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THE ROLE OF INACTIVATION IN THE EFFECTS OF *n*-ALKANOLS ON THE SODIUM CURRENT OF CULTURED RAT SENSORY NEURONES

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SUMMARY

1. The whole-cell patch-clamp technique has been used to investigate the actions of *n*-butanol, *n*-pentanol, *n*-hexanol and *n*-octanol on the sodium current of cells isolated from the dorsal root ganglia (DRGs) of neonatal rats and maintained in short-term tissue culture.

2. The influence of *n*-alkanols on the level of steady-state inactivation of the sodium current was investigated by a standard two-pulse protocol. All alkanols increased the level of resting inactivation and this was manifested as a hyperpolarizing shift of the relationship between the steady-state inactivation parameter (h_∞) and membrane potential. The mid-point of the h_∞ curve was moved by up to -30 mV.

3. The relationship between the shift in the mid-point of the inactivation curve (ΔV_h) and aqueous *n*-alkanol concentration has been derived for each *n*-alkanol. These are complex in shape and do not appear consistent with a hypothesis that the increase in inactivation results from 1:1 binding of an alkanol molecule to a single site on the channel protein.

4. The aqueous concentrations used ranged from 70 mM-*n*-butanol to 0·05 mM-*n*-octanol. However, equal fractional saturations of *n*-alkanols produced approximately equal shifts in the h_∞ curve, particularly in the range 0·01–0·07 saturated. This implies a hydrophobic site of action, with a standard free energy per methylene group for adsorption to the site from the aqueous phase of *ca* $-3{\cdot}2$ kJ/mol.

5. The increase in resting inactivation was not the sole means by which *n*-alkanols reduced the sodium current. The current was still reduced in cells pre-pulsed to sufficiently negative potentials to remove steady-state inactivation even in the presence of alkanols. The concentration required to reduce the current by 50% (ED_{50}) has been interpolated for each *n*-alkanol. From these data it was estimated that the standard free energy per methylene group for adsorption to the site of action was *ca* $-3{\cdot}1$ kJ/mol, similar to that calculated for the effect on inactivation. The concentration dependence of this residual block indicated the involvement of more than one *n*-alkanol molecule.

6. The *n*-alkanols increase the level of inactivation of rat DRG cell sodium channels at potentials around the resting membrane potential and this effect contributes to their local anaesthetic action. By contrast, *n*-alkanols have almost no

effect on the level of resting inactivation of squid axon sodium channels. There is thus a species difference in the actions of these structurally simple compounds. It is tentatively suggested that this may arise from a difference in both the protein and lipid portions of the membrane.

INTRODUCTION

Many local anaesthetics increase the fraction of excitable cell sodium channels which are in the inactivated state at potentials around the resting membrane potential. Two main hypotheses have been proposed to account for this effect. The 'modulated receptor hypothesis' (Hille, 1977) suggests that the drug binds to a site on the channel protein and that the inactivated state has the highest affinity for the drug. Drug binding thus stabilizes the inactive form of the channel. By contrast, the 'membrane thickness hypothesis' (Haydon & Kimura, 1981; Haydon & Urban, 1983*a*) proposes that the primary effect is an increase in the thickness of the membrane around the inactivation voltage sensor. At a given membrane potential the increase in thickness would reduce the transmembrane electrical field and the resultant effective depolarization should increase the fraction of inactivated channels.

n-Alkanols have virtually no effect on resting steady-state inactivation of the squid axon sodium current (Armstrong & Binstock, 1964; Oxford & Swenson, 1979; Haydon & Urban, 1983*b*), nor do they thicken solvent-free model lipid bilayers (Elliott & Haydon, 1979, 1984). These observations would appear to support the thickness hypothesis. However, the effects of *n*-alkanols on sodium current inactivation are species dependent. In the frog (Venitz & Schwarz, 1980; Hirche, 1985) and, to a lesser extent, the crayfish (Swenson & Narahashi, 1980) *n*-alkanols do increase steady-state inactivation; but little is known about the mechanism of this effect. For example, there are few data available concerning the concentration dependence of the shift of inactivation or the effects of different chain length *n*-alkanols. Moreover, there is little information from preparations of greater clinical relevance, such as mammals, although the suppression of action potential amplitude in rat dorsal root ganglia cells by *n*-hexanol is modified by membrane potential in a manner consistent with an increase in resting sodium current inactivation (Elliott & McElwee, 1987).

