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Some Effects of Short-Chain Phospholipids and *n*-Alkanes on a Transient Potassium Current (I_A) in Identified *Helix* Neurons

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Abstract. Many effects of short-chain phospholipids and *n*-alkanes on the squid axon sodium current (I_{Na}) are consistent with mechanisms involving changes in membrane thickness. Here, we suggest that the actions of short-chain phospholipids on an A-type potassium current (I_A) in two-microelectrode voltage clamped *Helix* D1 and F77 neurons are incompatible with such simple mechanisms. Diheptanoyl phosphatidylcholine (diC_7PC, 0.2 and 0.3 mM) caused substantial (58 and 79%), and in some cases partially reversible, increases in I_A amplitude. These were correlated with hyperpolarizing shifts of up to −7 mV in the voltage dependence of current activation. The voltage dependence of steady-state inactivation was also moved in the hyperpolarizing direction. These effects are the opposite of those described for squid I_{Na}. 0.5 Saturated *n*-pentane and saturated *n*-hexane caused significant (−3 and −6 mV) hyperpolarizing shifts in the voltage dependence of I_A inactivation, qualitatively consistent with their effects on squid I_{Na}, while the voltage dependence of activation was moved slightly to the left or unchanged. Hydrocarbons had variable effects on peak current amplitude, although saturated *n*-pentane produced a clear suppression. DiC_7PC caused a 25% increase in the time constant of macroscopic I_A inactivation (τ_b) but 0.5 saturated *n*-pentane and saturated *n*-hexane reduced τ_b by 40%. The effects of these agents on current-clamped cells were broadly consistent with their opposing actions on τ_b—phospholipids tended to reduce excitability and *n*-alkanes tended to increase it. Possible mechanisms of I_A perturbation are discussed.

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Introduction

Voltage-gated ion channels are presumed to change their gating state in response to alterations in the strength of the electrical field across the cell membrane. Haydon and co-workers suggested that the effects of a range of lipophilic compounds on the voltage dependence of squid axon sodium and delayed rectifier potassium currents (I_{Na} and I_K) could in part be explained by perturbant-induced changes in the membrane field (Haydon & Kimura, 1981; Haydon & Urban, 1983*a,b*, 1986). Haydon and colleagues drew particular attention to the oppositely directed effects of *n*-alkane hydrocarbons and short-chain phospholipids on I_{Na} gating. *n*-Alkanes induced hyperpolarizing shifts in Na current steady-state activation (m_∞) and inactivation (h_∞) gating parameters, while short-chain phospholipids moved the m_∞ and h_∞ curves in the depolarizing direction. Furthermore, these changes were shown to be consistent with the observation that hydrocarbons thicken, while short-chain phospholipids thin, lipid membranes (Haydon & Urban, 1983*a,b;* Elliott et al., 1985; Hendry, Elliott & Haydon, 1985). However, the generality of such a thickness hypothesis was not tested by demonstrating its relevance to a different voltage-gated ion channel in another preparation.

We have now performed that test by determining the effects of *n*-alkanes and short-chain phospholipids on the activation and inactivation characteristics of a fast transient potassium current (commonly called the A current or I_A (Connor & Stevens, 1971)) in D1 and F77 neuronal cell bodies from parietal ganglia of the snail *Helix aspersa.* There were two reasons for choosing I_A.

First, it can readily be isolated from other voltage-gated currents in these cells. Second, it exhibits both fast, voltage-dependent activation and an inactivation process which, while substantially slower than that shown by most sodium currents, still occurs on the subsecond timescale. I_A therefore provides an interesting comparison with I_{Na}.

The results of our investigation were not as expected. Both types of perturbant affected I_A, but the actions of the short-chain phospholipids, in particular, bore no resemblance to their effects on the squid Na current and were not consistent with the predicted effect of a thickness decrease on the transmembrane field. Thus, while 0.2 and 0.5 mM diheptanoyl phosphatidylcholine reduced the peak squid I_{Na} amplitude and moved the voltage dependence of channel gating parameters in the depolarizing direction, application of 0.2 and 0.3 mM solutions to snail ganglia increased I_A amplitude and moved its gating parameters in the hyperpolarizing direction. This observation does not by itself invalidate hypotheses concerning voltage-gated ion channels in the squid giant axon. However, it does add to the growing body of evidence demonstrating the high degree of specificity of action of lipophilic compounds on neuronal ion channels.

Some of this work has been published in abstract form (Winpenny, Elliott & Harper, 1991, 1992*a*).

Materials and Methods

Winpenny, Elliott and Harper (1992*b*) contains a detailed description of the methods used in this study, including a full account of the identification of cells and current. Recordings were made from cells situated on the rostro-dorsal aspect of the left and right parietal ganglia of the pulmonate snail *H. aspersa* (Blades Biological, Edenbridge, UK). These corresponded to D1 (left parietal ganglion) and F77 (right parietal ganglion) of the key given by Kerkut et al. (1975). No differences were detected between the responses of these two cells and data from each have thus been pooled.

Solutions

Ganglia were bathed in control solution containing (in mM): NaCl, 80; KCl, 4; $CaCl_2$, 10; $MgCl_2$, 5; glucose, 10; HEPES, 5 (all Analar grade (BDH, Poole, UK)). The pH was adjusted to 7.8 by the use of Trizma base (Sigma, Poole, UK). This recipe was taken from the work of Taylor (1987). Phospholipids were obtained from Sigma (*ca.* 99% pure) and used as soon as possible after dispersion in saline. DiC_7PC was supplied dissolved in chloroform which was evaporated under vacuum before making up the experimental solutions. The *n*-alkanes used were puriss grade from Fluka (Glossop, UK). Solutions were delivered to a 1 ml volume experimental chamber by gravity, through Teflon tubing, and at a flow rate of around 4 ml/min. Precautions were taken to minimize evaporative loss of hydrocarbons when making up solutions and during delivery. The temperature of the bathing solution was controlled at a temperature of 12 ± 2°C, with a maximum deviation within an individual experiment of 0.5°C and a maximum range per compound of 2.5°C. These temperatures were used to improve the resolution of the peak current and are compatible with sustained life for the animal or cell.

