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Triton Channels Are Sensitive to Divalent Cations and Protons

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Abstract. Addition of Triton X-100 to planar bilayers composed of dioleoyl phosphatidyl choline, diphytanoyl phosphatidyl choline or mono-oleoyl glycerol induces single channel-like events when electrical conductivity across the bilayer is measured. Addition of divalent cations or protons causes channels to disappear; single channel conductance of remaining channels is not significantly altered; addition of EDTA or alkali (respectively) reverses the effect. It is concluded that sensitivity to divalent cations and protons need not be dependent on specific channel proteins or pore-forming toxins, but may be a feature of any aqueous pore across a lipid milieu.

Key words: Phospholipid bilayers — Triton X-100 — Ion channels — Calcium — Zinc — Protons

Introduction

Many endogenous channels (e.g., sodium channel: Woodhull, 1973; Gilly & Armstrong, 1982*a;* Hille, 1992; potassium channel: Gilly & Armstrong 1982*b;* calcium channel: Nachshen, 1984; Prod'hom, Pietrobon & Hess, 1987; Pietrobon, Prod'hom & Hess, 1988; glutamate receptor: Westbrook & Mayer, 1987; Smart, 1990; chloride channel: Wolosin & Forte, 1985; Woll et al., 1987; Nagel, Natochin & Crabbe, 1988; communicating junctions: Rose & Loewenstein, 1975; Obaid, Socolar & Rose, 1983) are sensitive to divalent cations like Ca^{2+} or Zn^{2+} and to protons. The same is true of pores induced across the cell plasma membrane or across liposomes and planar bilayers by hemolytic viruses (Burnet, 1949; Pasternak & Micklem, 1974; Patel & Pasternak, 1985), by bacterial (Avigad & Bernheimer, 1976; Thelestam & Mollby, 1980; Bashford et al., 1984, 1986; Liu & Blumenthal, 1988; Menestrina, Bashford & Pasternak, 1990; Wilmsen, Pattus & Buckley, 1990) or animal (Bashford et al., 1986; Mironov et al., 1986; Alder et al., 1991) toxins or by immune molecules (Gotze, Haupt & Fisher, 1986; Boyle, Langone & Borsos, 1979; Bashford et al., 1984; Bashford et al., 1988*a*). At low concentration (below critical micellar concentration), detergents like Triton X-100 also induce divalent cation-sensitive pores across the plasma membrane of different cells (Avigad & Bernheimer, 1976; Bashford et al., 1986, 1988*b,* Madigan, Whitbread & Katz, 1990; Alder et al., 1991). The relative efficacy of inhibition by divalent cations and protons is generally $H^+ > Zn^{2+} > Ca^{2+} > Mg^{2+}$, with approximate pK or pM^{2+} values (concentration at which leakage across the membrane or bilayer is inhibited by 50%) around 10^{-5}, 10^{-4}, 10^{-3} and 10^{-2} M, respectively. In the case of induced pores, these concentrations vary more with the amount of agent, than with the type of agent, added (e.g., Micklem et al., 1988). Exceptions to the above generalization occur when divalent cations such as Ca^{2+} (lymphocyte perforin: Henkart, 1985) or protons (diphtheria toxin: Sandvig & Olsnes, 1980; influenza virus: Maeda & Ohnishi, 1980) are required for the *formation* of pores: in this case the apparent sensitivity is much decreased [e.g., Ca^{2+} and perforin: Bashford et al. (1988*a*)], whereas sensitivity to a non-activating cation is unaffected [e.g., Zn^{2+} and perforin: Bashford et al. (1988*a*)] or increased [e.g., Zn^{2+} and *Escherichia coli* haemolysin: Menestrina et al. (1990)].

The question therefore arises as to where the ligand(s) for binding cations with efficacy $H^+ > Zn^{2+} > Ca^{2+} > Mg^{2+}$ are: on membrane proteins, on membrane lipids, on both, or on neither? To attempt an answer to

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this question, we have investigated the proton and divalent cation sensitivity of pores induced by a non-charged agent, namely Triton X-100, across lipid bilayers of defined composition: we find that, regardless of whether the lipid bilayer is charged or not, the sensitivity to closure by protons and divalent cations is $H^+ > Zn^{2+} > Ca^{2+} > Mg^{2+}$. We therefore conclude that selectivity of inhibition by protons and divalent cations does not necessarily depend on the presence of specific ligands on proteins *or* lipid. These results have been reported in brief at meetings (Lev et al., 1992; Pasternak et al., 1992).

Materials and Methods

Materials

Diphytanoyl phosphatidylcholine (DPhPC) was obtained from Avanti, dioleoyl phosphatidylcholine (DOPC) and mono-oleoyl glycerol (GMO) from Sigma. Triton X-100 was obtained either from Merck (Germany) for initial experiments in St. Petersburg, Russia or from BDH Chemicals, Poole (UK) for later experiments in London. The impurities in the BDH sample are given as: water, 0.1%; sulfated ash, 0.1%; free alkali (NH_3), 0.002%; free acid (CH_3COOH), 0.002%. At the highest concentration of triton used (10^{-5} M), the contamination of free acid is approximately 10^{-7} M. The conductance of a 0.1% solution of BDH triton in double distilled water was not significantly different from water itself (and 10^{-5} M triton—the highest concentration used—is approximately 100-fold less than 0.1%). Since essentially similar results were obtained with regard to channel formation, ion selectivity and proton and divalent cation sensitivity in St. Petersburg and London, and with triton concentrations varying from 10^{-5}–10^{-10} M, it is unlikely that contaminations in triton account for the present results.