This paper reports an investigation into the effects of four *n*-alkanols on the sodium current of sensory neurone cell bodies from DRGs of neonatal rats. The main finding is that *n*-alkanols increase steady-state inactivation of this mammalian sodium current and that action contributes to, but does not totally account for, the suppression of current at the resting membrane potential. The effects of different chain lengths are consistent with a hydrophobic site of action for both the inactivation-linked and residual current reduction but the concentration dependence of the shift in inactivation suggests the presence of more than one site of action.

A preliminary account of some of this work has been presented to the Physiological Society (Elliott & Elliott, 1989).

METHODS

Cell culture

The cultures were prepared from dorsal root ganglia of 1- to 3-day-old Wistar rats, dissociated essentially as described by Forda & Kelly (1985). The rats were anaesthetized by ether inhalation and then killed by cervical dislocation. Cells were plated onto poly-L-lysine-coated 35 mm culture dishes at a density of $5–9 \times 10^4$ cells/plate. The incubation medium contained Eagle's minimum essential medium (with Earle's salts), 10% fetal calf serum, 4 mM-L-glutamine, 20 mM-KCl, 1 μg/ml 7 S nerve growth factor (Sigma, Poole, Dorset, UK), 50 units/ml penicillin and 0·5 mg/ml streptomycin. Medium and supplements were obtained from Gibco (Paisley, Renfrewshire, UK). The cells were maintained in a humidified atmosphere of 95% air–5% CO_2 at 37 °C and used for recording during days 1–4 after plating.

Electrophysiological recording

The culture dish was also used as the bath for recording currents. The culture medium was initially replaced with an extracellular recording solution (ERS) containing (in mM): NaCl, 65; choline chloride, 70; KCl, 5; $CaCl_2$, 2; $MgCl_2$, 2; glucose, 5; Na–HEPES, 5; HEPES, 5; $CoCl_2$, 5. The pH was adjusted where necessary to 7·4 by the addition of NaOH. Eight preliminary experiments on the effects of hexanol were carried out in an ERS containing 140 mM-Na^+ and no choline. The pipette contained (in mM): CsF, 100–110 (total Cs^+ = 140 mM); $MgCl_2$, 2; $CaCl_2$, 1; EGTA, 11; NaCl, 27; HEPES, 10. The pH was adjusted to 7·2 (and the total Cs^+ concentration to 140 mM) by the addition of CsOH. *n*-Alkanol solutions were delivered to the bath by gravity and solutions were exchanged by total replacement of the bathing medium. Fifteen to twenty times the bath volume was allowed to run through during each solution change to ensure complete replacement. The time taken for this exchange was more than sufficient to reach a steady current reading.

The whole-cell patch-clamp technique (Hamill, Marty, Neher, Sakmann & Sigworth, 1981) was employed to measure ionic currents using a List EPC-7 amplifier (Darmstadt, FRG). Patch pipettes were pulled from Brand glass haematocrit tubing (Horwell, London, UK), fire-polished and in the majority of experiments used uncoated. (Coating with Sylgard in the conventional manner reduced the fast capacitative transient but had no significant effect on the noise level, the slow transient or the results; we therefore followed the example of Gonoi & Hille (1987) and used uncoated pipettes.) The measured pipette resistance was 1–3 MΩ and the seal resistance 5–40 GΩ. The reference electrode was a blunt-tipped patch pipette filled with pipette solution. Any potential difference between the two electrodes was nulled before seal formation and all potentials quoted are with reference to this zero. Currents were recorded on videotape via a suitably modified Sony PCM 701-ES (HHB Hire & Sales Ltd, London, UK) for subsequent analysis using a Gould 1425 digital oscilloscope (Ilford, Essex, UK). The current amplitude was measured relative to the plateau phase near the end of the test voltage pulse. Examination of leakage-subtracted records showed this to be essentially zero current for pulses around 0 mV. The position of the mid-point of the h_∞ curve was not very sensitive to the choice of the zero-current level (analysis of a particularly leaky record, not used here, showed that V_h varied by less than 2 mV with different zero-current levels). Voltage command protocols were generated by an Amstrad PC1640 microcomputer and applied through a Data Translation DT2801-A interface board (Wokingham, Berks, UK), using software kindly donated by Mr J. Dempster of Strathclyde University.

