyenes amphotericin B and nystatin [2] which are commonly used in human antifungal therapy, although their mode of action appears comparable. All large macrolide ring polyenes are membrane active compounds, able to form channels, the selectivity of which is dependent on the antibiotic structure, concentration and conditions of action. Aromatic heptaenes form cation-selective channels [3–5] which exhibit some inter-cationic selectivity [6, 7].

Our previous studies of the action of aromatic heptaenes on both biological and model systems [2, 8] have shown that perimycin A has apparently very low activity for cholesterol-containing membranes. This antibiotic has very low hemolytic activity on human erythrocytes, although it is very efficient in inhibiting growth of microorganisms such as yeast cells. This is in contrast to vacidin A which is as active in cholesterol- as in ergosterol-containing membranes.

A comparative study of the ionophoric action of a series of natural or semisynthetic derivatives of aromatic polyenes (vacidin A derivatives) has shown [8] that there is a relationship between the presence of a free carboxyl group in the C18 position of the macrolide ring and the hemolytic activity of the compound. This carboxyl group, present in vacidin A, confers hemolytic activity to these molecules. In the absence of this group (perimycin A, vacidin A-methyl ester) the hemolytic activity is very low. In order to interpret these observations, it was suggested that the free carboxyl group is important in determining the permselectivity of polyene induced pathway to cations and more precisely the permeability to protons [9]. Therefore, a comparative study of the effect of perimycin A and vacidin A on human erythrocytes was carried out. The results of this study show that, although both polyenes induce a high permeability to potassium, the resulting volume change in isotonic potassium chloride medium, which eventually leads to hemolysis, depends on the ability of the polyene to induce proton permeability.

MATERIALS AND METHODS

The sources of vacidin A and perimycin A were reported previously [2]. FCCP (carbonyl cyanid-*p*-trifluoromethoxyphenyl hydrazone) was from Boehringer-Mannheim (Hamburg, F.R.G.), valinomycin from Sigma Chemical Co. (Poole, U.K.) and DiS-C_3-5-(diisopropyl dicarbocyanide) from Eastern–Kodak Co. (Rochester, NY).

† To whom correspondence should be addressed.

Human erythrocytes were isolated from blood as previously described [8]. After a final washing in 150 mM choline chloride buffered with 3 mM Tris at pH 7.4, cells were kept on ice and used within a few hours.

Hemolytic activity measurements. 2×10^7 cells/ml were suspended in a 150 mM KCl solution buffered with 3 mM Tris at pH 7.4 and equilibrated at 37° in a shaking water-bath. Then the desired quantities of the tested polyene were added as microliters of its concentrated solution in dimethylsulfoxide. The solvent by itself had no effect on cells at the concentration used.

Kinetics of lysis. At time intervals, aliquots of the cell suspension were centrifuged (1500 *g*, 2 min) and hemoglobin was measured in supernatants at 540 nm. 100% hemolysis was taken as the absorbance obtained after osmotic shock. Dose–response curves were established in the same condition after 60 min incubation with increasing concentration of polyenes. In experiments in which the effect of FCCP was measured, microliters of a concentrated ethanolic solution of this protonophore were added before polyene addition. The final FCCP concentration was 5×10^{-5} M, otherwise indicated.

K^+-release. Kinetics and dose–response curves were established by suspending red cells (2×10^7 cell/ml) in a 150 mM sodium chloride solution buffered with 3 mM Tris at pH 7.4. K^+-release was measured at time intervals (kinetics) or after 60 min of incubation (dose–response curves) with the polyene at 37°, by flame photometry in the supernatant. 10^8 cells/ml of unbuffered choline chloride has been used for the measurement at 22° of the kinetic of K^+ release parallel to the measurements of proton efflux (see below).

Volume changes were measured by monitoring the light scattered at a right angle at 660 nm by the cell suspension, using a Perkin–Elmer spectrofluorimeter, in temperature controlled conditions (22°). Calibration curves were obtained by suspending 10^7 cells/ml of saline solution of osmolarity ranging from 200 to 600 mOsmoles. In the range studied a linear relationship was obtained between light scattering and volume change. For swelling measurements, 10^7 cells were suspended per ml of 140 mM potassium chloride buffered with 3 mM Tris at pH 7.4 and containing 20 mM sucrose to prevent hemolysis. For shrinking experiments 140 mM choline chloride was used instead of potassium chloride.

Kinetics of proton efflux. 10^8 cells were suspended per ml of unbuffered 150 mM choline chloride solution adjusted at pH 7.4 with KOH. The pH of the suspension was monitored using a pH meter equipped with a combined glass electrode (Radiometer, Copenhagen) connected to a recorder (TZ 4200 Laboratorni Pristoje, Praha). The measurements were carried out on cell suspension in equilibrium with atmospheric carbon dioxide at 22°.