Electrophysiological Recording

A conventional two-microelectrode voltage clamp was used (Axoclamp 2A, Axon Instruments, CA). Microelectrodes were pulled from 1.5 mm OD borosilicate glass. The potential-recording electrode contained 3 M KCl while the current-passing electrode was filled with filtered 2 M K citrate. Electrode resistances were between 5 and 15 MΩ. A grounded steel foil shield was usually placed between the electrodes to minimize capacitative coupling. The membrane potential was measured differentially between the KCl microelectrode (through an Ag/AgCl pellet) and an Ag/AgCl pellet in the bathing solution. Membrane currents were measured using a virtual ground circuit connected to the bath through an Ag/AgCl wire. The Axoclamp was used in current-clamp mode between periods of voltage clamp to obtain measurements of resting potentials, action potentials and the cell input resistance. The voltage difference between the potential microelectrode and the bath electrode was measured at the end of each experiment, having been zeroed before impalement. Experiments were discarded if this potential proved to be greater than ±5 mV. The voltage clamp changed the membrane potential from 10 to 90% of its final value in a step within 700 μsec and settled at the command value within 4 msec. The measured series resistance was 21 ± 3 kΩ (n = 5), which resulted in a maximum voltage error of around 3 mV for a 150 nA current. Therefore, no compensation was applied.

Data Acquisition and Analysis

Readings of the membrane current and potential were recorded on videotape through a modified Sony PCM 701 while being simultaneously viewed on a digital storage oscilloscope. Records obtained under current clamp were subsequently displayed on the oscilloscope for analysis of action potential frequency, time to the peak of the first action potential and input resistance. Voltage clamp data were transferred to a microcomputer by means of a Data Translation DT2801-A interface board, controlled by software kindly donated by Dr. J. Dempster of Strathclyde University (Dempster, 1993). Records were analyzed using the same software.

Two types of voltage-clamp experiment were routinely performed. In one, the voltage dependence of A current inactivation was determined by applying a 1 sec test pulse to −30 mV from holding potentials in the range −110 to −50 mV. Test pulses were applied at a frequency not greater than 0.2 Hz so the cell was at each new holding potential for at least 4 sec preceding the test pulse. Checks were made to ensure that a steady level of inactivation was achieved before the test pulse. A steady-state inactivation parameter was then calculated for each holding potential by dividing the associated peak (with time) test pulse current by that achieved following the most negative holding potentials. This inactivation parameter was termed b_∞. The potential at which b_∞ was 0.5 was termed V_b and shifts in that potential, ΔV_b.

In the other type of experiment, the holding potential was maintained at −100 mV and various test pulses in the range −70 to −20 mV were applied. The frequency of stimulation was again not greater than 0.2 Hz. Suitably scaled current responses to hyperpolarizing voltage pulses were used to correct the current records for leakage and zero current was taken as the steady-state value of such leakage-subtracted traces. The effects of test compounds on the following parameters were then determined: the amplitude of the peak (with time) current at a test potential of around −30 mV (sometimes interpolated from current-voltage plots); the time constant of macroscopic in-

activation (τ_b), as calculated by fitting a single exponential function to the falling phase of the current; the time-to-peak current (t_c) and, lastly, the voltage dependence of current activation. An activation parameter, a_∞, was determined by first extrapolating an exponential fit to the falling phase of the current back to zero time. This was done for currents obtained at a number of test pulse potentials. The amplitude of each extrapolated current was then expressed as a fraction of that produced by the most positive test potential (usually *ca.* −20 mV) and these normalized a_∞ values were then plotted against test pulse potential. The potential at which a_∞ was 0.5 was termed V_a and shifts in that potential were called ΔV_a. It should be noted that this activation parameter was calculated from normalized current amplitudes, not conductance values. Therefore, a_∞ would not be expected to saturate with depolarization (*see e.g.,* Belluzzi, Sacchi & Wanke, 1985; Treistman & Wilson, 1987). In addition, the analysis then depends on the use of the same maximum depolarizing pulse for control, test and reversal solutions. This condition was met to within *ca.* 2 mV when account was taken of the measured potential difference between the voltage and bath electrodes at the end of each experiment.

Each experiment involved the application of a single concentration of a given compound to a separate identified cell and usually comprised control, test and reversal phases. The final control readings were taken once the A current had reached a steady-state—some 15 to 30 min after impalement—and the superfusing solution was then switched to one containing the test compound. Clear indications of a current potentiation by the phospholipids were apparent some 30 min following the change to test solution, and what appeared to be the maximum effect was not achieved until around 60 min of lipid superfusion. The lipid-induced increase in amplitude was often difficult to reverse. Reversal readings were taken after 90 min of lipid-free superfusion. Extending the washout time to 120 min did not significantly improve the level of recovery. The time course of action of the hydrocarbons tested were similar to those of the *n*-alkanols used previously (Winpenny et al., 1992*b*). The first signs of an effect were apparent after 5 min of application, a steady level was reached after *ca.* 20 min and the results presented were taken after 30 min. Extending the washout period beyond the usual 30 min did not improve reversal.

Error values were calculated as the standard error of the mean ($n \geq 3$) or the range. Least-squares fits plus their associated standard errors were calculated, and statistical tests were performed, with the aid of Statgraphics (Statistical Graphics, MD) software or Vcan software kindly donated by Dr. J. Dempster.

Results

Current-Clamp Experiments

Superfusion of *Helix* D1 and F77 neurons with solutions containing 0.2 mM dioctanoyl phosphatidylcholine (diC_8PC) or 0.5 mM diC_7PC consistently produced a dramatic decrease in the resting membrane potential, i.e., a depolarization from satisfactorily negative values in control solution to around 0 mV in test solutions. In two out of three experiments with 0.4 mM diC_7PC, the resting membrane potential decreased from −55 and −59 mV, respectively, to 0 mV. However, a successful experiment was performed on the third cell. By contrast, squid giant axons invariably survived superfusion with 0.2 mM diC_8PC or 0.52 mM diC_7PC (Hendry et al., 1985) and neonatal rat dorsal root ganglion neurons could be patch clamped in 0.5 mM diC_7PC (A.A. Elliott and J.R. Elliott, *unpublished results*). The deleterious effects of such concentrations of phospholipids on D1 and F77 neurons impaled with two microelectrodes caused us to restrict our investigations primarily to 0.2 and 0.3 mM diC_7PC, following preliminary experiments with 0.05 and 0.07 mM diC_8PC. *Helix* neurons survived superfusion with saturated solutions of *n*-alkanes (*n*-pentane, *n*-hexane and *n*-octane).

The actions of phospholipids and *n*-alkanes on various properties of current-clamped cells are summarized in Table 1. It should be noted that these cells were electrically silent unless stimulated or perturbed. The main points of contrast between the actions of phospholipids and those of the *n*-alkanes were as follows. First, the phospholipids increased the time to the peak of the first action potential while the *n*-alkanes decreased this parameter. Second, higher concentrations of phospholipids tended to decrease action potential firing frequency while the *n*-alkanes tended to increase firing frequency. The latter effect was not simply a consequence of the change in time to the peak of the first action potential, the inter-spike interval also changed. These effects are illustrated in Fig. 1, which demonstrates the actions of (*A*) 0.3 mM diC_7PC and (*B*) saturated *n*-hexane. The apparent variation in action potential height is an artifact resulting from the limited number of data points (1,024) available for display on the digital oscilloscope (measurements of the time to peak of the first action potential were made on an expanded time scale which allowed accurate resolution of the peak).

