BRIEF COMMUNICATION

PANCURONIUM INACTIVATES ALAMETHICIN-INDUCED CONDUCTANCE IN ARTIFICIAL MEMBRANES

JAMES J. DONOVAN AND RAMON LATORRE, *Department of Physiology, Harvard Medical School, Boston, Massachusetts 02115 U.S.A.*

ABSTRACT The effect of pancuronium on alamethicin-induced currents was studied in negatively charged lipid bilayer membranes. Pancuronium induces inactivation of the alamethicin-induced current. Inactivation is only observed if this compound is added to the compartment containing alamethicin. Moreover, the process of inactivation is reduced or abolished if pancuronium is added to the alamethicin-free side of the membrane. The time needed to recover from inactivation is greatly reduced if the aqueous solution in the alamethicin-free compartment is stirred. These data suggest that pancuronium permeates through the membrane when the alamethicin-induced conductance is "turned on," binds to the other membrane surface, and changes the surface potential.

INTRODUCTION

The interaction of pancuronium (PC), a bis-quaternary ammonium compound with a rigid steroidal structure, with sodium channels in squid axons, has been reported recently by Yeh and Narahashi (1977). PC selectively blocks the sodium conductance and induces inactivation after the sodium inactivation has been removed by pronase. Moreover, Armstrong and Yeh (1978) have found that in axons where inactivation has been destroyed by pronase, the on-gating current is unchanged by PC, but the fast component of the off-gating current is eliminated. Thus, PC appears to hold the activation gates open and immobilize the gating charge.

We report here the effect of the drug PC on the alamethicin-induced conductance in lipid bilayer membranes. Alamethicin is a linear polypeptide with a molecular weight of 1,700 (Martin and Williams, 1976). The main component (85%) of natural alamethicin is the F-30 fraction containing one titratable group with a pK of 5.5 (Payne et al., 1970). When present on one side of a lipid bilayer membrane, alamethicin induces voltage-dependent conductance phenomena similar to those found in excitable biological membranes. In particular, the conductance of a membrane treated with alamethicin increases e-fold for a potential change of about 5 mV (Gordon and Haydon, 1975; Eisenberg et al., 1973). There is strong evidence that the alamethicin-induced conductance arises as a consequence of the formation of channels through the membrane (Gordon and Haydon, 1972; Eisenberg et al., 1973).

METHODS

We formed the membranes by apposition of two separate monolayers, as has been previously described in detail (Montal and Mueller, 1972; Alvarez Latorre, 1978). In the present experiments, the monolayers were formed with a mixture of glycerol monooleate (GMO) (Nucheck, Prep. Inc., Elysian, Minn.) and phosphatidylserine (PS) (Supelco, Inc., Bellefonte, Pa.), 2:1 by weight. The aqueous solutions in both compartments consisted of unbuffered 0.1 M KCl, pH 5.5. Alamethicin (Upjohn Co., Kalamazoo, Mich.) was added to only one compartment in a concentrated ethanolic solution to a final concentration of 3–5 $\times$ 10^{-7} g/ml. An equivalent amount of ethanol had no effect on the membrane conductance. Both compartments were stirred for one hour in the presence of the antibiotic before any electrical measurements were performed. PC bromide ($3\alpha,17\beta$-diacetoxy-$2\beta,16\beta$-dipiperidino-5α-androstane dimethobromide) and decamethonium bromide ($[CH_3]_3$ $N[CH_2]_{10}$ $N[CH_3]_3$ Br_2) (both kindly supplied by J. Z. Yeh, Northwestern University), unless otherwise stated, were added to only one compartment in aqueous solutions of various concentrations. Electrical measurements used Ag/AgCl electrodes in a four-electrode system. Positive potential and currents are defined as the alamethicin-containing compartment positive.