The cells selected for recording appeared spherical and were *ca* 20 μm in diameter, in an effort to minimize space-clamp problems. However, a significant proportion of cells tested proved to have an inadequate space clamp and were discarded. Compensation for the series resistance between the amplifier and the cell membrane was applied in all experiments. This was usually 70% for control and reversal runs and slightly less (still > 50%) for test runs. The mean control current amplitude (cells pre-pulsed to remove resting inactivation) was 3·4 nA. With 70% compensation of a series resistance of 4 MΩ such current magnitude would lead to a *ca* 4 mV absolute error in the potential. The series resistance is reported to have only a small effect on the position of the h_∞ curve (Goldman & Schauf, 1972). Consistent with this, experiments in which the peak current was reduced by replacing 140 mM-sodium ERS with the normal 70 mM-sodium ERS gave a depolarizing shift of the mid-point of the h_∞ curve of only 1–2 mV.

Experiments were performed at room temperature (23 ± 2 °C). The chemicals used in the

intracellular and extracellular recording solutions were Analar grade from BDH Chemicals (Poole, Dorset, UK) or equivalent from Fluorochem Ltd (Glossop, Derbyshire, UK) and the *n*-alkanols were 99% pure from Sigma.

RESULTS

The control current

The cells were clamped at a holding potential of −60 mV. The steady-state inactivation parameter was measured by a modification of the procedure described by Hodgkin & Huxley (1952). A 200 ms pre-pulse to potentials in the range −140 to 0 mV was immediately followed by a 20 ms test pulse to the potential which evoked the maximum current (usually *ca* 0 mV). h_∞ was then calculated by dividing the current achieved following a given pre-pulse by the maximum test pulse current achieved (this was usually produced by pre-pulses around −110 mV). Checks were made to ensure that the duration of the pre-pulse (200 ms) was sufficient to allow the fast inactivation system to reach a steady state at all pre-pulse potentials. The pre-pulse potential at which h_∞ is 0·5 is termed V_h.

The intracellular and extracellular recording solutions were designed to reduce the contribution of currents other than that carried by sodium to the observed ionic current. Thus intracellular K^+ was replaced by Cs^+ to eliminate potassium currents and Ca^{2+} currents were suppressed by external Co^{2+} and internal F^-. The external Co^{2+} should also reduce the contribution of TTX-insensitive sodium currents (Kostyuk, Veselovsky & Tsyndrenko, 1981). The resulting currents have the form and voltage dependence expected of sodium currents (see Fig. 1), were reduced as expected by partial replacement of the external sodium by choline and were essentially eliminated by 0·3 μM-tetrodotoxin (TTX). The potential for zero current flow moved 13·5 mV ($n = 2$) in the hyperpolarizing direction when the external Na^+ concentration was reduced from 140 to 73 mM. The shift predicted by the Nernst equation for a sodium-selective channel is 16·4 mV. (The potential for zero current flow was not, however, consistent with an intracellular sodium concentration of 27 mM (the pipette concentration), although when the sodium concentration in the pipette was reduced to 10 mM the expected reversal potential was achieved. This may indicate that the normal intracellular Na^+ concentration of these immature DRG cells is lower than that reported, for example, for cells from mature autonomic ganglia (Belluzzi & Sacchi, 1986; Ikeda, Schofield & Weight, 1986; Schofield & Ikeda, 1988).)