Methodology

Planar bilayer membranes (BLM) were prepared according to the modified Schindler (1980) procedure. They were formed from two lipid monolayers without organic solvent across a 10–20 μm diameter hole generated by a hot wire through a 10 μm thick Teflon film; formation of BLM was monitored by an increase in capacitance. Electrical measurements were made in voltage clamp conditions. Current was measured with Ag/AgCl electrodes connected to an operational amplifier (OP 121 Burr-Brown). Data were either recorded on a chart recorder or on video tape by using a Biologic PCM Instrumentation Recorder and subsequently analyzed by using Cambridge Electronic Design Patch and Voltage-Clamp software. The compartment connected to virtual ground was called *cis;* voltage signs refer to the *cis* compartment; at positive voltages cations move from *cis* to *trans.* KCl buffered to pH 7.4 with 5 mM HEPES or tris HCl was used. All experiments were performed at room temperature.

Triton X-100 from a stock aqueous solution was added to a dispersion of lipid vesicles in buffered KCl solution and the system allowed to equilibrate for 5–15 min before formation of the lipid bilayer. Single channel activity was usually observed on formation of the membrane. Channel activity was never observed with DPhPC or GMO alone; samples of DOPC that showed channel activity were discarded. Bilayers of DPhPC, DOPC or GMO, to which triton was added only by injection into the aqueous medium, showed no channel activity. Channel activity disappeared if the membrane broke and was reformed. To generate further Triton X-100 channels, 0.5 μl aliquots of stock Triton X-100 solutions were added by Hamilton syringe to each chamber. Practically all bilayer membranes formed after this treatment showed that Triton X-100 induced conductivity. In either case, Triton X-100 was present in the *cis* and *trans* compartments to a final concentration which was enough for the formation of single channels in the BLM and varied from 10^{-10}–10^{-9} M in the first case, and from 10^{-7}–10^{-5} M in the second one.

Reversal potential (Ψ) was determined in a fivefold gradient of KCl. Selectivity (t_+) was calculated from the reversal potential where t_+ is defined as

$$t_+ = 1/2[1 + \Psi/\{(RT/F)\ln([K^+]trans/[K^+]cis)\}]$$

where R, T and F have their usual meanings.

Results

DPhPC Bilayers

Addition of low concentrations of Triton X-100 to planar bilayers composed of DPhPC results in the appearance of 'single channel'-like events (Fig. 1). The amplitude of the conductance steps varies from experiment to experiment, from approx. 10 pS to > 1,000 pS (0.1 M KCl). Small conductance steps often change spontaneously to large steps and occasionally the reverse is true; there is no correlation with the amount of triton added (10^{-10}–10^{-5} M). *I/V* plots are nearly linear even when the solutions bathing the membrane have differing KCl concentrations. Measurement of the reversal potentials under these conditions (e.g., 0.1 and 0.5 M KCl *cis* and *trans*) reveals selectivity for K^+ over Cl^- ranging from zero (t_+ of 0.5) to occasionally highly cation selective (t_+ of 0.98); the average for 16 experiments was 0.60 ± 0.15.

In the presence of divalent cations, conductance is decreased, due to successive disappearance of 'single-channel'-like fluctuations (Fig. 1); the conductance of residual channels in the presence of divalent cations (modal value 280 pS) is similar to that of channels prior to the addition of divalent cations (modal value 240 pS) (Fig. 1*E*). Addition of EDTA reverses the effect. Lowering pH has an effect similar to the addition of divalent cations (Fig. 1*D*). When the relative efficacy of H^+ and divalent cations in all experiments analyzed is compared (Fig. 2), it is clear that $H^+ > Zn^{2+} > Ca^{2+}$ or Mg^{2+} [50% inhibition at approx. 10^{-8} M (H^+) 10^{-4} M (Zn^{2+}) and 10^{-2} M (Ca^{2+} or Mg^{2+})]. There is no correlation between sensitivity to protons and M^{2+} and the amplitude of the conductance or its cation selectivity.

Triton-induced channels are observed when the membrane is bathed in a solution of $CaCl_2$ (Fig. 3). Single channel fluctuations can be seen; these are abolished at low pH and restored at neutrality (Fig. 3).

DOPC Bilayers

DOPC bilayers give results essentially similar to those obtained with bilayers composed of DPhPC: single