Voltage-Clamp Experiments

The effects of various short-chain phospholipids and *n*-alkanes on I_A are detailed in Table 2. Specific examples of these effects are given in Figs. 2–4. The time-to-peak current was not affected to an extent which warranted detailed investigation or discussion and thus does not appear in Table 2. Figure 2 shows the action of (*A*) 0.2 mM diC_7PC and (*B*) saturated *n*-hexane on A currents and on the current-voltage relationship (*C* and *D*). The phospholipid caused an increase in peak current amplitude which was partially reversible. The maximum current potentiation by a phospholipid (seen in an experiment with 0.3 mM diC_7PC) was to 225% of the control value, with a reversal to 172% of control.

The effects of *n*-alkanes on peak amplitude were less clear-cut. Saturated *n*-pentane caused a dramatic reduction which was in one experiment reversed, but both decreases and occasional increases were found with 0.5 saturated *n*-pentane and saturated *n*-hexane. These different responses were not correlated with cell type. When meaned, the data for saturated *n*-hexane and 0.5

Table 1. Effects of short-chain phospholipids and *n*-alkanes on current clamped *Helix* D1 and F77 neurons

Substance	Concentration or fractional saturation	Resting membrane potential (mv)			Input resistance (MΩ)			Time to peak of first AP (msec)			AP frequency*
		c	t	r	c	t	r	c	t	r	
DiC_7PC	0.2 mM	−54	−50	−51	6.0	6.7	6.5	287	461	302	↓↑↑↔
DiC_7PC	0.3 mM	−55	−66	−69	9.9	9.0	7.9	298	621	353	↓↓↓↓
DiC_7PC	0.4 mM	−47	−39	−37	9.8	3.6	4.2		104	60	↓
DiC_8PC	0.05 mM	−47	−47	−46	5.3	3.9	3.5	52	70	72	↓
DiC_8PC	0.07 mM	−59	−56	−61	10	7.9	10	514	no AP	664	no AP
n-Pentane	0.5 saturated	−54	−56	−53	8.9	7.9	6.5	283	92	183	↑↑↓
n-Pentane	Saturated	−54	−65	−60	8.8	6.8	7.5	172	62	70	↑↔
n-Hexane	Saturated	−54	−55	−57	7.1	6.9	6.2	271	177	328	6↑ 1↓ 3↔

AP = action potential; c = control; t = test; r = reversal. * Change in AP frequency indicated by the direction of the arrow; (↑) increased; (↓) decreased; (↔) no change. One arrow per experiment.

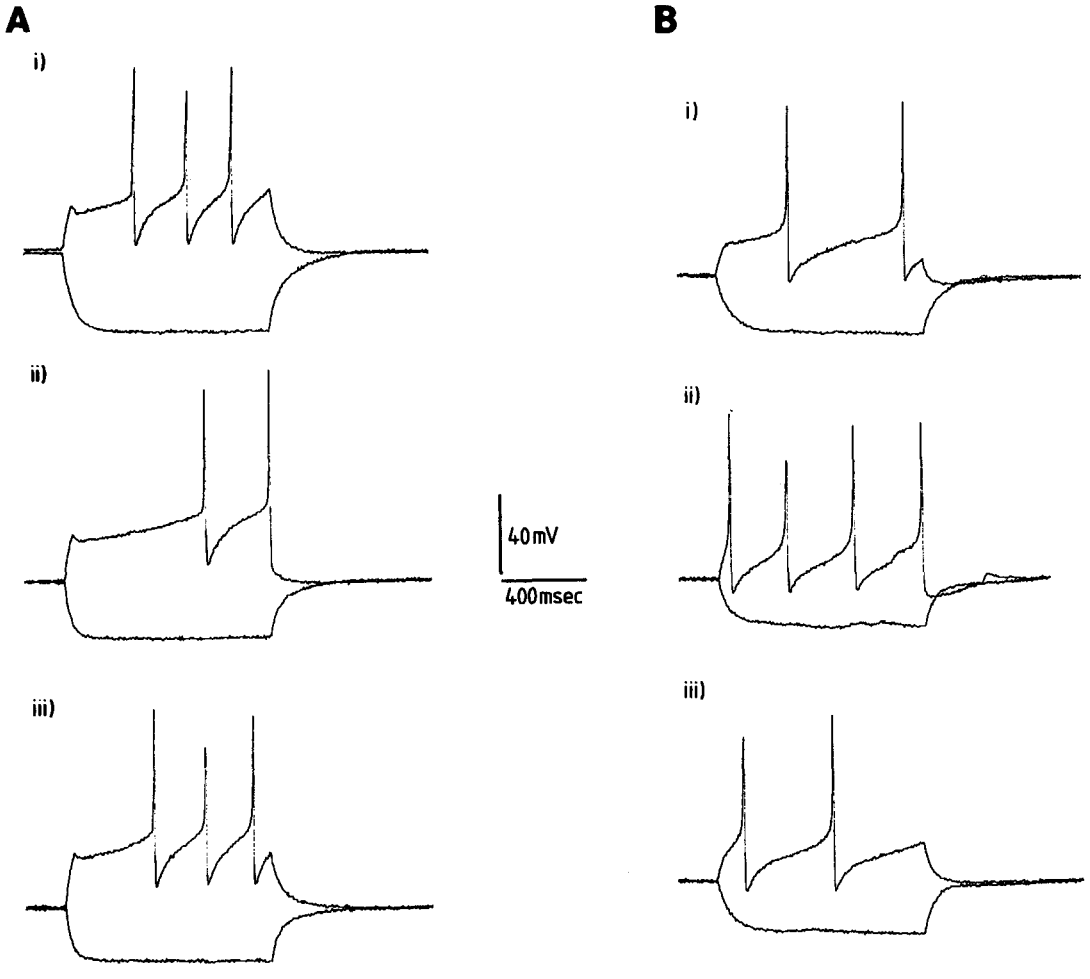


Fig. 1. The effect of 0.3 mM diC_7PC (*A;* cell JPW293550) and saturated *n*-hexane (*B;* cell JPW35) on the voltage response to a +5 nA (upper trace of each pair) and −5 nA (lower trace) current pulse of 1 sec duration. Records are shown under control (*i*), test (*ii*) and reversal (*iii*) conditions for each cell. In the experiments shown, the phospholipid hyperpolarized the neuron (from a resting membrane potential (rmp) of −62 mV under control conditions to −72 mV under test conditions) while the hydrocarbon caused a depolarization (from a control rmp of −57 mV to a test rmp of −50 mV). However, the rmp's after washout were −76 and −50 mV, respectively. The lack of reversal of resting membrane potential and the good reversal of firing frequency indicate that changes in the latter were not simply the result of alterations in the former.