Previous studies of the voltage dependence of steady-state inactivation of the sodium current in mammalian DRG neurones have reported a bimodal distribution of V_h values (Kostyuk *et al.* 1981; Petersen, Pierau & Weyrich, 1987), one associated with TTX-sensitive sodium channels and the other with TTX-insensitive channels. Figure 2*A* shows a frequency histogram of control V_h values from the 112 *n*-alkanol experiments reported here. The records were taken within 2 min of achieving the whole-cell configuration. There seems no reason to suggest that the distribution is bimodal and in fact it is satisfactorily fitted by a normal distribution. Figure 2*B* gives the corresponding data for records obtained after recovery from alkanol treatment.

The mean value of V_h for the control data in Fig. 2 is −42·8 mV. The mean value

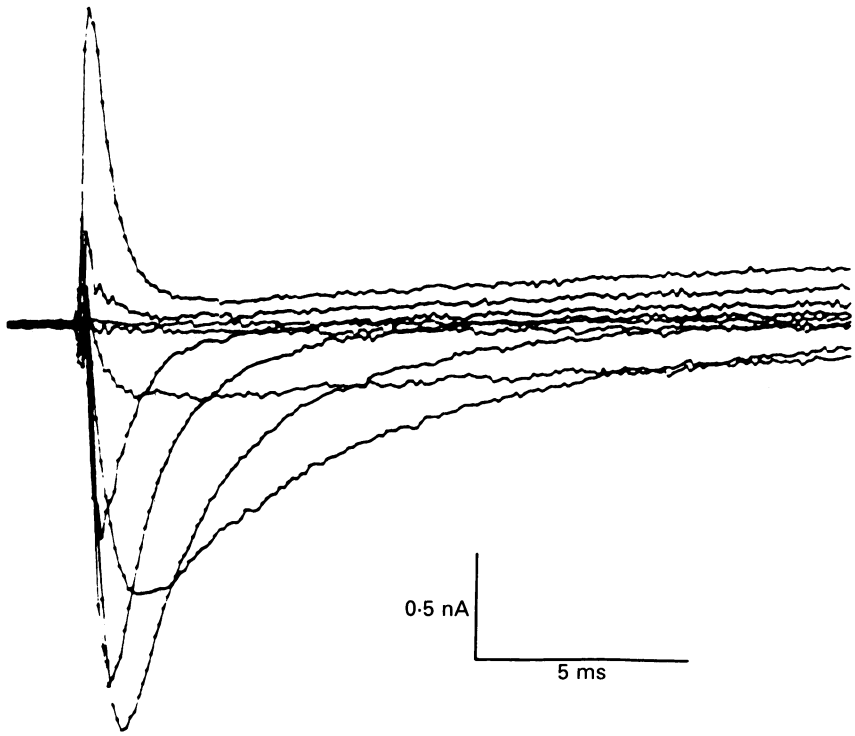

Fig. 1. A family of sodium currents from a rat dorsal root ganglion cell. The holding potential was −60 mV and the various currents were elicited by 20 ms test pulses first to −35 mV and then in 10 mV steps to 45 mV. 'Leakage currents' have been digitally subtracted by adding to each record the current trace produced by an equal hyperpolarizing pulse from −60 mV. Cell 195882.