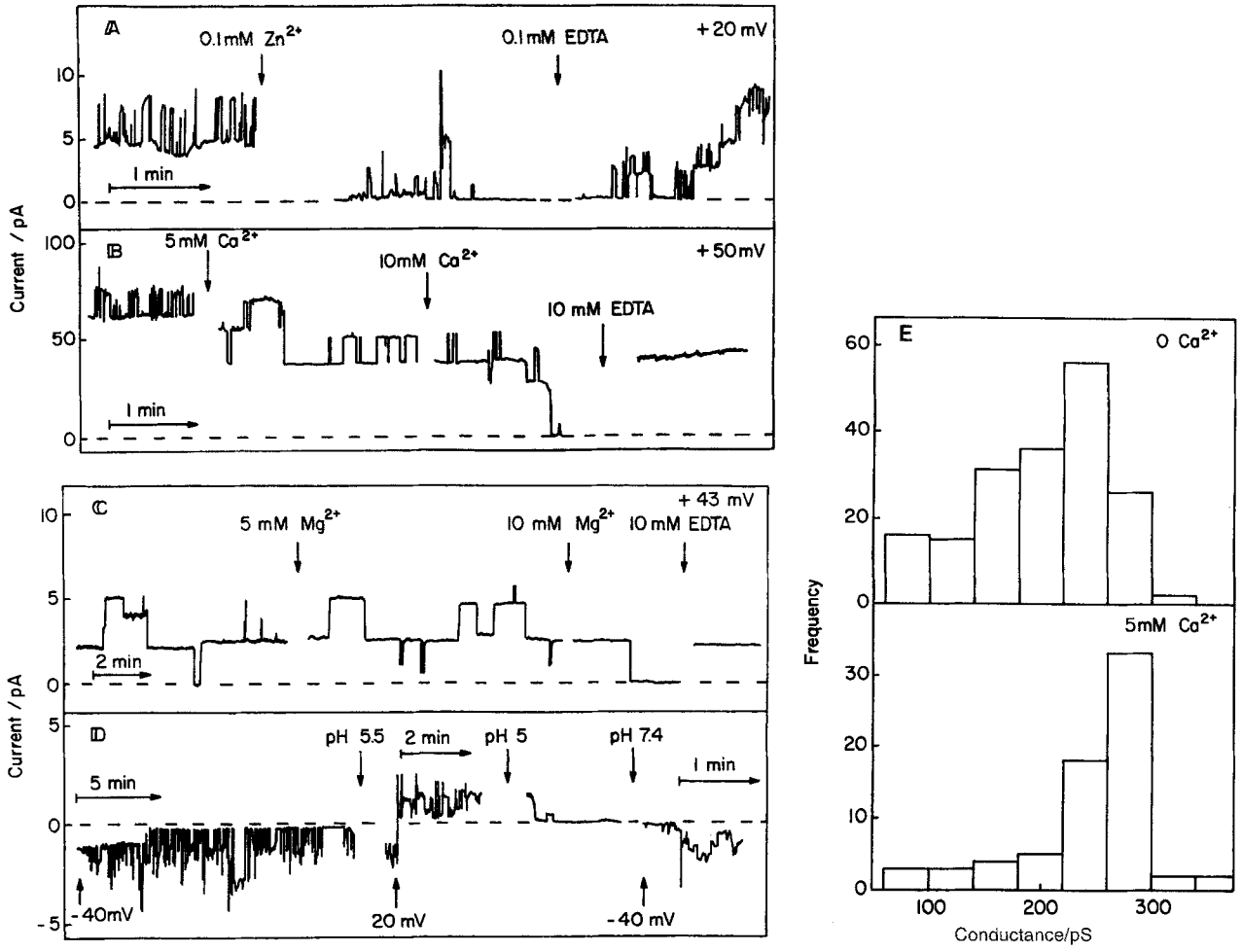

Fig. 1. Effect of protons and divalent cations on Triton-X-100-induced channels in DPhPC bilayers. Triton X-100 (5.10^{-6} M), in 0.005 M HEPES (*A,C,D*) or 0.005 M tris (*B*) pH 7.4 and 0.1 M KCl (*cis*)/0.5 M KCl (*trans*) (*A,C,D*) or 0.1 M KCl (*cis* and *trans*) (*B*). The cation selectivity, t_+, was 0.5 (*A*), 0.7 (*C*) and 0.6 (*D*). $ZnSO_4$, $CaCl_2$, $MgCl_2$, EDTA and KCl/KOH (*cis* and *trans*) were added to give the final concentration indicated. (*E*): Amplitude distribution of the Triton-X-100-induced channels, presented in *B* without and with 0.005 M $CaCl_2$ (*cis* and *trans*). The ordinate frequency indicates the number of occasions on which each conductance level on the abscissa (in pS) was observed in records containing 182 (upper panel) and 60 (lower panel) resolvable levels, respectively.

channel-like events, of varying size, are observed (Fig. 4). Addition of Ca^{2+} decreases such fluctuations, which are restored by EDTA (Fig. 4).

GMO Bilayers

Addition of triton to bilayers of uncharged lipids gives the same result as addition to phospholipid bilayers. Triton-treated bilayers composed of GMO, for example, show single channel-like fluctuations (Fig. 5*A*). Fluctuations are of varying size (~20 to > 1,000 pS; *not shown*) and selectivity (mean t_+ 0.75 ± 0.1); an example of a 30 pS pore having selectivity t_+ 0.7 is illustrated in Fig. 5*B*. Note the linearity of this *I/V* plot between ± 100 mV; at voltages greater than this, current increases. Addition of Ca^{2+} reduces channel activity (Fig. 5*A*). Protons have the same effect. For example, in the experiment illustrated in Fig. 5*C*, conductance at pH 4 is zero (trace *i*), whereas at pH 7, two predominant conductance states are seen: a flickering high conductance (trace *ii*) and a stable, low conductance state (trace *iii*). Analysis of these conductance states (Fig. 5C, lower panels) reveals modal values of 7 pA (trace *ii*) and 3 pA (trace *iii*).

The relative efficacy of protons and divalent cations from all experiments analyzed is shown in Fig. 6. As with bilayers composed of DPhPC (Fig. 2) or DOPC (*not shown*), $H^+ > Zn^{2+} > Ca^{2+}$ or Mg^{2+} [50% inhibition at approx. 10^{-8} M (H^+), 10^{-4} M (Zn^{2+}) and 10^{-2} M (Ca^{2+} or Mg^{2+})]. As with DPhPC bilayers, there is

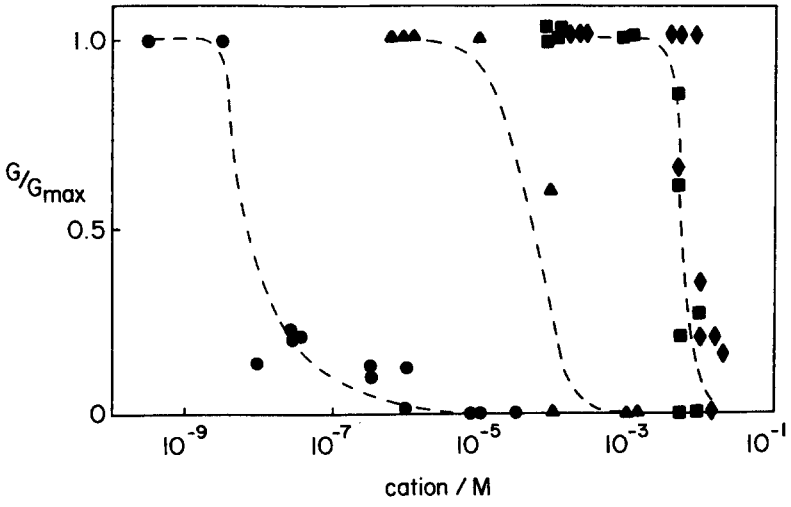

Fig. 2. Effect of H^+, Zn^{2+}, Ca^{2+} and Mg^{2+} on Triton-X-100-induced conductance in DPhPC bilayers. Triton X-100, (5.10^{-6} M), in 0.005 M HEPES pH 7.4 and 0.1 M KCl (*cis*)/0.5 M KCl (*trans*) with H^+ (●), Zn^{2+} ($ZnSO_4$ or $ZnCl_2$, ▲), Ca^{2+} (■) or Mg^{2+} (◆) at the concentration indicated. Integral conductance G (pS/min) is presented relative to the maximum (G_{max}) observed in each titration. Each curve combines data of 3–4 experiments with G_{max} ranging from 20 to 1,300 pS.