Membrane potential determination. 3 ml of a 150 mM choline chloride solution buffered with 3 mM Tris at pH 7.4, and containing 2×10^{-7} M of DiS-C_3-5 were introduced in the cuvette of the spectrofluorimeter. After temperature equilibration, 3×10^7 cells were introduced. When a stable level of fluorescence corresponding to the distribution of the dye between cells and medium was reached, the tested polyene was introduced and the kinetics of fluorescence intensity change were recorded. Excitation and emission wavelengths were, respectively, 620 and 670 nm.

RESULTS

Hemolytic activity and potassium release

Typical time courses of hemoglobin release induced by perimycin A and vacidin A are given in Fig. 2. It appears clearly from this figure that there is a very large difference between the hemolytic activity of the two antibiotics. The hemolytic activity of perimycin A is very low, as compared to vacidin A: 20 μM of perimycin A are necessary to obtain in 60 min approximately the same level of hemoglobin release than the level obtained with 0.02 μM of vacidin.

The hemolytic activity of both polyenes is increased in the presence of the protonophore FCCP. In preliminary experiments, the effect of FCCP on both hemolysis and potassium release induced by the two polyenes was studied as a function of its concentration. These experiments showed firstly that beyond 10^{-5} M the FCCP effect becomes maximum, and secondly that this effect is the same whether FCCP is added prior to polyene addition or after.

The effect of FCCP on the kinetic of hemolysis induced by perimycin A and vacidin A are given in Fig. 3a and b, respectively.

The stimulatory effect is very important in the case of perimycin A. For instance, whereas 0.1 μM of perimycin A does not promote any significant hemolysis in 6 hr, the same concentration in the presence of FCCP results in 65% hemolysis in less than one hr.

The fact that when FCCP is introduced after 5 hr of incubation with perimycin A, the hemolytic process starts immediately demonstrates that, although inactive, perimycin is actually present in the membrane. The stimulatory effect of FCCP on the hemolytic activity of vacidin A, although significant, is much less important (Fig. 3b). The increase in the hemolytic rate induced by FCCP is mainly visible at low vacidin A concentration.

The activities of the two polyenes in inducing K^+ release do not exhibit such a large difference as their hemolytic activities. Perimycin A and vacidin A have a rather similar efficiency in inducing K^+ release. As an example, the kinetics of K^+ release obtained at the same concentration of 0.05 μM are reproduced in Fig. 4. Perimycin A is only about two times less efficient than vacidin A. Moreover, the kinetics observed are quite similar in the presence of FCCP.

The study of the kinetics of K^+ efflux in general indicates that within about 60 min of incubation with either one of the polyenes, a maximal level of K^+ release is obtained, which depends on the polyene concentration. FCCP increases the rates but does not modify the level of this maximum. Similar kinetics have been already obtained on lipidic vesicles. On this system, it has been shown [19] that channels forming ionophores such as gramicidin D, amphotericin B or vacidin A, at low concentration exhibit a biphasic kinetics of action, which reflects the fact

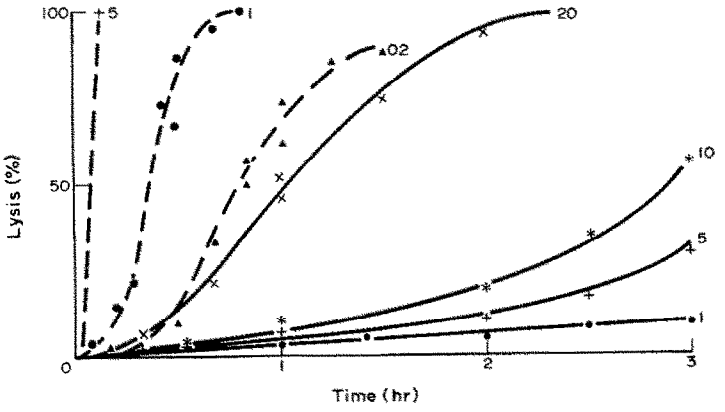


Fig. 2. Time-course of hemolysis induced by perimycin A (solid lines) and vacidin A (dotted lines). Polyene concentration as indicated in μM.