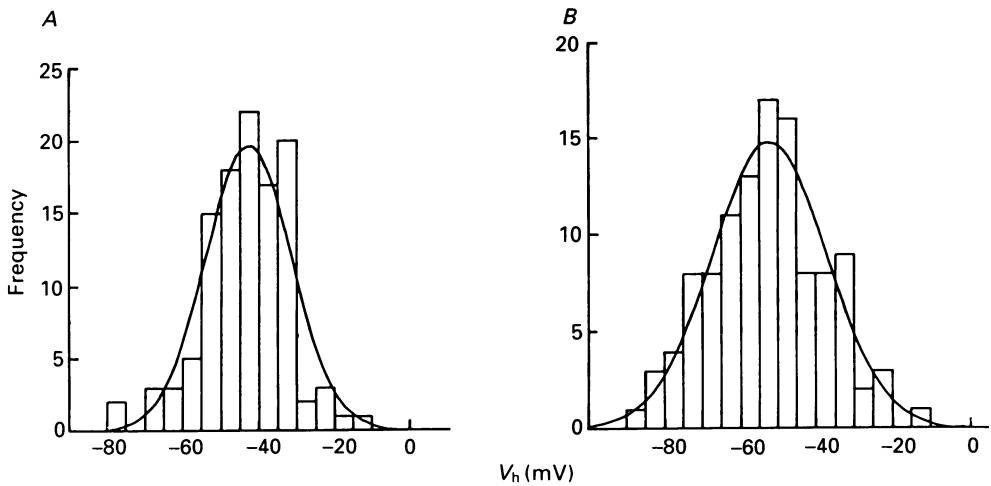

Fig. 2. Frequency distributions of the potential for the mid-point of the h_x curve (V_h) for the 112 control records (*A*) and the corresponding records obtained after recovery from alkanol treatment (*B*). The bin width is 5 mV. The lines indicate a normal distribution.

for the corresponding reversal data is −52·4 mV, almost 10 mV hyperpolarized. A hyperpolarizing drift with time (20–30 min) was found in the absence of treatment with *n*-alkanols and is commonly encountered in whole-cell patch-clamp studies (e.g. Fernandez, Fox & Krasne, 1984). This drift complicates the analysis of alkanol effects on inactivation. One way to avoid such a complication would be to wait in control solution until the control curve had reached a steady position before applying the test solution. However, our hydraulic manipulator was not suitable for such long experiments and we therefore only report those experiments for which we have control, test and reversal records. All effects of the *n*-alkanols have been calculated as test result relative to the mean of the control and reversal results.

Shifts in steady-state inactivation

Figure 3 gives sample results of experiments to investigate the effects of 50 mM-*n*-butanol (*A*) and 5 mM-*n*-hexanol (*B*). Both the current records and the relationships between h_∞ and membrane potential are shown. The reversible reduction of current is obvious, as is the fact that some inhibition remained even when test h_∞ was one. There is also a clear hyperpolarizing shift of the h_∞ curve, which partially reserved (see above). *n*-Butanol, particularly at high concentrations, often caused an increased plateau 'leakage' current as shown in Fig. 3*A*. This was not seen with the other *n*-alkanols.

Figure 4 shows for each *n*-alkanol the relationship between the mean shift in the mid-point of the h_∞ curve (ΔV_h) and the aqueous *n*-alkanol concentration (in mM). Except where indicated the results are averages of at least three experiments and the error bars give the standard error of the mean (S.E.M.). Experimental success rate was relatively low for the highest concentrations of each *n*-alkanol. This was because exposure to high alkanol concentrations, which caused the largest current suppressions and shifts, appeared to increase the probability of losing the cell during the recovery stage. It is therefore possible that the results achieved for the high concentrations resulted from a particularly resistant subpopulation of DRG cells. Figure 5 plots ΔV_h against the aqueous fractional saturation of alkanol. *n*-Alkanol solubilities were taken from Bell (1973) and corrected to 140 mM-salt (a small correction) using the data of Aveyard & Heselden (1975). Equal fractional saturations of *n*-alkanols produce approximately equal shifts in the h_∞ curve, particularly below about 0·07 saturated, but the pentanol data do not show a sharp increase in ΔV_h above 0·07 saturated. The line given in Fig. 5 was fitted by eye and is described by the relationship:

$$y = \frac{9{\cdot}5x^3}{5{\cdot}5 \times 10^{-6} + x^3} + 1{\cdot}7 \times 10^5 x^4,$$

where y is the mean voltage shift and x is the alkanol fractional saturation. This complicated function was chosen because it follows what appear to us to be common features of the data in Fig. 4. These are a sigmoidal rise in effect to a plateau at around −9 to −10 mV, followed by an upturn. The latter phase is seen most clearly in the data for *n*-hexanol and *n*-octanol. A linear fit provides a much simpler mathematical relationship between shift and concentration but we feel that the common deviations from linearity shown by all four *n*-alkanols are unlikely to result