Table 2. Effects of short-chain phospholipids and *n*-alkanes on the A current in voltage clamped *Helix* D1 and F77 neurones

Substance	Concentration or fractional saturation	I_p^t / I_p^c	I_p^r / I_p^c	ΔV_a^{t-c} (mV)	ΔV_a^{r-c} (mV)	ΔV_b^{t-c} (mV)	ΔV_b^{r-c} (mV)	k^t/k^c	k^r/k^c	τ_b^t/τ_b^c	τ_b^t/τ_b^c
DiC_7PC	0.2 mM	1.58 ± 0.14	1.41 ± 0.18	−3.3 ± 0.6	−4.1 ± 0.9	−5.5 ± 2.2	−3.3 ± 1.7	1.0 ± 0.1	1.1 ± 0.1	1.28 ± 0.10	1.29 ± 0.16
DiC_7PC	0.3 mM	1.79 ± 0.21	1.61 ± 0.10	−4.1 ± 1.2	−4.4 ± 0.5	−5.0 ± 1.5	−4.0 ± 0.7	0.9 ± 0.1	1.1 ± 0.2	1.25 ± 0.08	0.98 ± 0.09
DiC_7PC	0.4 mM	1.36	1.13	−3.0	0	−5.0	−4.0	0.9	0.8	1.34	1.17
DiC_8PC	0.05 mM	1.21	1.13	−2.5	−2.0	0.6	0.2	0.6	0.7	1.06	1.07
DiC_8PC	0.07 mM	1.67	1.48	−5.0	−4.0	−6.1	−3.8	0.8	0.8	1.03	1.0
n-Pentane	0.5 Saturated	1.05 ± 0.23	1.18 ± 0.34	−2.7 ± 1.2	−4.8 ± 2.3	−5.7 ± 1.8	−6.0 ± 2.0	0.9 ± 0.2	1.0 ± 0.3	0.58 ± 0.08	0.96 ± 0.26
n-Pentane	Saturated	0.30 ± 0.25	1.0 (n = 1)	NA	NA	NA	NA	NA	NA	NA	NA
n-Hexane	Saturated	0.87 ± 0.08	0.97 ± 0.08	0.1 ± 0.6	−1.0 ± 1.0	−2.7 ± 0.5	−1.8 ± 1.2	1.2 ± 0.1	0.9 ± 0.2	0.57 ± 0.05	0.62 ± 0.06

c = control; t = test; r = reversal. NA indicates not available. Mean values are given ± SEM, or range, where possible.

saturated *n*-pentane indicated relatively little effect on peak amplitude. The current traces shown in Fig. 2 also illustrate the oppositely directed actions of diC_7PC and saturated *n*-hexane on the time course of decay or macroscopic inactivation of I_A. These effects are explored further in Fig. 3 which gives examples of the single exponential fits used to estimate the time constant of decay (τ_b) and also shows meaned data for 0.3 mM diC_7PC and saturated *n*-hexane. The lipid can be seen to increase, and the *n*-alkane to reduce, τ_b at all potentials. The two experiments with diC_8PC did not show a marked effect on τ_b but *n*-pentane mimicked *n*-hexane in causing a marked acceleration of macroscopic inactivation.

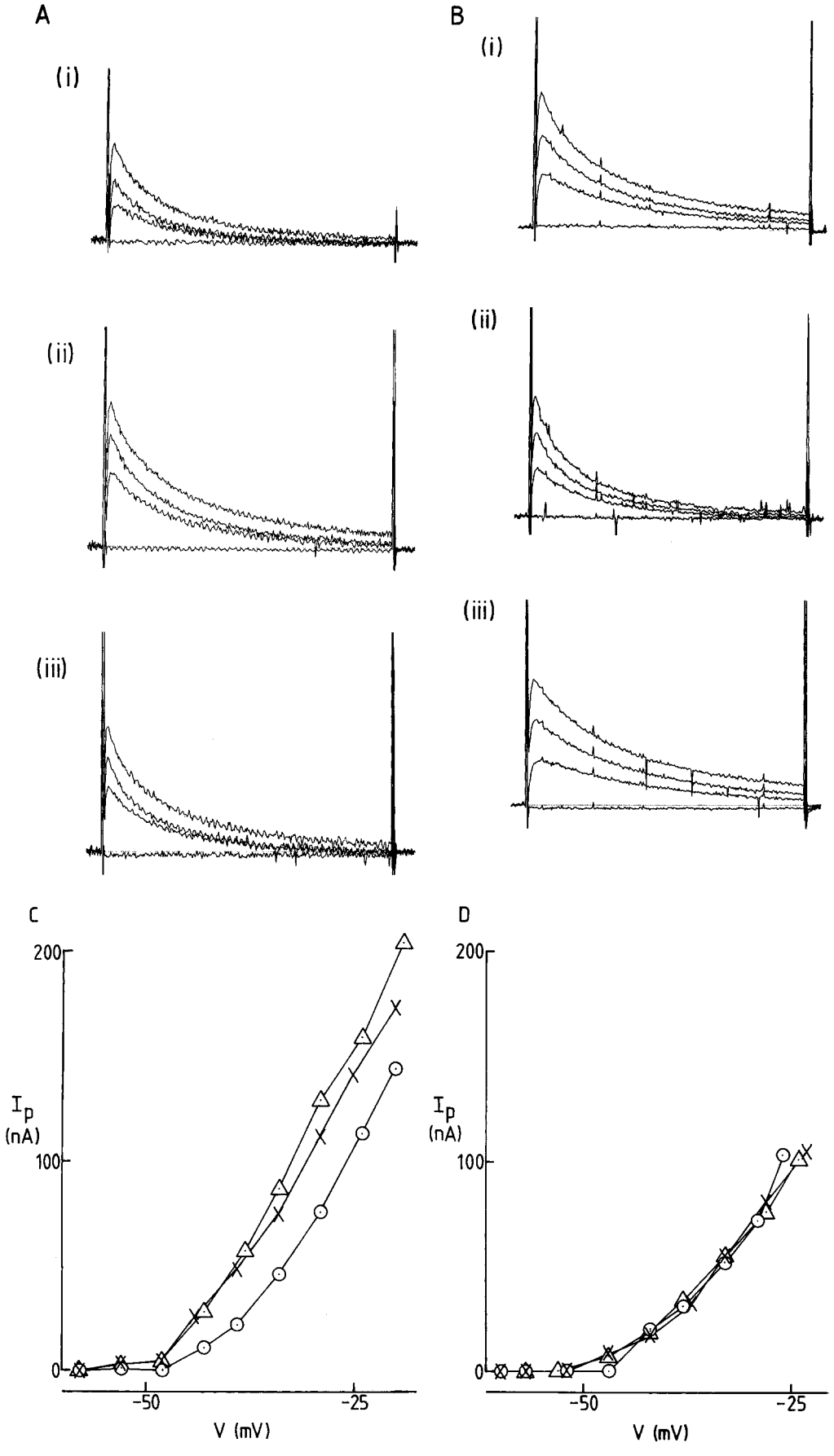
Figure 4 gives examples of the effects of (*A* and *C*) 0.3 mM diC_7PC and (*B* and *D*) saturated *n*-hexane on the voltage dependence of A current activation (a_∞) and inactivation (b_∞) parameters. The relationships between b_∞ and potential have been fitted by a simple Boltzmann function to give estimates of the potential for 50% inactivation (V_b) and the midpoint slope factor (k). The data of Fig. 4 and Table 2 indicate that short-chain phospholipids caused a hyperpolarizing shift in V_b. Cells treated with *n*-pentane and *n*-hexane also exhibited a small hyperpolarizing shift in V_b. This effect was, in general, poorly reversed. There was no consistent change in the midpoint slope. Short-chain phospholipids also caused hyperpolarizing shifts in the voltage dependence of A current activation. These were quantified by visual estimation of the midpoint potential (V_a). In general, hydrocarbons had a smaller effect on V_a than did the short-chain phospholipids.