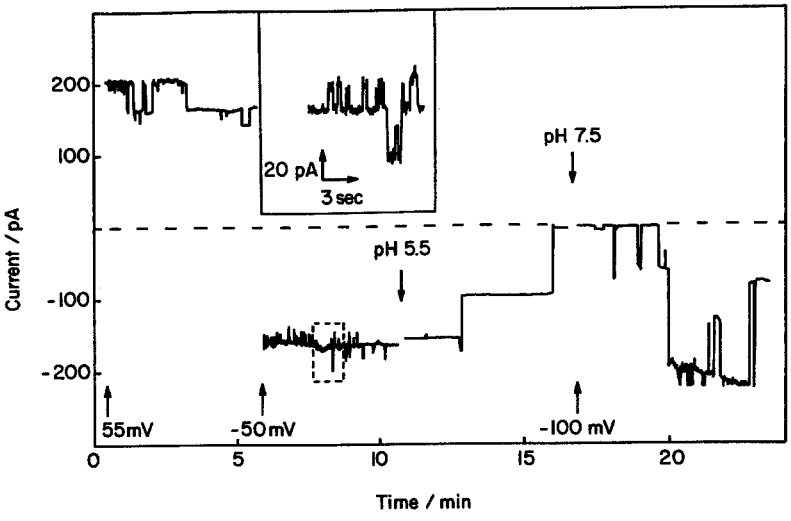

Fig. 3. Effect of H^+ on Triton-X-100-induced channels in DPhPC bilayers in the presence of calcium. Triton X-100 (5.10^{-6} M), in 0.005 M tris pH 7.4 and 0.05 M $CaCl_2$ (*cis*)/0.1 M $CaCl_2$ (*trans*), t_+ 0.5. HCl/KOH were added to give the final pH indicated.

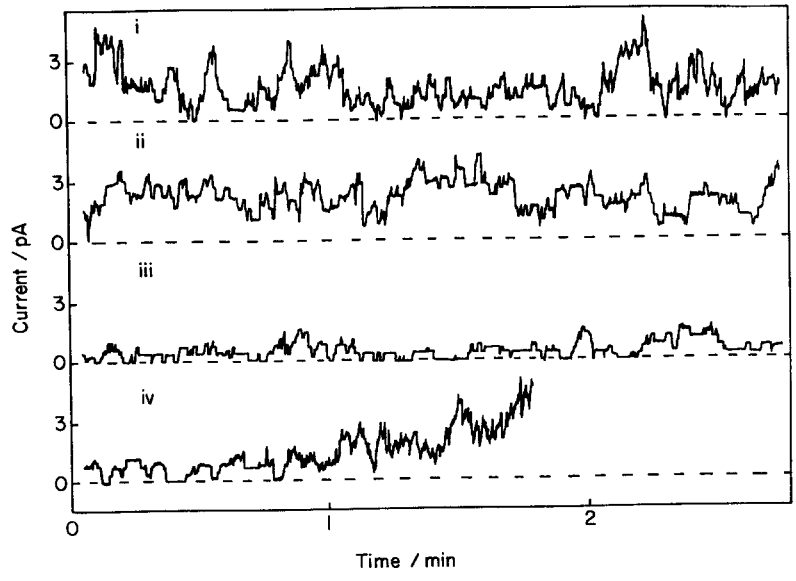

Fig. 4. Effect of Ca^{2+} and Mg^{2+} on Triton-X-100-induced channels in DOPC bilayers. Triton X-100 (10^{-10} M), in 0.005 M tris pH 7.4 and 0.1 M KCl (*cis* and *trans*) without (*i*) or with 10^{-3} M $CaCl_2$ (*ii*), 10^{-2} M $CaCl_2$ (*iii*) and 10^{-2} M $CaCl_2$ with 10^{-2} M EDTA (*iv*). Applied potential was 50 mV. $CaCl_2$, $MgCl_2$ and EDTA were added *cis* and *trans*.