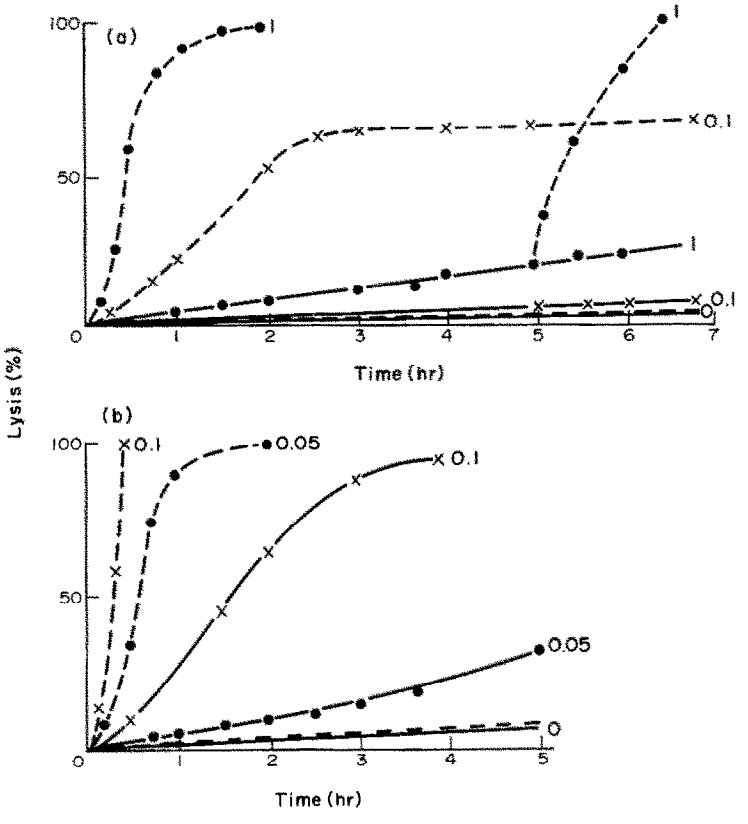


Fig. 3. Time-course of hemolysis induced by perimycin A (a) and vacidin A (b) in presence and absence of protonophore (FCCP, 2.5×10^{-5} M)-polyene concentration as indicated in figures, in μM. Solid line: polyene alone; dotted line: polyene + FCCP. Temperature 37°.

that only part of the vesicles population is permeabilized rapidly upon ionophore addition, the second part, the importance of which is greater the lower the concentration, is permeabilized secondarily at a very slow rate which depends on the rate of exchange of ionophore between vesicles. A similar mechanism may take place with red cells. The statistical distribution of ionophore molecules might result in the formation of subpopulations very differently permeabilized.

Data obtained for the hemolytic and permeabilizing activities of perimycin A and vacidin A are summarized in Fig. 5a and b, under the form of dose–response curves of hemolysis and K^+ release measured after one hr of incubation with the two polyenes with or without FCCP. The parameter of one hr of incubation was chosen rather than the $t_{1/2}$ classically used. It makes possible the direct comparison of the two polyenes which exhibit such a large difference in hemolytic activity. The measurement of

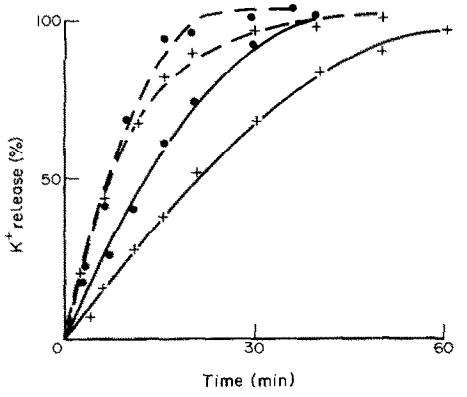


Fig. 4. Time-course of potassium efflux induced by vacidin A and perimycin alone and in the presence of protonophore. Polyene concentration 0.05 μM, FCCP 10^{-5} M. Solid line: antibiotic alone; dotted line: antibiotic + FCCP. Crosses: perimycin A; black dots: vacidin A.

Table 1. Effect of protonophore on potassium release and on hemolytic activity of perimycin A and vacidin A

	K_{50}/(μM)		H_{50}/(μM)	
Antiobiotic	−FCCP	+FCCP	−FCCP	+FCCP
Perimycin A	0.045	0.045	16	0.2
Vacidin A	0.025	0.025	0.24	0.04

K_{50} is the concentration of antibiotic causing 50% intracellular potassium release from 2×10^7 cells/ml in isoosmotic buffered solution of choline chloride after 1 hr incubation at 37°.

H_{50} is the concentration of antibiotic causing 50% hemolysis of 2×10^7 cells/ml in isoosmotic buffered solution of potassium chloride after 1 hr incubation at 37°.

These values were determined graphically from the dose–response curves presented in Fig. 5. FCCP was used at final concentration 2×10^{-5} M.