RESULTS AND DISCUSSION

Fig. 1 *a* shows the time course of the alamethicin-induced current in response to a potential step of 85 mV in the absence of PC. The current rises monotonically to a constant steady-state value. The time-dependent current response is well described by a single exponential with a time constant of 25 ms. When PC is added to the same side as the alamethicin, the shape of the current response changes: it increases rapidly to a maximum and then decreases to a lower, steady-state value (Fig. 1 *b*). Thus, when PC is present in the same side as the alamethicin, the alamethicin-induced current "inactivates."

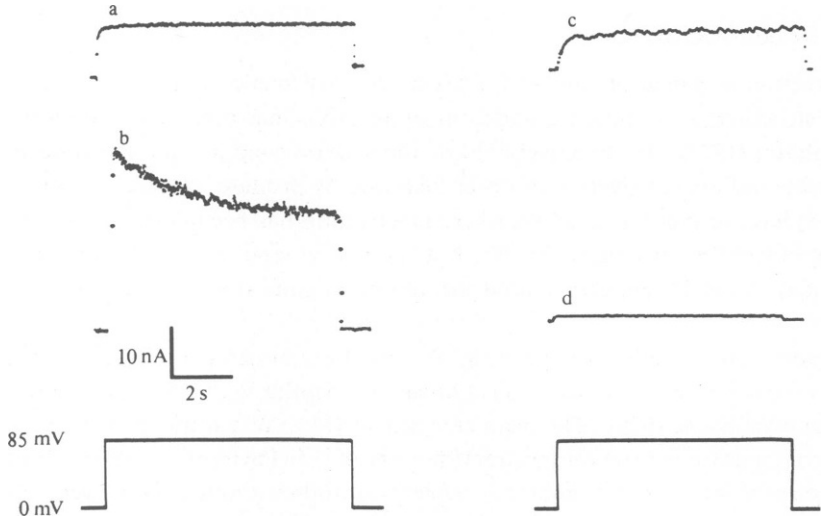

FIGURE 1 The alamethicin-induced conductance in response to a step change in potential in the presence and absence of PC. In each case, the potential step is from zero to 85 mV. (*a*) No PC is present. (*b*) After adding PC to the alamethicin-containing compartment only, [PC] = 1 mM. (*c*) After adding PC to both sides, [PC] = 1 mM. (*d*) After adding PC to the alamethicin-free side only, [PC] = 1 mM. GMO/PS membrane formed in 0.1 M KCl. Alamethicin concentration was 4.0 $\times$ 10^{-7} g/ml. Membrane area was 3.5 $\times$ 10^{-4} cm^2.