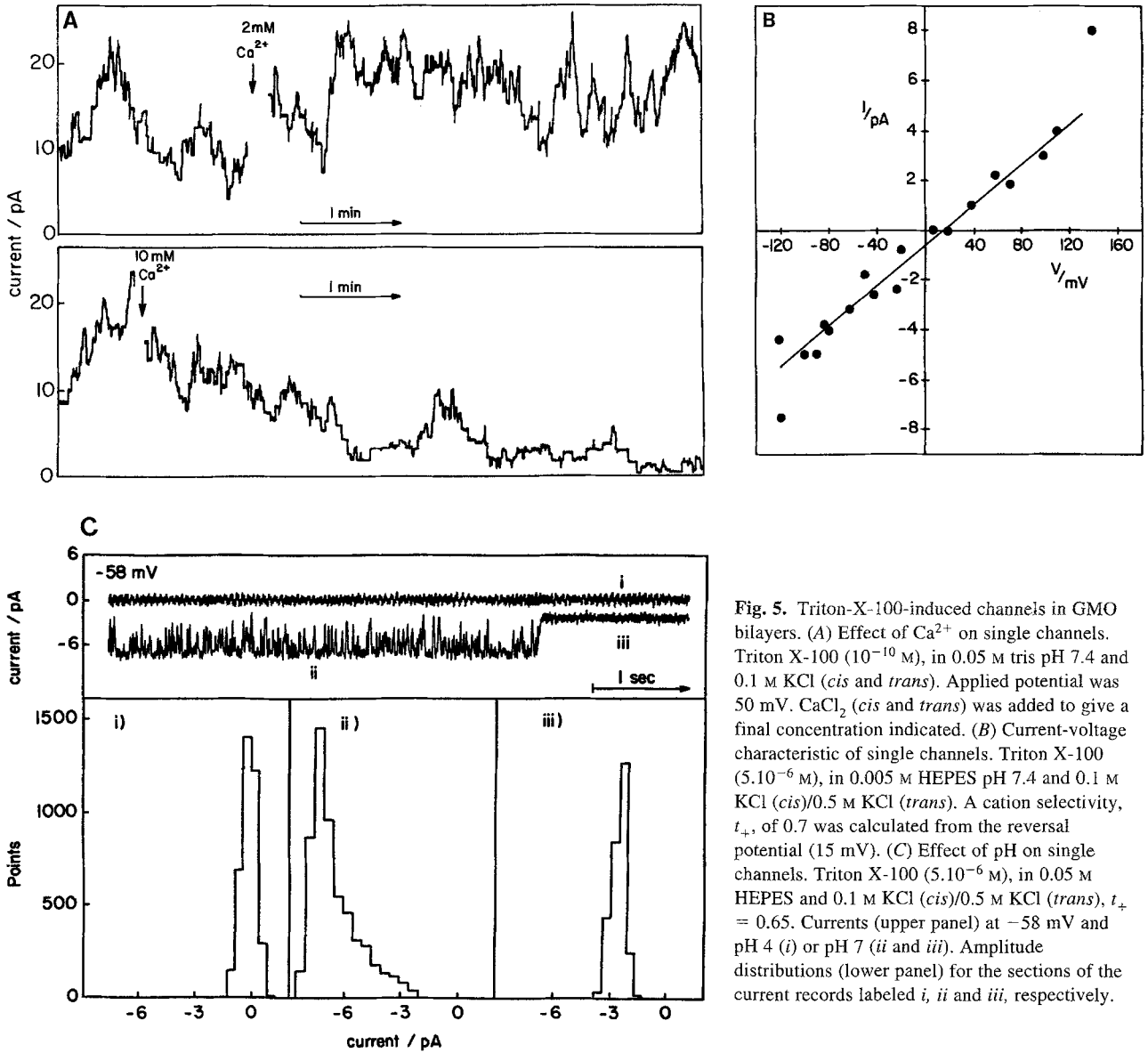

Fig. 5. Triton-X-100-induced channels in GMO bilayers. (*A*) Effect of Ca^{2+} on single channels. Triton X-100 (10^{-10} M), in 0.05 M tris pH 7.4 and 0.1 M KCl (*cis* and *trans*). Applied potential was 50 mV. $CaCl_2$ (*cis* and *trans*) was added to give a final concentration indicated. (*B*) Current-voltage characteristic of single channels. Triton X-100 (5.10^{-6} M), in 0.005 M HEPES pH 7.4 and 0.1 M KCl (*cis*)/0.5 M KCl (*trans*). A cation selectivity, t_+, of 0.7 was calculated from the reversal potential (15 mV). (*C*) Effect of pH on single channels. Triton X-100 (5.10^{-6} M), in 0.05 M HEPES and 0.1 M KCl (*cis*)/0.5 M KCl (*trans*), $t_+ = 0.65$. Currents (upper panel) at −58 mV and pH 4 (*i*) or pH 7 (*ii* and *iii*). Amplitude distributions (lower panel) for the sections of the current records labeled *i, ii* and *iii*, respectively.

no correlation between sensitivity to protons or M^{2+} and the amplitude of the conductance or its cation selectivity.

Discussion

The results of this investigation allow two conclusions to be drawn. First, discrete channels of varying conductance (10 to > 1,000 pS; 0.1 M KCl) and selectivity (t_+ 0.5 [unselective] to 0.9 [cation selective]) can be induced across bilayers of phospholipid or GMO by low concentrations of a detergent such as Triton X-100. Similar channels have been observed with triton and other non-ionic detergents by Tanaka, Furman and Barchi (1986). That channels are never anion selective is compatible with the observations of Van Zutphen et al. (1972) and Gotlib et al. (1992), who observed transient high cation selectivity ($K^+ \gg Na^+$) with lipid membranes—isolated from natural sources—after addition of triton. Possible explanations for cation selectivity are similar to explanations for sensitivity to protons and M^{2+} and are discussed below. The structures of triton-induced channels are at present unclear, but may resemble those seen by atomic force microscopy of lipid detergent bilayers (Lacapere, Stokes & Chatenay, 1992). Since there is no correlation between conductance and selectivity—in contrast to channels formed by toxins such as pneumolysin (Korchev, Bashford & Pasternak, 1992) or to pores across certain synthetic, track-etched filters (Y.E. Korchev and T.K. Rostovtseva, *unpublished results*), for which high selectivity correlates with

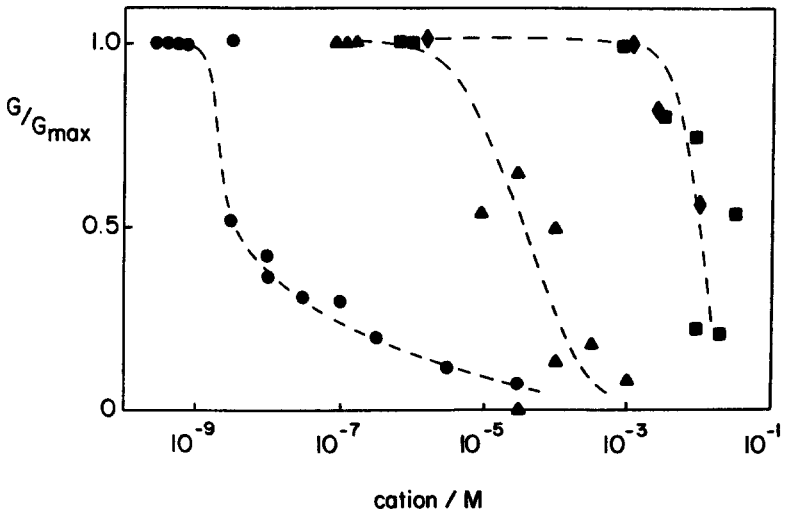

Fig. 6. Effect of H^+, Zn^{2+}, Ca^{2+} and Mg^{2+} on Triton-X-100-induced conductance in GMO bilayers. Triton X-100 (5.10^{-6} M), in 0.005 M HEPES pH 7.4 and 0.1 M KCl (*cis*)/0.5 M KCl (*trans*), with H^+ (●), Zn^{2+} ($ZnSO_4$ or $ZnCl_2$, ▲), Ca^{2+} (■) or Mg^{2+} (◆) at the concentration indicated. Integral conductance G (pS/min) is presented relative to the maximum (G_{max}) observed in each titration. Each curve combines data of 3–4 experiments with G_{max} ranging from 20 to 1,300 pS.