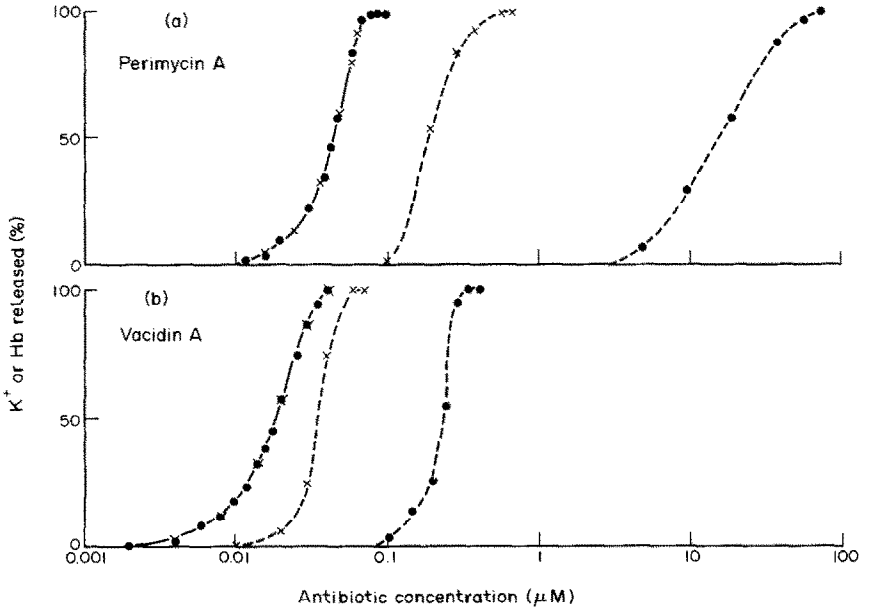


Fig. 5. Dose–response curves, measured after 60 min incubation at 37° with increasing concentration of perimycin A (a) and vacidin A (b). Solid lines: potassium efflux; dotted lines: hemolysis. Black dots: polyene alone; crosses: polyene + FCCP (2.5×10^{-5} M).

$t_{1/2}$ for perimycin A is unpracticable. Moreover, the $t_{1/2}$ parameter implicitly refers to simple, monoexponential kinetics. The kinetics observed with perimycin A and vacidin A are more complex.

The concentration corresponding to 50% K^+ release, K_{50} and 50% hemoglobin release, H_{50}, obtained from the dose–response curves are listed in Table 1.

Vacidin A and perimycin A appear to be similarly efficient in inducing K^+-permeability. The K_{50} of vacidin A is less than two times smaller than the perimycin A K_{50}. The purely kinetical effect of FCCP on K^+-release cannot be observed on dose–response curves established after 60 min of incubation, a long enough period of time for equilibrium to be reached. The hemolytic concentration range of perimycin A is two orders of magnitude higher than that of vacidin A. In the presence of FCCP, both dose–response curves are shifted to a lower concentration range, but the shift is much more important for perimycin A than for vacidin A. The H_{50} values (Table 1) in the presence of FCCP decrease about six-fold for vacidin A and eighty-fold for perimycin A. The protonophore stimulates much more the hemolytic activity of perimycin A than that of vacidin A.

Volume changes

In order to interpret the large difference between

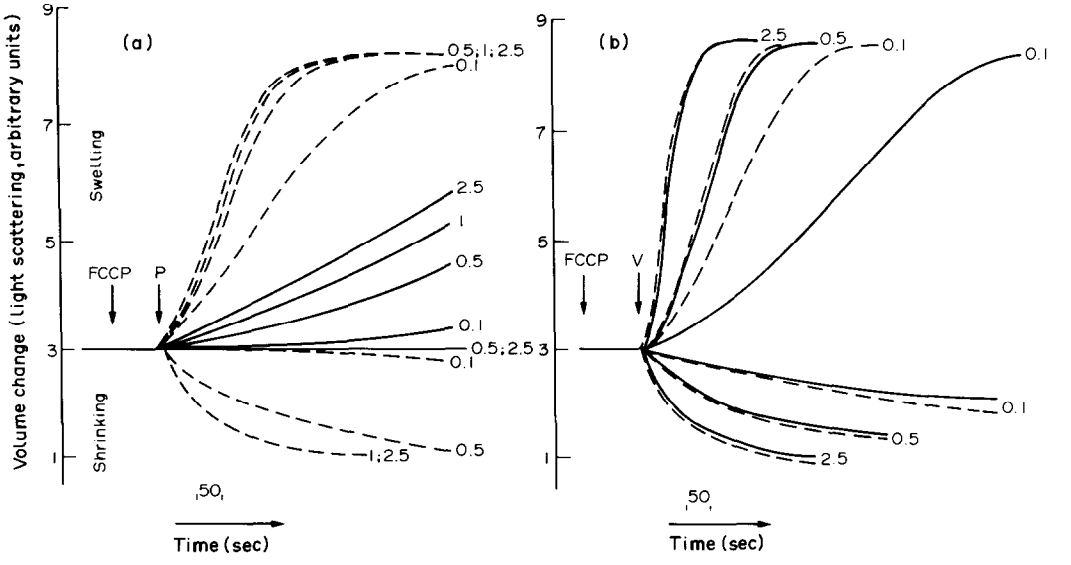


Fig. 6. Time-course of volume changes induced by perimycin A (a) and vacidin A (b). Solid line: polyene alone; dotted lines: polyene + FCCP (2.5×10^{-5} M). Polyene concentrations as indicated in μM. Shrinking in isoosmotic choline chloride. Swelling in isoosmotic potassium chloride.

the hemolytic activity of perimycin A and vacidin A and between the stimulatory effect of FCCP on these activities, cell volumes changes were measured. Cell volumes changes are directly related to net flux of salt [10]. Shrinking of red cells suspended in isotonic choline chloride is the result of the efflux of KCl and swelling of cells in isotonic potassium chloride is the result of potassium chloride uptake, leading eventually to hemolysis under the influence of the oncotic pressure of hemoglobin. In the present experiment, hemolysis was prevented by balancing hemoglobin oncotic pressure by 20 mM sucrose in the suspension medium.