Discussion

Current Potentiation

The short-chain phospholipids diC_7PC and diC_8PC reduced the peak squid axon sodium current (Haydon & Urban, 1983*b*; Hendry et al., 1985). In axons voltage clamped to remove resting inactivation, that peak current inhibition was the result of: (i) a depolarizing shift in the voltage dependence of current activation, such that a given voltage pulse opened fewer channels; (ii) an increase in the time constant of activation, which increased the speed of inactivation relative to that of activation and (iii) in some cases, a decrease in the maximum conductance (as obtained by Hodgkin-Huxley analysis). In addition, the voltage dependence of steady-state inactivation was moved in the depolarizing direction. Given these data, the phospholipid-induced potentiation of *Helix* I_A came as a surprise to us. The first question we consider is the origin, in terms of analyzable parameters of the A current, of that potentiation.

I_A current-voltage relationships were produced from experiments with a holding potential of −100 mV. At that potential, all A channels were available for conduction in both control and test solutions, i.e., shifts of up to −10 mV in the steady-state inactivation curve would not affect the peak current amplitude. However, shifts in the activation-voltage relationship would alter the peak current produced by a pulse to around −30 mV, as this was on the rising phase of the *I-V* curve (*see* Fig. 2). A hyperpolarizing shift in the current-voltage relationship or the related activation-voltage relationship


A
(i)
(ii)
(iii)
B
(i)
(ii)
(iii)
C
200
100
0
I_p
(nA)
-50
-25
V (mV)
D
200
100
0
I_p
(nA)
-50
-25
V (mV)

would increase the peak current produced by a pulse to −30 mV. As shown in Figs. 2 and 4 and in Table 2, short-chain phospholipids did indeed cause a hyperpolarizing shift in the A current- and activation-voltage relationships. The correlation between the shift in the midpoint of our activation curves (ΔV_a) and the change in peak current amplitude at −30 mV is shown in Fig. 5. We have not calculated a linear or other fit to this relationship because, in the absence of a confirmed model for even the control current, we do not know the correct predicted relationship. However, the degree of correlation is readily apparent. Therefore, at least part of the current potentiation by phospholipids may be attributed to a negative shift in the voltage dependence of channel activation.

Short-chain phospholipids also increased the time constant of A current inactivation and this could, in theory, produce an increase in the peak current amplitude by slowing the decay of the macroscopic current relative to the rising phase. However, the normal pace of macroscopic inactivation is sufficiently slow that the alterations in τ_b produced by phospholipids would have little effect on peak amplitude. This contrasts with the squid axon sodium current, where the more brisk inactivation relative to current rise leads to a substantial dependence of peak current amplitude on the time constants of inactivation and activation.

Shifts in the Voltage Dependence of Channel Gating Parameters

As explained in the Introduction, the first aim of this study was to test the general relevance of a membrane thickness hypothesis for shifts in voltage-dependent channel gating parameters by determining the effects of short-chain phospholipids and *n*-alkanes on an A channel. *n*-Pentane and *n*-hexane produced negative shifts in the squid axon Na and K current gating parameters, while short-chain phospholipids caused positive shifts in axonal Na current gating parameters. When coupled with evidence from high frequency measurements of membrane capacitance that *n*-alkanes thickened and short-chain phospholipids thinned the axonal membrane, with a time course comparable to that of changes in the ionic currents (Haydon, Requena & Urban, 1980; Hendry et al., 1985), these findings were consistent with the hypothesis that a thickness-mediated alteration in the membrane electrical field underlay the changes in steady-state gating parameters. However, it should be said that Haydon and co-workers recognized that the correspondence in effects between axonal sodium and potassium channels was not perfect. For example, a different type of hydrocarbon, cyclopentane, moved the Na activation (and inactivation) curves in the hyperpolarizing direction but caused depolarizing shifts in the K activation curve. An interaction between thickness increases and surface potential changes were thought perhaps to account for such variations (Haydon & Urban, 1986).

We have now shown that short-chain phospholipids produced hyperpolarizing shifts in both the inactivation and activation gating parameters of the A current in *Helix* D1 and F77 cells. These results are not consistent with the proposal that their major effects on the voltage dependence of channel gating were exerted through a membrane thickness decrease. Nor do we wish to suggest that adsorption of diC_7 or diC_8 phospholipids to these cells increased the membrane thickness. Therefore, we must look elsewhere for an explanation of our I_A results.

There are a number of ways in which adsorption of a short-chain phospholipid could in theory alter the normal free energy balance of the A channel-cell membrane system and thus change the voltage dependence of channel gating. One still involves the potential profile across the membrane but considers the influence of the surface dipole potential on the membrane field rather than that of the membrane thickness (*see e.g.*, Haydon, Elliott & Hendry, 1984, for a full explanation). A hyperpolarizing shift in gating parameters is consistent with a reduction in the resting field strength. Such a change would follow either an increase in the internal membrane dipole potential or a reduction in the external potential. The dipole potential of cholesterol/egg phosphatidylcholine monolayers (2:1 bulk phase mole ratio) is some 28 mV higher than that of pure egg phosphatidylcholine monolayers (Haydon & Elliott, 1986). It is thus possible that an increase in phosphatidylcholine content may have reduced the potential of a cholesterol-rich area near the snail A channel. Alternatively, adsorption of a zwitterionic lipid to an area normally rich in negatively charged lipids such as phosphatidylserine would increase the surface potential.