low conductance—it is possible that high conductance channels having high selectivity represent a narrow annulus between a large "plug" and the surrounding bilayer, the "plug" perhaps made up of triton ± lipid in micellar form (e.g., Alonso & Goni, 1983; Gotlib et al., 1992). Triton-induced channels show little voltage dependency (at least between ± 100 mV), as noted also by Tanaka et al. (1986); in this regard they resemble channels formed by diphtheria toxin, melittin, heat shock proteins, transit (signal) peptides or viral fusion peptides (*see* Pasternak, 1991) as well as pores across synthetic, track-etched filters (Lev et al., 1992, 1993; Pasternak et al., 1993), rather than channels formed by agents such as *Staphylococcus aureus* α toxin (Menestrina, 1986), lymphocyte perforin (Young et al., 1986; Bashford et al., 1988*b*), perfringolysin (Menestrina et al., 1990), aerolysin (Wilmsen, Pattus & Buckley, 1990) or pneumolysin (Korchev et al., 1992).

Second, conductivity decreases on the addition of divalent cations and protons (efficacy $H^+ > Zn^{2+} > Ca^{2+}$ or Mg^{2+}), regardless of the composition of the lipid bilayer (DOPC, DPhPC or GMO). That low (mM) concentrations of a divalent cation such as Ca^{2+} decrease channel activity in 0.1 M KCl, while pure $CaCl_2$ at (0.05/0.1 M) shows fluctuations as high as 2 nS (Fig. 3) is reminiscent of other situations (e.g., Ca^{2+} channel; Pietrobon et al., 1988). An effect of low pH on triton-induced conductivity across bilayers has previously been reported by Schlieper and de Robertis (1977). The conductance of single channels is relatively unaffected by protons or divalent cations (e.g., Fig. 1*E;* Fig. 4; Fig. 5*A*); rather, the decrease in conductivity is due to the disappearance of channels as a result of closure, dispersal or some other effect (*cf.* Lev et al. 1993). The similarity of action of divalent cations on leakage through triton-induced lesions in cells (Avigad & Bernheimer, 1976; Bashford et al. 1986, 1988*b;* Madigan et al., 1990) and liposomes (Alder et al., 1991), is to be noted. Since Triton X-100 does not possess ligands selective for divalent cations and protons, these would appear to be on the lipid. Yet neither DOPC, DPhPC nor GMO possess such sites (dissociation constant of Ca^{2+} from phosphatidylcholine bilayers is around 50–1000 mM (McLaughlin, Grathwohl & McLaughlin, 1978; Akutsu & Seelig, 1981), not < 10 mM) and one must conclude that they are contributed either by impurities or that they are due to some other cause. Impurities include traces of free fatty acid or oxidation products (in the case of DOPC or GMO) that could be well below the limits of detection (since single channel activity is essentially a consequence of single molecular events); if GMO is deliberately oxidized, for example, single channels with t_+ of 0.8 appear (Rostovtseva & Lev, 1986). Other causes include the possibility of a negative surface charge, since negative surface potentials have been reported for neutral lipid membranes (e.g., Tatulian, 1983; Rostovtseva, Osipov & Lev, 1987). Furthermore, water molecules may be polarized at a hydrophobic surface (Lee, McCammon & Rossky, 1984), creating, in effect, a negative charge on the aqueous side of a layer of water molecules and the greater solubility of cations (e.g., K^+) compared with anions (e.g., Cl^-) in nonaqueous solvents (Levitt, 1988) may lead to similar consequences. While such effects may account for the selectivity of channels for cations over anions, it is difficult to envisage how they are able to confer differing sensitivity to H^+, Zn^{2+} and Ca^{2+}. Moreover, triton-induced channels are as sensitive to inhibition by divalent cations whether they show no cation selectivity (t_+ 0.5) or high cation selectivity (t_+ 0.9), which suggests that selectivity and sensitivity result from different molecular interactions.

In conclusion, our results extend previous observations of ion current fluctuations in the absence of

channel proteins (Antonov et al., 1980; Yoshikawa et al., 1988; Woodbury, 1989) to another property of endogenous ion channels (Hille, 1992) and toxin-induced pores (Bashford et al., 1986): their sensitivity to protons and divalent cations.

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References

Akutsu, H., Seelig, J. 1981. Interaction of metal ions with phosphatidylcholine bilayer membranes. *Biochemistry* **20:**7366–7373

Alder, G.M., Arnold, W.M., Bashford, C.L., Drake, A.F., Pasternak, C.A., Zimmerman, U. 1991. Divalent cation-sensitive pores formed by natural and synthetic melittin and by triton X-100. *Biochim. Biophys. Acta* **1061:**111–120

Alonso, A., Goni, F.M. 1983. Effect of detergents and fusogenic lipids on phospholipid phase transitions. *J. Membrane Biol.* **71:**183–187

Antonov, V.F., Petrov, V.V., Molnar, A.A., Prevoditelev, D.A., Ivanov, A.S. 1980. The appearance of single-ion channels in unmodified lipid bilayer membranes at the phase transition temperature. *Nature* **283:**585–587

Avigad, L.S., Bernheimer, A.W. 1976. Inhibition by zinc of hemolysis induced by bacterial and other cytolytic agents. *Infect. Immun.* **13:**1378–1381