The results are given in Fig. 6a and b for perimycin A and vacidin A, respectively. The concentration range studied for both polyenes was 0.1 to 2.5 μM. As shown before (Fig. 5), in this concentration range the permeabilization to K^+ of the whole cell population is obtained. The volume change induced by vacidin A is increasingly fast with its concentration. The maximum shrinkage is obtained within 2 min at 2.5 μM and within 15 min at 0.1 μM. FCCP increases the shrinking rate, this action being significant only at low vacidin A concentration.

In potassium chloride, the maximum swelling is reached in less than 10 min; the level of this maximum depends on vacidin A concentration. FCCP has no significant effect: neither the rate, nor the maximum level are influenced. As expected perimycin A is much less efficient in inducing volume changes. This polyene induces slowly cell shrinking when alone, but much more rapidly in the presence of FCCP. In potassium chloride perimycin A alone is not able to promote any swelling even at the highest concentration. In the presence of FCCP, the swelling is obtained rapidly at a rate equivalent to that obtained with vacidin A, except at the lowest concentration (0.1 μM). These results show that the effect of FCCP is symmetrical. The protonophore increases the rate of both KCl influx and efflux, without modifying the final equilibrium level of loss or gain of this salt. The fact that FCCP exhibits a much greater stimulating effect on perimycin A than on vacidin A action, suggests that vacidin A is able to induce a proton flux in exchange for potassium, whereas perimycin is not. According to this assumption, the potassium permeability induced by perimycin A must polarize strongly the red cell membrane and this should not happen with vacidin A. In order to test this hypothesis, proton movements and membrane polarization induced by the polyenes were measured.

Proton movements across the red cell membrane were followed by monitoring the pH variation in cell suspensions in unbuffered isotonic choline chloride. Increase in pH is interpreted as a proton uptake by red cells in exchange for K^+ [10]. The K^+ efflux was concurrently measured. It must be noted that this set of experiments has been carried out at a cell concentration of 10^8 cells/ml instead of 10^7 cells/ml in the previous ones. As a result, the polyene concentration indicated cannot be compared directly on a quantitative basis. However, binding measurements of a series of amphotericin B derivatives on red cells [11] have shown that polyenes bind to cells proportionally to their concentration in the external medium. The membrane/water partition coefficients measured are not very high and not very dependent on temperature. If it is assumed that perimycin A and vacidin A behave similarly, it may be concluded that for a given total concentration of polyene introduced in suspensions containing 10^7 cells/ml or 10^8 cells/ml, the actual polyene concentration reached at the membrane level is only about two times higher in the former than in the latter suspension. Therefore, the conclusion which can be drawn on the basis of the comparison of experiments made at different

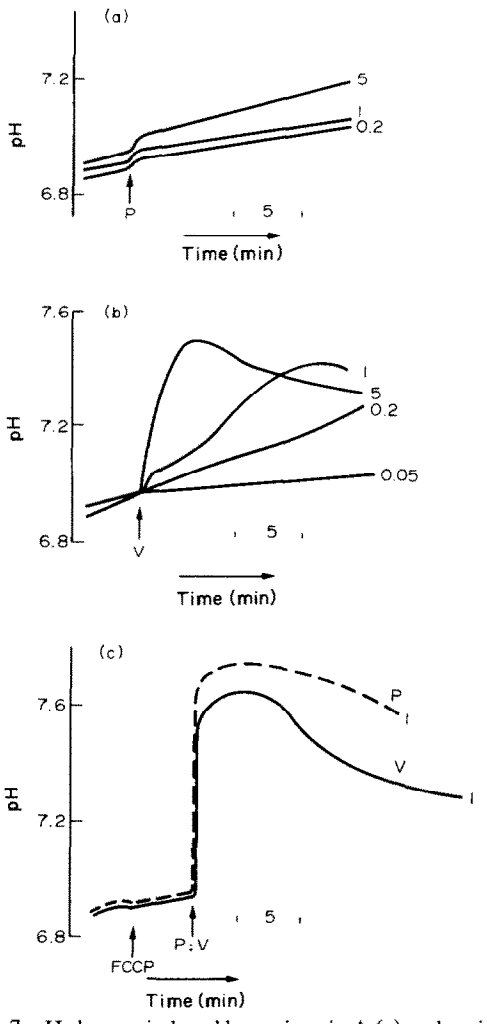


Fig. 7. pH changes induced by perimycin A (a) and vacidin A (b) alone, and in presence of FCCP (2.5×10^{-5} M) (c). Polyene concentrations as indicated in μM. P: perimycin A; V: vacidin A.