Fig. 2. The influence of 0.2 mM diC_7PC (*A* and *C;* cell JPW301000) and saturated *n*-hexane (*B* and *D;* cell JPW343270) on the A current and peak current-voltage relationship. Currents were evoked by a 1 sec depolarizing pulse from a holding potential of −100 mV. The traces presented were obtained by depolarizations to around −67, −34, −29 and −24 mV (*A*) and −62, −38, −33 and −28 mV (*B*). Groups of leak subtracted records are shown under control (*i*), test (*ii*) and reversal (*iii*) conditions. The dotted line (often obscured by the first trace) indicates the zero current level. The scale bars indicate: (*A*) 100 nA and 100 msec; (*B*) 50 nA and 100 msec. The current-voltage relationships (*C* and *D*) show the dependence of the peak (with time) value of the A current (I_p) on the pulse potential (*V*). The lines simply connect adjacent points. The symbols represent: (⊙) control; (△) test and (X) reversal conditions.

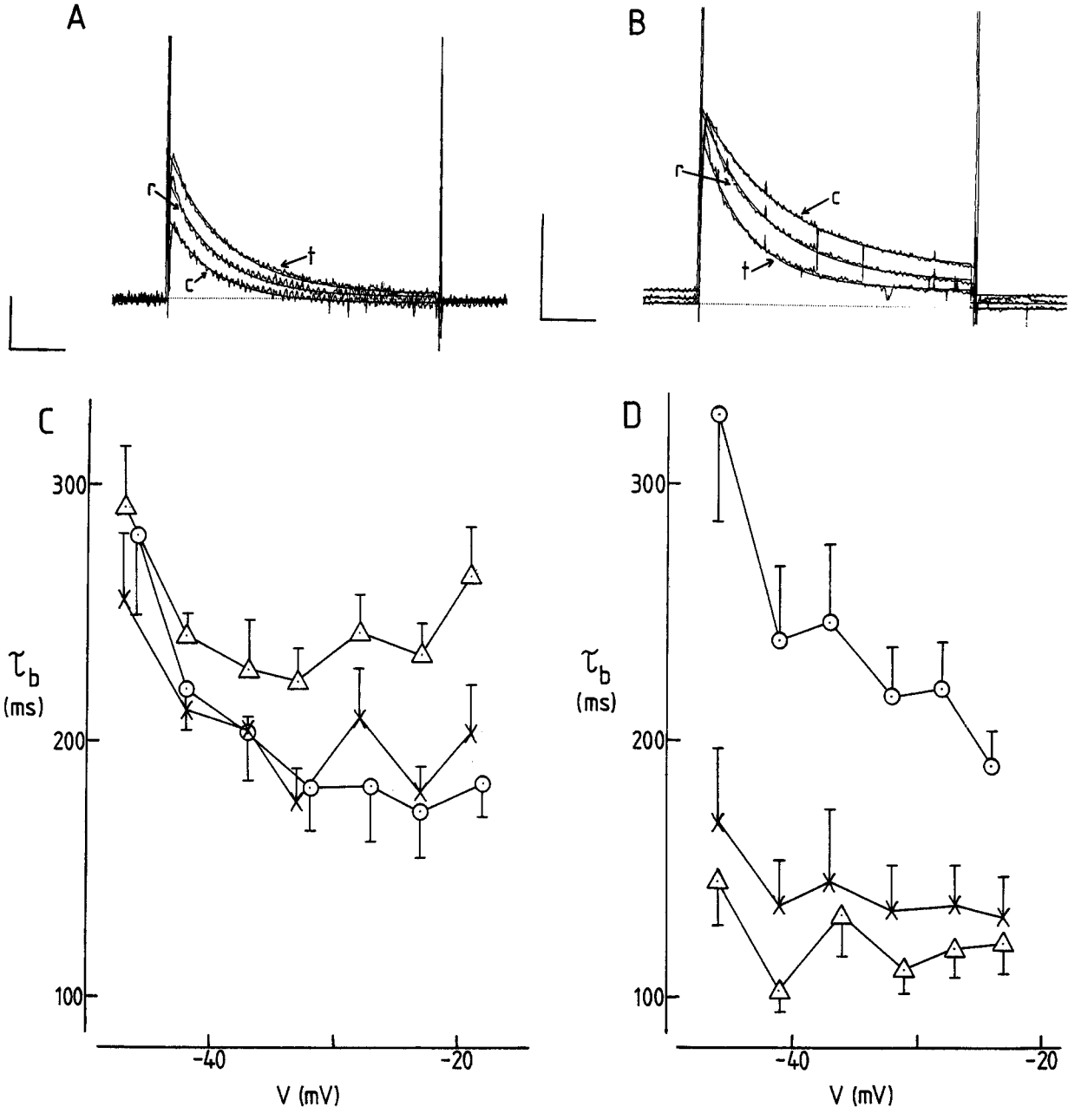


Fig. 3. The effects of diC$_7$PC and *n*-hexane on the time course of macroscopic current inactivation. An inactivation time constant, τ_b, was obtained by fitting a single exponential function ($I_A = A_1\exp(-t/\tau_b)$ + ss where: I_A is the current amplitude; t is the time in msec; A_1 is the extrapolated amplitude at time zero and ss is the extrapolated amplitude at infinite time) to the falling phase of the current. Examples of such fits are given for the currents shown in Fig. 2 (cells JPW301000 and JPW343270) which were elicited by voltage pulses to *ca.* −30 mV. *A* shows the effect of 0.2 mM diC$_7$PC, which was to increase τ_b from 187 msec under control conditions (trace *c*) to 228 msec in the test solution (trace *t*). τ_b then fell to 198 msec following reversal (trace *r*). In *B*, saturated *n*-hexane is seen to reduce τ_b from 320 to 185 msec, with reversal to 252 msec. The scale bars next to the current traces show 50 nA and 200 msec. Meaned data for 0.3 mM diC$_7$PC and saturated *n*-hexane are given in *C* and *D*. The symbols represent: (⊙) control; (△) test and (X) reversal conditions. The lines simply connect adjacent points and the error bars are the standard error of the mean for n = 4 to 10.