Bashford, C.L., Alder, G.M., Menestrina, G., Micklem, K.J., Murphy, J.J., Pasternak, C.A. 1986. Membrane damage by hemolytic viruses, toxins, complement and other cytotoxic agents: a common mechanism blocked by divalent cations. *J. Biol. Chem.* **261:** 9300–9308

Bashford, C.L., Alder, G.M., Patel, K., Pasternak, C.A. 1984. Common action of certain viruses, toxins, and activated complement: pore formation and its prevention by extracellular Ca^{2+}. *Biosci. Rep.* **4:**797–805

Bashford, C.L., Alder, G.M., Graham, J.M., Menestrina, G., Pasternak, C.A. 1988*a*. Ion modulation of membrane permeability: Effect of cations on intact cells and on cells and phospholipid bilayers treated with pore-forming agents. *J. Membrane Biol.* **103:**79–94

Bashford, C.L., Menestrina, G., Henkart, P.A., Pasternak, C.A. 1988*b*. Cell damage by cytolysin. Spontaneous recovery and reversible inhibition by divalent cations. *J. Immunol.* **141:**3965–3974

Boyle M.D., Langone J.J., Borsos T. 1979. Studies on the terminal stages of immune hemolysis. IV. Effect of metal salts. *J. Immunol.* **122:**1209–1213

Burnet, F.M. 1949. Haemolysis by Newcastle disease virus. *Nature* **164:**1008

Gilly, W.F., Armstrong, C.M. 1982*a*. Slowing of sodium channel opening kinetics in squid axon by extracellular zinc. *J. Gen. Physiol.* **79:**935–964

Gilly, W.F., Armstrong, C.M. 1982*b*. Divalent cations and the activation kinetics of potassium channels in squid giant axons. *J. Gen. Physiol.* **79:**965–966

Gotlib, V.A., Lebedeva, N.E., Stakhov, S.V., Rostovtseva, T.K., Lev, A.A. 1992. Lateral phase separation in lipid bilayers in the presence of non-ionic detergent triton X-100. *Membrane Biol.* **9:**629–674 (*in Russian*)

Gotze, O., Haupt, I., Fisher, H. 1968. Immune haemolysis: reaction of the terminal complement component. *Nature* **217:**1165–1167

Hille, B. 1992. Ionic Channels of Excitable Membranes. Second edition. Sinauer Associates, Sunderland, MA

Henkart, P.A. 1985. Mechanism of lymphocyte-mediated cytotoxicity. *Annu. Rev. Immunol.* **3:**31–58

Korchev, Y.E., Bashford, C.L., Pasternak, C.A. 1992. Differential sensitivity of pneumolysin-induced channels to gating by divalent cations. *J. Membrane Biol.* **127:**195–203

Lacapere, J.-J., Stokes, D.L., Chatenay, D. 1992. Atomic force microscopy of three-dimensional membrane protein crystals. *Biophys. J.* **63:**303–308

Lee, C.Y., McCammon, J.A., Rossky, P.J. 1984. The structure of liquid water at an extended hydrophobic surface. *J. Chem. Phys.* **80:**4448–4455

Lev, A.A., Korchev, Y.E., Rostovtseva, T.K., Bashford, C.L., Pasternak, C.A. 1992. Lipid impregnated nuclear filters as a new model for studies of surface conductance and single channel phenomena. *In:* Biophysics of Membrane Transport, Proceedings of Eleventh School J. Kuczera, and S. Przestalski, editors. pp. 321–349. Agricultural University of Wroclaw, Poland

Lev, A.A., Korchev, Y.E., Rostovtseva, T.K., Bashford, C.L., Edmonds, D.T., Pasternak, C.A. 1993. Rapid switching of ion current in narrow pores: implications for biological ion channels. *Proc. R. Soc. Lond. B* **252:**187–192

Levitt, D.G. 1988. Solvation effects on the transport of ions across cell membranes. *In:* The Chemical Physics of Solvation, Part C, Solvation Phenomena in Specific Physical, Chemical, and Biological Systems R.R., Dogonadze, E. Kalman, A.A. Kornishev, and J. Ulstrup, editors. pp. 741–750. Elsevier, Amsterdam

Liu, J., Blumenthal, K.M. 1988. Membrane damage by *Cerebratulus lacteus* cytolysin A-III. Effects of monovalent and divalent cations on A-III hemolytic activity. *Biochim. Biophys. Acta* **937:**153–160

Madigan, S.T., Whitbread, J.A., Katz, E.R. 1990. A *Dictyostelium discoideum* mutant exhibiting calcium dependent, high level detergent resistance. *J. Bact.* **172:**2785–2787

Maeda, T., Ohnishi, S.-I. 1980. Activation of influenza virus by acidic media causes hemolysis and fusion of erythrocytes. *FEBS Lett.* **122:**283–287

McLaughlin, A., Grathwohl, C., McLaughlin, S. 1978. The adsorption of divalent cations to phosphatidylcholine bilayer membranes. *Biochim. Biophys. Acta* **513:**338–357

Menestrina, G. 1986. Ionic channels formed by *Staphylococcus aureus* α-toxin: Voltage-dependent inhibition by divalent and trivalent cations. *J. Membrane Biol.* **90:**177–190

Menestrina, G., Bashford, C.L., Pasternak, C.A. 1990. Pore-forming toxins: experiments with *S. aureus* α-toxin, *C. perfringens* Θ-toxin and *E. coli* haemolysin in lipid bilayers, liposomes and intact cells. *Toxicon* **28:**477–491

Micklem, K.J., Alder, G.M., Buckley, G.D., Murphy, J., Pasternak, C.A. 1988. Protection against complement-mediated cell damage by Ca^{2+} and Zn^{2+}. *Complement* **5:**141–152

Mironov, S.L., Sokolov Y.V., Chanturiya, A.N., Lishko, V.K. 1986. Channels produced by spider venoms in bilayer lipid membrane: mechanisms of ion transport and toxic action. *Biochim. Biophys. Acta* **862:**185–198