cell concentration remains valid, at least qualitatively. The pH variations induced by perimycin A and vacidin A in these conditions are presented in Fig. 7a and b, respectively. Upon addition of vacidin A, pH increases and the amplitude of this variation increases with concentration. At 0.05 μM, the pH variation is at the limit of significance after 15 min, although the K^+ released after this period corresponds to 50% of the cell content. At 5 μM, the pH increases by more than 0.5 units in 5 min during which 100% of the K^+-content of the cell is released. It appears that concomitantly to K^+-efflux, vacidin A promotes a proton influx. However, since protons can leak back rather rapidly through the natural system of pH equilibration in red cells [12] the proton influx measured by the pH variations in the external medium is largely under-estimated. As a matter of fact, the pH change is transitory and it comes back to an equilibrium value, about pH 7.3, after 30 min.

The pH change induced by perimycin A (Fig. 7a), was significant only at the highest concentration used (5 μM). However, even the lowest concentration of perimycin (0.2 μM) was sufficient to induce K^+-permeability although at a slower rate than vacidin A. At a concentration of 1 μM, after 15 min, 100% of intracellular potassium was released into the medium. This indicates that perimycin A, unlike vacidin A, is not able to promote a significant proton influx although it forms an efficient pathway for potassium. This is further evidenced in Fig. 7c in which the pH changes induced by both vacidin A and perimycin A (1 μM) in the presence of protonophore are presented. Similar, abrupt pH shift up to 1 pH unit is observed under the action of both antibiotics. The shift is transient and after some time pH is coming back to equilibrium value.

Membrane potential

On the basis of determinations presented above it can be concluded that as far as K^+ permeability is concerned the differences between vacidin A and perimycin A resides mainly in the kinetics of potassium flux. Since the effect of perimycin A on the permeability to proton is much smaller than that of vacidin A, the K^+-efflux induced by this polyene in the absence of protonophore seems to be delayed by membrane hyperpolarization. This should not be observed for vacidin A which is able to mediate a H^+/K^+ exchange.

In order to check this hypothesis, the effect of perimycin A and vacidin A on the membrane potential was measured by fluorescence, using DiS-C_3-5 as a probe [13]. The effect of both polyene antibiotics was compared to that induced by valinomycin, a K^+-selective ionophore.

The results obtained are in agreement with the expectations (Fig. 8).

Perimycin A (0.1 μM) causes a large hyperpolarization which develops within 2 min and is comparable to that induced by valinomycin (at 2 μM). Vacidin A, used at the same concentration as perimycin A, induces a much smaller change in the potential.

DISCUSSION

The hemolytic activity of perimycin A is about one hundred times lower than that of vacidin A. This large difference between the two polyenes cannot be ascribed to a lower ionophoric efficiency, since perimycin A appears practically as efficient as vacidin A in inducing K^+ permeability. Besides, both polyenes exhibit a cation over anion selectivity even greater than amphotericin B or nystatin [14, 15] and it may be reasonably assumed that like these two non-aromatic polyenes, perimycin A and vacidin A do not have any direct effect on the anion transport systems of the red blood cells [16]. The poor hemolytic activity of perimycin A, as compared to vacidin A, appears to be clearly related to its inability to induce a proton permeability across the red cell membrane, as shown in the present report. It is well known that for red cells in which a large cationic permeability has been induced and which are suspended in isotonic (chloride) medium, the limiting factor of the rate of the net flux of salt which produces volume changes is the chloride permeability: the

