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 (dC7PC, 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 INa. 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 INa, 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. dC7PC caused a 25% increase in the time constant of macroscopic $I_A$ inactivation ($\tau_p$) but 0.5 saturated n-pentane and saturated n-hexane reduced $\tau_p$ by 40%. The effects of these agents on current-clamped cells were broadly consistent with their opposing actions on $\tau_p$—phospholipids tended to reduce excitability and n-alkanes tended to increase it. Possible mechanisms of $I_A$ perturbation are discussed.

Key words: Voltage clamp — Snail neuron — Potassium current — Ion channels — A current — Membranes

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 (INa and IK) could in part be explained by perturbant-induced changes in the membrane field (Haydon & Kimura, 1981; Haydon & Urban, 1983a, b, 1986). Haydon and colleagues drew particular attention to the oppositely directed effects of n-alkane hydrocarbons and short-chain phospholipids on INa gating, n-Alkanes induced hyperpolarizing shifts in Na current steady-state activation ($m_\alpha$) and inactivation ($h_\alpha$) gating parameters, while short-chain phospholipids moved the $m_\alpha$ and $h_\alpha$ 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, 1983a,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 $I_A$. 

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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 $I_{Na}^v$.

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, 1992a).

**Materials and Methods**

Winpenny, Elliott and Harper (1992b) 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 *Helix aspersa* (Blades Biological, Edenbridge, UK). These corresponded to D1 (left parietal ganglion) and F77 (right parietal ganglion) of the key given by Kersey 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$_2$PC 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 mKCl while the current-passing electrode was filled with filtered 2 mK 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 KC1 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 μsec. 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 $b_{INa}$. The potential at which $b_{INa}$ was 0.5 was termed $V_{b,INa}$ and shifts in that potential, $\Delta V_{b,INa}$.

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-