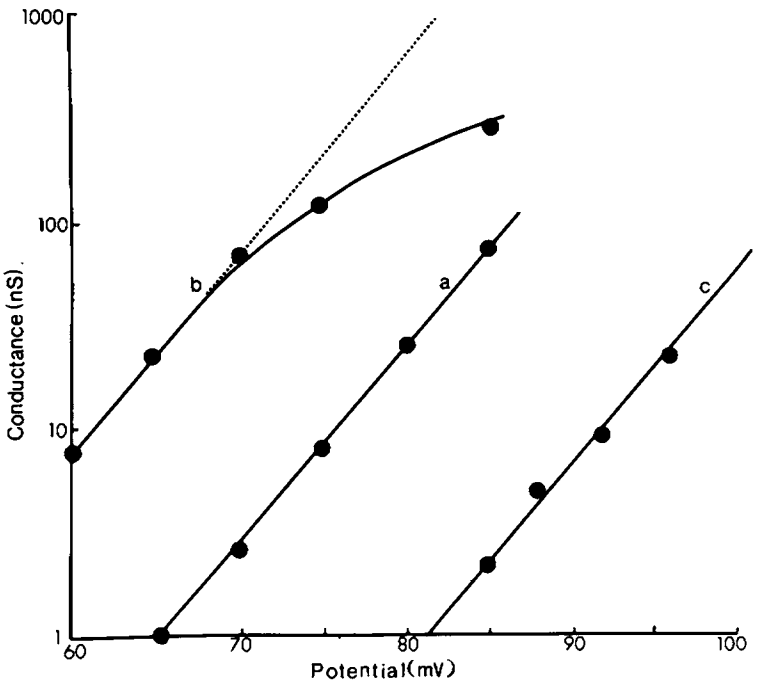

FIGURE 2 The steady-state conductance characteristics in the absences and presence of PC. (*a*) No PC is present. (*b*) PC is present on the side containing alamethicin at a concentration of 1 mM. (*c*) PC is present on the alamethicin-free side at a concentration of 1 mM. GMO/PS membrane formed in 0.1 M KCl. Alamethicin concentration was 4.0×10^{-7} g/ml. Membrane area was 3.5×10^{-4} cm^2.

Moreover, we have found that the amount of inactivation induced by PC, when present in the alamethicin-containing compartment, is a function of the PC concentration in the alamethicin-free compartment. Specifically, if the PC concentration in the latter compartment is increased, the inactivation is reduced or abolished (Fig. 1 *c*). The general shape of the time course of the current does not change in the presence of PC on the alamethicin-free side only (Fig. 1 *d*), but the magnitude of the steady-state alamethicin-induced current is greatly reduced.

The steady-state alamethicin-induced conductance (G_{ss}) versus potential in the absence of PC is shown in Fig. 2. In the absence of PC, the log of the steady-state conductance versus potential curve (Fig. 2 *a*) is well described by a straight line which follows the expression: $\ln(G_{ss}) \sim \gamma e V_m / kT$, where e is the electronic charge; V_m is the transmembrane potential, and k and T have their usual meanings, In GMO/PS membranes, $\gamma = 4.4$.

The effects of PC on alamethicin-induced conductance shown in Fig. 1 are also evident in Fig. 2. When PC is added to the alamethicin-containing side, the log G_{ss}-V curve is shifted to the left (Fig. 2 *b*). The curve is linear at low conductances, but at higher conductances, it bends towards the potential axis. The deviation from the dotted line is due to the appearance of the inactivation process shown in Fig. 1 *b*. Fig. 2 *b* also shows that the degree of inactivation is proportional to the alamethicin-induced conductance. When PC is added only to the alamethicin-free side of the membrane, the log G_{ss}-V curve shifts to the right

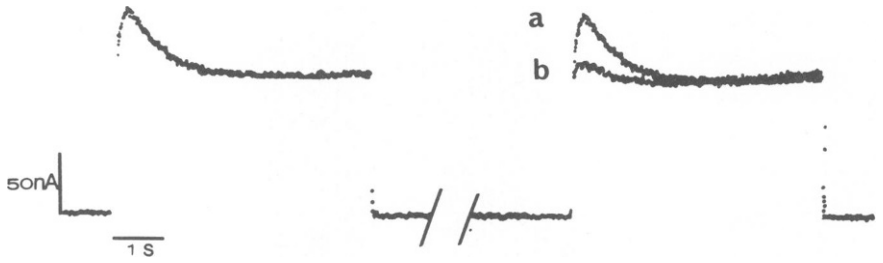

FIGURE 3 Recovery from inactivation caused by PC added to the alamethicin-containing side. Two identical pulses of 80 mV were applied to the membrane, separated by different time intervals. The time intervals were 60 s for *a*, and 30 s for *b*. The alamethicin-free compartment was stirred throughout each time interval. GMO/PS membrane formed in 0.1 M KCl. Alamethicin concentration was 5.3×10^{-7} g/ml. Membrane area was 3.5×10^{-4} cm^2. [PC] = 1 mM.

(Fig. 2 *c*). The curve does not deviate from a straight line because no inactivation is seen (Fig. 1 *d*).