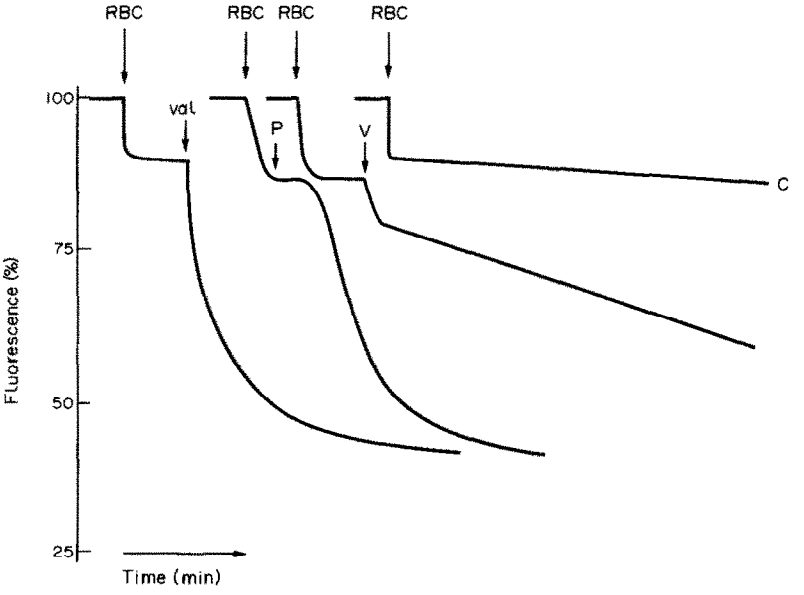


Fig. 8. Fluorescence tracing from 2×10^{-7} Dis-C_3-5. Ordinate: 100% fluorescent dye alone in isoosmotic choline chloride. RBC: addition of 10^7 cells/ml. Val: addition of valinomycin (2 μM); P: addition of perimycin (0.1 μM). V: addition of vacidin A (0.1 μM); C: control.

electrogenic movement of chloride is very slow. This has been shown in particular by experiments carried out with the K^+-selective carrier valinomycin [10, 13, 17] which is not able by itself to promote volume change and hemolysis, but does so rapidly in the presence of protonophore such as FCCP. The same mechanism can account for the difference between the hemolytic activities of perimycin A and vacidin A. Like valinomycin, perimycin A induces a large permeability to K^+ but not to H^+. Thus the rate of volume change, which eventually leads to hemolysis in isotonic KCl medium, is determined by the conducting flux of Cl^-, which is very slow in spite of the strong membrane hyperpolarization. The addition of FCCP allows an electroneutral H^+/K^+ exchange to occur. This promotes an intracellular pH modification which switches on the Jacobs–Steward cycle and the very fast HCO_3^-/Cl^- exchange transport system. This system permits a rapid flux of Cl^- following the K^+, whereas the pH variation is limited by a rapid recirculation of proton and bicarbonate by the very efficient physiological system. At variance with perimycin A, vacidin A is able to perform the H^+/K^+ electroneutral exchange and thus to promote rapid volume changes. Nonetheless, the protonophoric efficiency of vacidin A is lower than that of FCCP, since this protonophore increases the volume change rate significantly.

In a previous report [8], it has been shown that the hemolytic activity of aromatic heptanes was the result of their ability to increase specifically the permeability of the red cell membranes to cation and not to a generalized membrane disorganization (detergent-like effect). Moreover, it was proposed that the hemolytic activity depends on the presence on the macrolide ring of these polyenes of a free, ionizable carboxyl group in the C_{18} position, like in vacidin A. The present data fully support this hypothesis, but also permit to ascribe to this C_{18} carboxyl group the ability of the polyene to induce proton permeability. The carboxyl group is unnecessary for K^+ permeability induction. Low hemolytic activity was exhibited by methyl-esters of vacidin A and others aromatic heptaenes [2, 8]. The decrease of hemolytic activity was not observed on a series of amphotericin B derivatives substituted on the carboxyl group [18].

On the other hand, it has been shown by a ^{31}P-NMR study [19] of aromatic and non aromatic heptaenes on lipid vesicles, that the C_{18} carboxyl group determines the type of permeability the heptaenes induce. In cholesterol-containing membrane, vacidin A and amphotericin B induce a ionic-permeability of the "channel" type, comparable to gramicidin D, whereas perimycin A induces a permeability of the "mobile carrier" type, comparable to valinomycin. Therefore, the C_{18} carboxyl group determines apparently the structure of the ionic pathway created by polyenes in membrane. In contrast all these heptaenes induce channel-type permeability in ergosterol-containing membranes. The question of the relationship between the structure of the pathway and its intercationic selectivity, especially regarding protons, as well as the role of carboxyl group for ergosterol/cholesterol discrimination are under study.

Concerning the biological effects of polyene macrolides, it can be considered that the toxicity of a K^+/H^+ exchanger may be very different from that of a cation selective ionophore such as valinomycin. Low permeability to proton is very important for the hemolytic effect, but this result on red cells cannot be simply generalized on other cell types. In particular, one of the most detrimental effect of poly-

enes, besides hemolytic anemia, is nephrotoxicity [20, 21].

The disturbances of these two types of antibiotics on kidney cells specialized in ion transport, and for which pH disequilibrium is potentially very important, remain to be investigated.

Acknowledgements—This investigation was supported by the Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw (Project CPBP 04 01) and in part by INSERM, France, Grant No. 041003.

We gratefully acknowledge Dr J. Bolard for providing his laboratory facilities and helpful discussions.