Nachshen, D.A. 1984. Selectivity of the Ca binding site in synaptosome Ca channels. Inhibition of Ca influx by multivalent metal cations. *J. Gen. Physiol.* **83:**941–967

Nagel, W., Natochin, Y., Crabbe, J. 1988. Effects of divalent cations in chloride movement across amphibian skin. *Pfluegers. Arch.* **411:**540–545

Obaid, A.L., Socolar, S.J., Rose, B. 1983. Cell-to-cell channels with two independently regulated gates in series: Analysis of junctional conductance modulation by membrane potential, calcium, and pH. *J. Membrane Biol.* **73:**69–89

Pasternak, C.A. 1991. Effect of divalent cations on membranes: reversible and irreversible closure of channels induced by membrane-inserting proteins and other agents. *Romanian J. Biophys.* **1:**3–11

Pasternak, C.A., Alder, G.M., Bashford, C.L., Korchev, Y.E., Pederzolli, C., Rostovtseva, T.K. 1992. Membrane damage: common mechanisms of induction and prevention. *FEMS Microbiol. Immunol.* **105:**83–92

Pasternak, C.A., Bashford, C.L., Korchev, Y.E., Rostovtseva, T.K., Lev, A.A. 1993. Modulation of surface flow by divalent cations and protons. *Colloids & Surfaces* **77:**119–124

Pasternak, C.A., Micklem, K.J. 1974. The biochemistry of virus-induced cell fusion. Changes in membrane integrity. *Biochem. J.* **140:**405–411

Patel, K., Pasternak, C.A. 1985. Permeability changes elicited by influenza and Sendai virus: separation of fusion and leakage by pH jump experiments. *J. Gen. Virol.* **66:**767–775

Pietrobon, D., Prod'hom, B., Hess, P. 1988. Conformational changes associated with ion permeation in L-type calcium channels. *Nature* **333:**373–376

Prod'hom, B., Pietrobon, D., Hess, P. 1987. Direct measurement of proton transfer rates to a group controlling the dihydropyridine-sensitive Ca^{2+} channel. *Nature* **329:**243–246

Rose, B., Loewenstein, W.R. 1975. Permeability of cell junction depends on local cytoplasmic calcium activity. *Nature* **254:**250–252

Rostovtseva, T.K., Lev, A.A. 1986. Lipid peroxidation induced single ion channels in the lipid bilayer membranes. Symposium on Activated Oxygen Species in Biological Systems, Varna. p. 81

Rostovtseva, T.K., Osipov, V.V., Lev, A.A. 1987. Dependence of single gramicidin channel conductance on potential induced by adsorbtion of 1-anilino- 8 naphthalene sulfonate anions on lipid membranes. *Biol. Membranes* **4:**955–964 (*in Russian*)

Sandvig, K., Olsnes, S. 1980. Diphtheria toxin entry into cells is facilitated by low pH. *J. Cell Biol.* **87:**828–832

Schindler, H. 1980. Formation of planar bilayers from artificial or native membrane vesicles. *FEBS Lett.* **122:**77–79

Schlieper, P., de Robertis, E. 1977. Triton X-100 as a channel-forming substance in artificial lipid bilayer membranes. *Arch. Biochem. Biophys.* **184:**204–208

Smart, T.G. 1990. Uncultured lobster muscle, cultured neurons and brain slices: the neurophysiology of zinc. *J. Pharm. Pharmacol.* **42:**377–387

Tanaka, J.C., Furman, R.E., Barchi, R.L. 1986. Skeletal muscle sodium channels. *In:* Ion Channel Reconstitution C. Miller, editor. pp. 277–305, Plenum, New York

Tatulian, S.A. 1983. Effect of lipid phase transition on the binding of anions to dimyristoylphosphatidylcholine liposomes. *Biochim. Biophys. Acta* **736:**189–195

Thelestam, M., Mollby, R. 1980. Interaction of streptolysin O from *Streptococcus pyogenes* and theta toxin from *Clostridium perfringens* with human fibroblasts. *Infect. Immunol.* **29:**863–872

Van Zutphen, Merola, A.J., Brierley, G.P., Cornwall, D.G. 1972. The interaction of non-ionic detergents with lipid bilayer membranes. *Arch. Biochem. Biophys.* **152:**755–766

Westbrook, G.L., Mayer, M.I. 1987. Micromolar concentrations of Zn^{2+} antagonise NMDA and GABA responses in hippocampal neurons. *Nature* **328:**640–643

Wilmsen, H.U., Pattus, F., Buckley, J.T. 1990. Aerolysin, a hemolysin from *Aeromonas hydrophila,* forms voltage-gated channels in planar lipid bilayers. *J. Membrane Biol.* **115:**71–81

Woll, K.H., Leibowitz, M.D., Neumcke, B., Hille, B. 1987. A high conductance anion channel in adult amphibian skeletal muscle. *Pfluegers Arch.* **410:**632–640

Wolosin, J.M., Forte, J.G. 1985. K^+ Cl^- conductances in the apical membrane from secreting oxyntic cells are concurrently inhibited by divalent cations. *J. Membrane Biol.* **83:**261–272

Woodbury, D.J. 1989. Pure lipid vesicles can induce channel-like conductances in planar bilayers. *J. Membrane Biol.* **109:**145–150

Woodhull, A.M. 1973. Ionic blockage of sodium channels in nerve. *J. Gen. Physiol.* **61:**687–708

Yoshikawa, K., Sakabe, K., Matsubara, Y., Ota, T. 1988. Self-excitation in a porous medium doped with sorbitan monooleate (SPAN-80) induced by a Na^+/K^+ concentration gradient. *Biophys. Chem.* **21:**33–39

Young, J.D.-E., Nathan, C.F., Podack, E.R., Palladino, M.A., Cohn, Z.A. 1986. Functional channel formation associated with cytotoxic T-cell granules. *Proc. Natl. Acad. Sci. USA* **83:**150–154