Functional A channels are thought to result from the assembly of a number (probably four) of individual subunits (*see e.g.*, Isacoff et al., 1990 and MacKinnon, 1991). If so, the influence of lipid chain length on membrane protein aggregation may be relevant. The basic proposition is that adsorption of lipids with a chain length different from normal may alter the balance between favorable lipid-protein interactions towards fa-

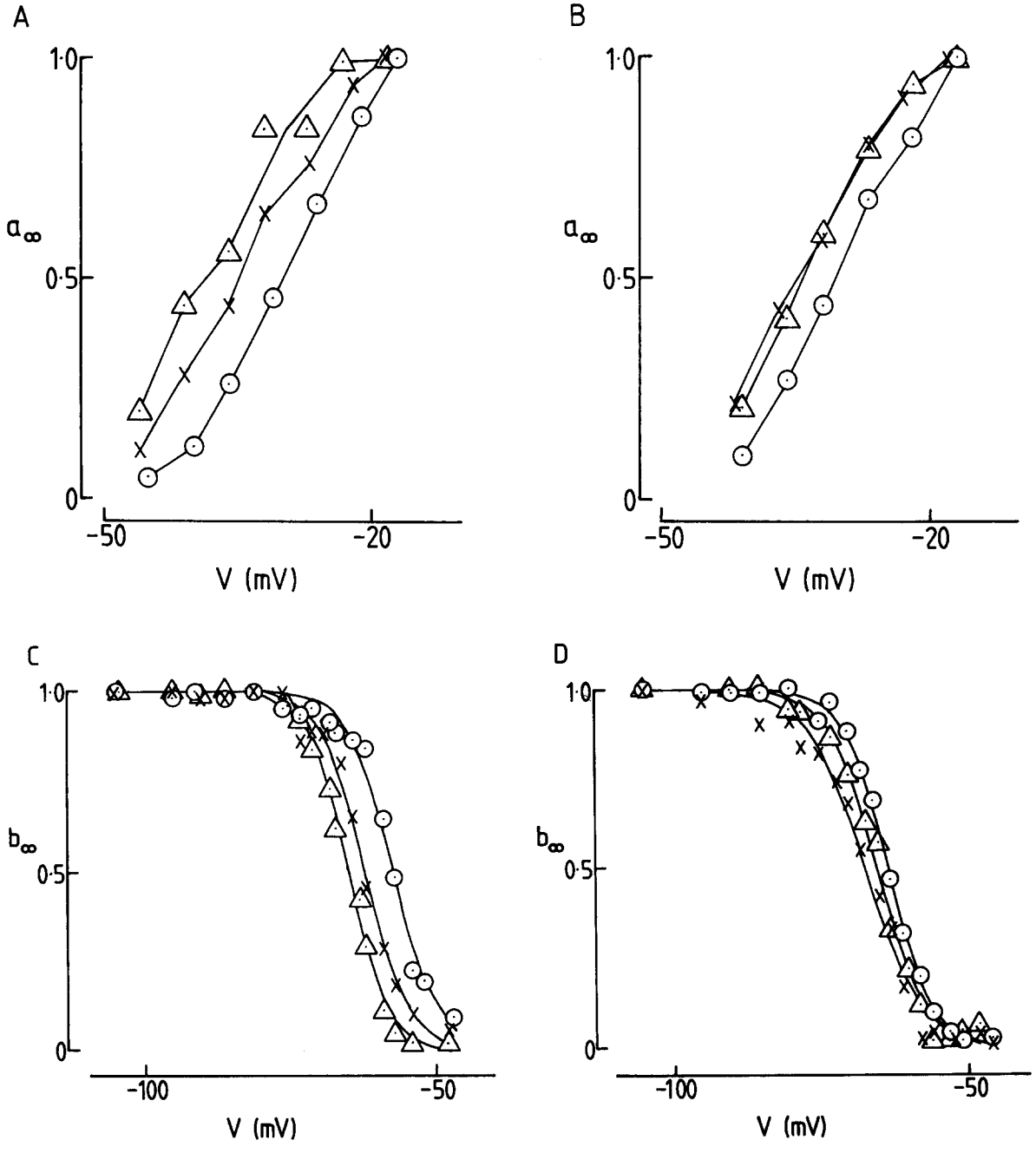


Fig. 4. The actions of 0.3 mM diC_7PC (*A* and *C;* cell JPW282550) and saturated *n*-hexane (*B* and *D;* cell JPW342550) on the voltage dependence of the A current activation parameter (a_∞) and steady-state inactivation parameter (b_∞). In *A* and *B* the lines connect adjacent points and the midpoint activation potential (V_a) was calculated by interpolation. *V* is the membrane potential during the current-activating test pulse. In *C* and *D*, the data were fitted to a simple Boltzmann function *viz.* $b_\infty = 1/(1 + \exp[(V - V_b)/k]$ where: b_∞ is the steady-state inactivation parameter; *V* is the membrane potential before the test pulse; V_b is the potential at which b_∞ is 0.5 and *k* is a midpoint slope parameter. In the examples given, diC_7PC shifted V_a by −7 mV, with a reversal of 2.5 mV and moved V_b −8 mV, with 3 mV reversal. *n*-Hexane shifted V_a by −3 mV but with no reversal (in fact, a further 1 mV hyperpolarizing shift) and moved V_b by − 2 mV with even less reversal (a further 2 mV hyperpolarizing shift). The symbols represent: (⊙) control; (△) test and (X) reversal conditions.

vorable protein-protein interactions and thus promote protein aggregation in the plane of the bilayer. The magnitude of this influence in model systems is uncertain (Lewis & Engelman, 1983; Pearson et al., 1983) and we have no data directly relevant to voltage-gated ion channels. However, we can say that the adsorption of phospholipids with a significantly different chain length from normal is likely to affect the surface free energy

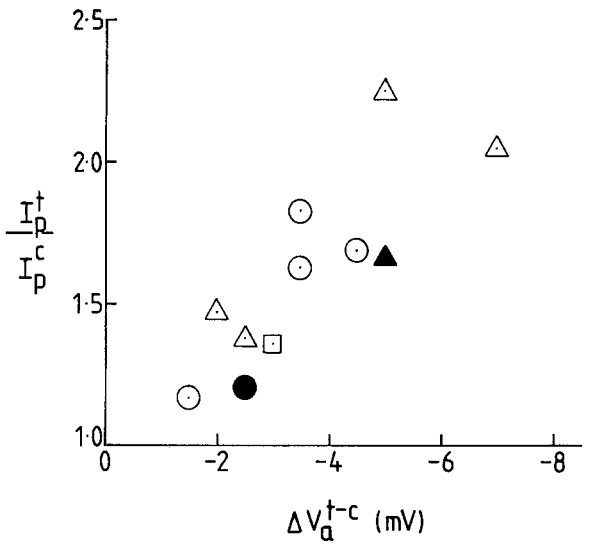


Fig. 5. The correlation between the effects of phospholipids on the peak A current amplitude and the shift in the voltage dependence of current activation. The ordinate indicates the peak current in test conditions (t) as a fraction of the control current (c) while the abscissa shows the shift in midpoint of the activation curve, again test relative to control. The symbols indicate: (○) 0.2 mM diC_7PC; (△) 0.3 mM diC_7PC; (□) 0.4 mM diC_7PC; (●) 0.05 mM diC_8PC; (▲) 0.07 mM diC_8PC.

of the membrane—either by an alteration in the surface tension (Elliott et al., 1983) or the deformation free energy (Huang, 1986; Helfrich & Jakobsson, 1990). Gruner and Shyamsunder (1991) have also drawn attention to the possible links between lipid geometry, lipid membrane curvature and the functioning of membrane proteins.