Fig. 3 shows that it takes time to recover from inactivation. Recovery of the alamethicin-induced current from the inactivation promoted by PC was studied by the double-pulse method. Two identical potential pulses were applied across the membrane at different time intervals, and the maximum amplitude of the current associated with the second pulse is taken as the degree of recovery from inactivation promoted by the first pulse. As shown in Fig. 3, the peak alamethicin-induced current associated with the second pulse reaches the same amplitude as the peak current associated with the first pulse when the time interval is greater than 1 min. This recovery time depends on stirring. Stirring of the solution in the alamethicin-free compartment leads to a recovery time on the order of 1–2 min (Fig. 3), but when stirring is stopped, this time increases to several minutes (not shown). Changes of the rate of stirring in the alamethicin-containing compartment do not change the recovery time.

We think that the inactivation of the alamethicin-induced current caused by PC can be interpreted as a change in the transmembrane potential, and we suggest the following model to explain the present results: when alamethicin channels open as a consequence of the applied potential, PC is able to permeate through the membrane. Since our GMO/PS membranes are negatively charged, permeation and subsequent binding of PC to the membrane surface not containing alamethicin will decrease the surface charge density. This decrease in surface density will, in turn, make the diffuse double layer potential less negative. Because alamethicin "sees" the transmembrane potential (i.e. the applied potential plus the difference in surface potential), the alamethicin-induced conductance will be turned off. This model is formally equivalent to that proposed by Heyer et al. (1976) to explain the inactivation promoted by long chain quaternary ammonium ions in membranes treated with monazomycin.

The fact that PC is able to shift the log G_{ss}-V curve to the right when added to the alamethicn-free side (Fig. 2 *c*) is consistent with the idea that this compound decreases the surface potential of that side. To test this point, we have studied the effects of PC on the nonactin-induced conductance (McLaughlin et al., 1970), and on the voltage-dependent capacitance (Alvarez and Latorre, 1978). The surface potential changes induced by PC calculated from these two independent methods are very similar and agree with the changes in surface

potential obtained from the shift to the right of the log G_{ss}-V curves. This effect is much larger than can be accounted for by a screening of the negative change of phosphatidlyserine, due to the divalent PC (3.5 mV or less under the conditions of our experiments), but can be explained if PC binds to the membrane surface and decreases the membrane surface charge density. The compound decamethonium is similar to PC in that it also has two positive charges, one at each end of the molecule, but it does not cause inactivation, even at concentrations up to 2 mM. Neither does it shift the log G_{ss}-V curve to the left when added to the alamethicin-free side at this concentration. Since it has no significant effect on the surface potential, it is not surprising that it does not cause inactivation.

The magnitude of the shift to the left along the voltage axis of the G_{ss}QV curve when PC is added to the alamethicin-containing side (Fig. 2 *b*) is less than the shift to the right when the same amount of PC is added to the alamethicin-free side (cf. Fig. 2). Since addition of PC to the alamethicin-containing side changes the surface potential of that side, both the concentration of potassium and that of alamethicin at the surface of the membrane change. At the pH of our experiments, 42% of the alamethicin present is negatively charged; therefore, addition of PC to the alamethicin-containing side will increase the alamethicin concentration and decrease the potassium concentration at the membrane surface. If one considers the high power dependence on both alamethicin and potassium concentration of the alamethicin-induced conductance (7 and 3, respectively, for GMO/PS membranes; Donovan and Latorre, 1979), and the actual amount of ionized alamethicin present, then the shift to the left can be explained in quantitative terms.[1]

The fact that the recovery time is reduced only when the solution in the alamethicin-free compartment is stirred is consistent with the notion that inactivation depends on PC crossing the membrane. Since PC binds reversibly, the recovery time is an indication of the time required for PC to be swept away from the membrane surface opposite to the one containing alamethicin. Upon stirring, the thickness of the unstirred layer decreases, so its permeability to PC increases and PC is swept away faster; hence, the initial surface potential returns to its original value more rapidly. Also consistent with a mechanism of inactivation that depends on an actual flux of PC when the alamethicin conductance is turned on is the fact that, when PC chemical gradient is abolished, the inactivation process disappears (e.g., Fig. 1 *c*).