REFERENCES

1. Omura S and Tanaka H, Production, structure and antifungal activity of polyene macrolides. In: *Macrolide antibiotics: Chemistry, Biology, and Practise* (Ed. Omura S.), pp. 351–404, Academic Press, New York, 1969.
2. Cybulska B, Ziminski T, Borowski E and Gary-Bobo CM, The influence of electric charge of aromatic heptaene macrolide antibiotics on their activity on biological and lipidic model membranes. *Mol Pharmacol* **24**: 270–265, 1983.
3. Holz RW, The effects of the polyene antibiotics nystatin and amphotericin B on thin lipid membranes. *Ann NY Acad Sci* **235**: 469–479, 1974.
4. Kasumow Kh M and Liberman EA, Ionic permeability of bimolecular membranes in the presence of polyenic antibiotics. *Biofizika (USSR)* **18**: 264–271, 1973.
5. Kasumow Kh M, Mekhtiev N Kh and Karakozov SD, Potential-dependent formation of single conducting ion channels in lipid bilayers induced by the polyene antibiotic levorin A_2. *Biochim Biophys Acta* **644**: 369–372, 1981.
6. Cybulska B, The role of structural factors in the modification of membranes permeability by polyene macrolide antibiotics. In: *Proceedings of Sixth School on Biophysics of Membrane Transport, Jastrzebia Gora, Poland, 4–13 May 1981* (Eds. Kuczera J, Gregorczyk C and Przestalski S), pp. 124–149. The Agricultural University of Wroclaw, 1981.
7. Kasumov Kh M, Samedowa AA and Shenin Yu D, Study on the mechanism of interaction between separate levorin and nystatin components and lipid membranes. *Antibiotiki i Medicinskaja Biotechnologia* **11**: 824–828, 1987.
8. Cybulska B, Mazerski J, Borowski E and Gary-Bobo CM, Haemolytic activity of aromatic heptaenes a group of polyene macrolide antifungal antibiotics. *Biochem Pharmacol* **33**: 41–46, 1984.
9. Cybulska B and Borowski E, What is responsible for the dissociation of permeabilizing and hemolytic activity of Perimycin A. In: *Molecular Aspects of Chemotherapy, Abstracts of Postsymposium of IUPAC 14th International Symposium on the Chemistry of Natural Products, Gdansk, Poland, 16–18 July, 1985* p. 52.
10. Hladky SB and Rink TJ, Use of ion transporters, pH measurements and light scattering with red blood cells. In: *Red Cells Membrane—A Methodological Approach* (Eds. Ellory JC and Young JD), pp. 335–358, Academic Press, 1982.
11. Szpornarski W, Wietzerbin J, Borowski E and Gary-Bobo CM, Interaction of ^{14}C-labelled amphotericin B derivative with human erythrocytes: relationship between binding and induced K^+ leak. *Biochim Biophys Acta* **938**: 97–106, 1988.
12. Mc Elroy Critz A and Crandall E, pH equilibration in human erythrocyte suspensions. *J Membr Biol* **54**: 81–88, 1980.
13. Rink TJ and Hladky SB, Measurement of red cell membrane potential with fluorescent dye. In: *Red Cells Membrane—A Methodological Approach*, (Eds. Ellory, JC and Young, JD), pp. 321–334, Academic Press, London, 1982.
14. Deuticke B, Lütkemeier P and Sistemich M, Ion selectivity of aqueous leaks induced in the erythrocyte membrane by crosslinking of membrane proteins. *Biochim Biophys Acta* **775**: 150–160, 1984.
15. Marty A and Finkelstein A, Pores formed in lipid bilayer membranes by nystatin differences in one sided and two sided actions. *J Gen Physiol* **65**: 515–5226, 1984.
16. Cass A and Dalmark M, Equilibrium dialysis of ions in nystatin treated cells. *Nature [New Biol]* **244**: 47–49, 1973.
17. Knauf PA, Law FV and Marchant PA, Relationship of net chloride flow across the human erythrocyte membrane to the anion exchange mechanism. *J Gen Physiol* **81**: 95–126, 1983.
18. Cheron M, Cybulska B, Mazerski J, Grzybowska J, Czerwinski and Borowski E, Quantitative structure–activity relationships in amphotericin B derivatives. *Biochem Pharmacol* **37**: 827–836, 1988.
19. Cybulska B, Herve M, Borowski E and Gary-Bobo CM, Effect of the polar head structure of polyene macrolide antifungal antibiotics on the mode of permeabilization of ergosterol and cholesterol containing lipidic vesicles studied by ^{31}P NMR. *Mol Pharmacol* **24**: 293–298, 1986.
20. Medoff G, Brajtburg J, Kobayashi GS and Bolard J, Antifungal agents useful in the therapy of systemic fungal infections. *Ann Rev Pharmacol Toxicol* **23**: 303–330, 1983.
21. Steinmetz PR and Palmisano J, Disorders of proton secretion by the kidney. In: *Physiology of Membrane Disorders*. (Eds. Andreoli ET, Hoffman JF, Fanestil DD and Shultz SG), pp. 957–983, Plenum, New York, 1986.