The above speculations as to mechanism regard the lipid membrane as a primary site of action, with alterations in channel function following from a general perturbation of membrane properties, and as originally formulated were aimed at explaining reductions in channel activity. They may, however, work equally well to explain increases in channel activity. But, it is quite possible, and indeed from the variety of ion channel effects we have shown, quite likely, that more specific actions, with a greater involvement of the protein, are responsible for the alterations in activity of neuronal voltage-gated ion channels (*see e.g.,* Michelangeli et al., 1991, for a detailed account of the influence of phospholipids on the kinetics of a membrane protein).

The effects of *n*-pentane and *n*-hexane on the voltage dependence of *Helix* A current inactivation were not inconsistent with a thickness mechanism, but were much smaller than those exhibited by axonal I_{Na}. In the absence of the detailed supporting evidence available for the axonal current, we would not rule out alternative mechanisms of gating shifts based on direct *n*-alkane-protein interactions. Protein-based mechanisms will be considered further in the next section.

Macroscopic Inactivation

Short-chain phospholipids and *n*-alkanes had opposing effects on the time constant of macroscopic inactivation, τ_b, as expressed by the time course of decline of the whole-cell current. The phospholipids increased, while *n*-pentane and *n*-hexane reduced, τ_b. This effect was the major influence of *n*-hexane on the A current and provided the clearest contrast between the actions of phospholipids and *n*-alkanes.

Aldrich, Hoshi and Zagotta (1990) described the single channel behavior of three variants of *Shaker* A-type potassium channels expressed in *Xenopus* oocytes. They were able to link differences in the time course of macroscopic inactivation to differences in the burst duration of single channel events. Variants with longer burst durations also showed a slower time course of macroscopic inactivation. It is thus possible that the *n*-alkane-induced decrease in τ_b of *Helix* I_A was the result of a reduction in single channel burst duration or effective open channel lifetime, while the phospholipids increased burst duration and thus τ_b. One model system which behaves in a manner compatible with this suggestion is that of gramicidin single channels in nominally solvent-free monoglyceride planar bilayers (Elliott et al., 1983). *n*-Alkanes reduced gramicidin channel lifetime, while substitution of C_{18} chain monoglycerides by lower chain length molecules increased channel lifetime.

The diC_7PC-induced increase in the time constant of potassium current inactivation is not unique. Treistman and colleagues (Treistman & Wilson, 1987; Treistman & Grant, 1990) have demonstrated a similar action by ethanol on the A current of certain *Aplysia* neurons. The lack of ethanol sensitivity of the corresponding current in other cells emphasizes the possible specificity of action of these apparently simple compounds. The classic K channel blocker tetraethylammonium (TEA) has also been shown to slow the decay of inactivating potassium currents. This effect may be coupled with either a decrease (Grissmer & Cahalan, 1989) or an increase (De Biasi et al., 1993) in peak current amplitude. Oxford and Wagoner (1989) reported a slowing of voltage-gated potassium current inactivation in GH_3 cells, with a corresponding increase in peak current amplitude, by internal application of *N*-bromoacetamide (NBA) or 4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonic acid (SITS). Oxford and Wagoner (1989) also reported a prolongation of single channel lifetime by internal NBA, consistent with our suggestion for the effects of short-chain phospholipids.

One explanation for the relatively slow onset and

offset of phospholipid effects, including that on τ_b, may be that these molecules had an internal site of action in *Helix* and that the rate-limiting step was determined by the rate of flip-flop from the external leaflet to the internal leaflet. This is likely to be slower than diffusion of an *n*-alkane or *n*-alkanol through the cell membrane (*see e.g.,* Dawidowicz, 1987). An additional hypothesis is that the active agent was a product of phospholipid metabolism (*see e.g.,* Billah & Anthes, 1990, and Ordway, Singer & Walsh, 1991, for details of phosphatidylcholine metabolism and the possible influence of such metabolites on ion channels), or that the phospholipid or its product initiated intracellular reactions such as a phosphorylation sequence. A number of voltage-gated ion channels are now known to be influenced by phosphorylation (*see e.g.,* Rogawski, 1985; Rudy, 1988; Augustine & Bezanilla, 1990) and further studies of the *Helix* channel along these lines may be fruitful.

Behavior under Current Clamp

The action potential firing behavior of a neuronal soma under current clamp is influenced by a number of inward and outward currents. However, the activation of I_A will tend to delay the rise in membrane potential towards threshold, regardless of whether that depolarization is in response to a physiological excitatory synaptic input or an experimental current injection. It will also increase the inter-spike interval in a train of action potentials (*see e.g.,* Rogawski, 1985). Other potassium currents have similar effects on the delay to the first action potential (Storm, 1988) but our reasons for identifying the current we studied as an A current have been given previously (Winpenny et al., 1992*b*). Taken overall, the results given in Table 1 indicate that short-chain phospholipids delayed the onset of action potential firing in response to a depolarizing current pulse and decreased firing frequency, while the *n*-alkanes reduced the time to the first spike and increased firing frequency. These effects are broadly consistent with our results under voltage clamp. The *n*-alkanes had little effect on peak A current amplitude (with the exception of saturated *n*-pentane) but they accelerated the decay of the current and thus reduced the amount of charge transferred from the cell. This would reduce the braking influence of I_A on cellular excitability. The phospholipids, however, would act to increase the loss of positive charge and thus potentiate the dampening effect of I_A activation.

Conclusions

Comparisons between the effects of short-chain phospholipids and *n*-alkanes on various parameters of the squid axon sodium current revealed similarities (peak current suppression and peak time constant reduction) and differences (depolarizing *vs.* hyperpolarizing shifts in the voltage dependence of current activation and inactivation). Our study of an A current in *Helix* neuronal somata also highlighted common and disparate features, but these were different from those found in the squid. Here, phospholipids and *n*-alkanes moved the current inactivation gating parameters in the hyperpolarizing direction but phospholipids increased current amplitude and the time constant of current decay while *n*-alkanes reduced one or both of these parameters. We found little reason to support a thickness-mediated mechanism for the effects of phospholipids on the voltage dependence of A channel gating, in contrast to the evidence in support of that theory with respect to squid axon I_{Na}.

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