The model proposed here can predict quantitatively the kinetic and steady-state aspects of inactivation, and a detailed theoretical analysis will be given elsewhere. Still remaining to be solved is the mechanism of permeation of pancuronium. Since PC is able to cross the lipid bilayer only when the alamethicin conductance is turned on, PC must permeate either through the lumen of the channel or across some structure directly related to it. It is surprising that the permeability of the alamethicin channels for PC is about 10 times larger than that of K; this is calculated on the basis of the inactivatòn data, assuming that the PC and K fluxes obey the constant field equation (Donovan and Latorre, 1979; Heyer et al., 1976).

[1]The high power dependence of the conductance on the salt concentration is probably due to a salting out of the alamethicin (Gordon and Haydon, 1975). We have used only the potassium concentration and ignored the chloride concentration because, for our negatively charged membranes, almost all of the ions at the membrane surface are potassium. The surface charge density of our membranes is 0.0044 charges/Å^2, so that at a KCl concentration of 0.1 M, the surface potential is −70 mV.

We thank Dr. J. Z. Yeh for suggesting the use of pancuronium and for many helpful discussions. We are grateful to Dr. M. Tieffenberg-Tosteson for useful suggestions and help with the manuscript.

This work has been supported by a National Institutes of Health grant, GM-27715. J. J. D. was supported by a National Science Foundation predoctoral fellowship.

Received for publication 11 September 1978 and in revised form 27 November 1978.

REFERENCES

ALVAREZ, O., AND R. LATORRE. 1978. Voltage-dependent capacitance in lipid bilayers made from monolayers. *Biophys. J.* **21**:1–17.

ARMSTRONG, C. M., and J. Z. YEH. 1978. Immobilisation of gating charge by a substance that simulates inactivation. *Nature (Lond.).* **273**:387–389.

DONOVAN, J. J., and R. LATORRE. 1979. Inactivation of the alamethicin-induced conductance caused by quaternary ammonium ions and local anesthetics. *J. Gen. Physiol.* In press.

EISENBERG, M., J. E. HALL, and C. A. MEAD. 1973. The nature of the voltage-dependent conductance induced by alamethicin in black lipid membranes. *J. Membr. Biol.* **14**:143–176.

GORDON, L. G. M., and D. A. HAYDON. 1972. The unit conductance channel of alamethicin. *Biochim. Biophys. Acta.* **255**:1014–1018.

GORDON, L. G. M., and D. A. HAYDON. 1975. Potential-dependent conductances in lipid membranes containing alamethicin. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **270**:433–447.

HEYER, E. J., R. U. MULLER, and A. FINKELSTEIN. 1976. Inactivation of monazomycin-induced voltage-dependent conductance in thin lipid membranes. I. Inactivation by long chain quaternary ammonium ions. *J. Gen. Physiol.* **67**:703–729.

MARTIN, D. R., and R. J. P. WILLIAMS. 1976. Chemical nature and sequence of alamethicin. *Biochem. J.* **153**:181–190.

MCLAUGHLIN, S. G. A., G. SZABO, G. EISENMAN, and S. M. CIANI. 1970. Surface charge and the conductance of phospholipid membranes. *Proc. Natl. Acad. Sci. U.S.A.* **67**:3361–3566.

MONTAL, M., and P. MUELLER. 1972. Formation of bimolecular membranes from lipid monolayers and a study of their electrical properties. *Proc. Natl. Acad. Sci. U.S.A.* **69**:3561–3566.

PAYNE, J. W., R. JAKES, and B. S. HARTLEY. 1970. The primary structure of alamethicin. *Biochem. J.* **117**:757–766.

YEH, J. Z., and T. NARAHASHI. 1977. Kinetic analysis of pancuronium interaction with sodium channels in squid axon membranes. *J. Gen. Physiol.* **69**:293–323.