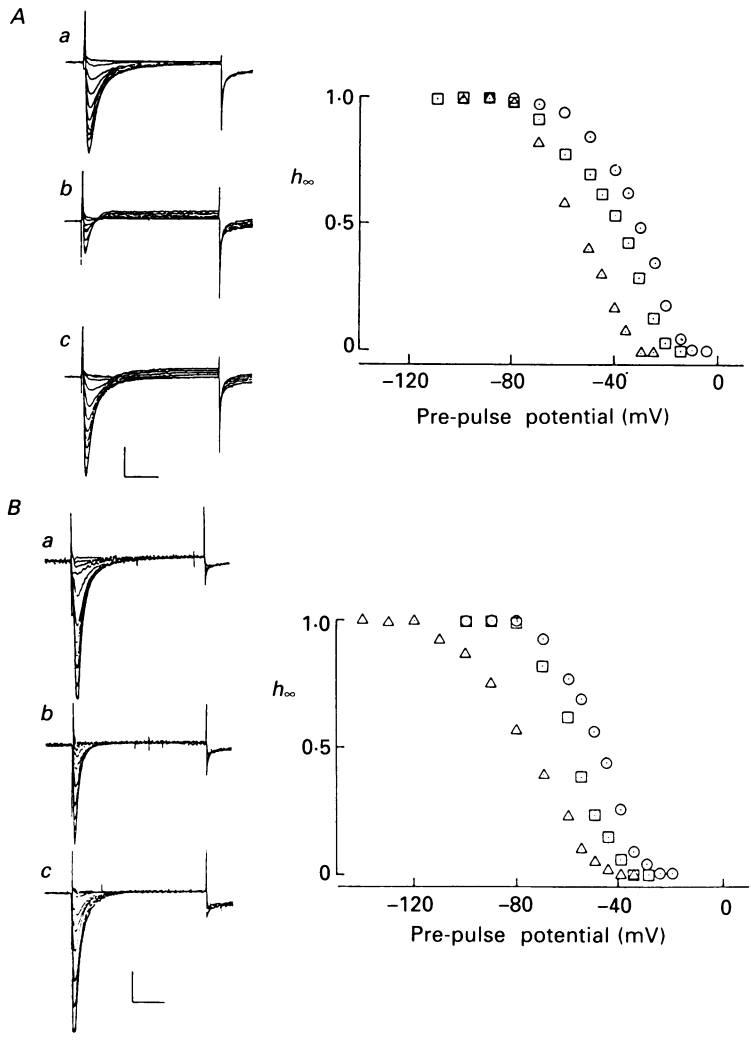

Fig. 3. The effects of 50 mM-*n*-butanol (*A*) and 5 mM-*n*-hexanol (*B*) on sodium current steady-state inactivation. The current traces were obtained under control (*a*) test (*b*) and reversal conditions (*c*). The vertical calibration bars show 1 nA (*A*) and 2 nA (*B*) and the horizontal bars both indicate 5 ms. The butanol experiment (cell 286886) was in 70 mM-sodium ERS and the hexanol experiment (cell 106875) was in 140 mM-sodium ERS. The plots of h_x *versus* membrane potential have been derived from the various current records shown, as explained in the text. ⊙, control; △, test; and ⊡, reversal conditions.

from chance and, therefore, are meaningful. Regression analysis indicates that a function such as that shown in Fig. 5 is a better description of the data than a linear fit, but that is hardly surprising, given five free parameters.

Current reduction

Figure 6 illustrates the influence of the steady-state inactivation system on the reduction of peak sodium current by *n*-alkanols. The ordinate gives the mean current reduction (test relative to the mean of control and reversal, a value of 1 indicating

total suppression) and the abscissa the aqueous n-alkanol concentration on a logarithmic scale. In all cases the test pulse was to the potential required to obtain the maximum current. This was usually moved a few millivolts in the depolarizing direction by n-alkanols but we have not systematically investigated that feature. Each test pulse followed a pre-pulse which was: A, sufficiently negative to remove resting inactivation even in the presence of n-alkanol (i.e. the starting value of h_∞ was one); B, to -60 mV (close to the resting membrane potential in physiological

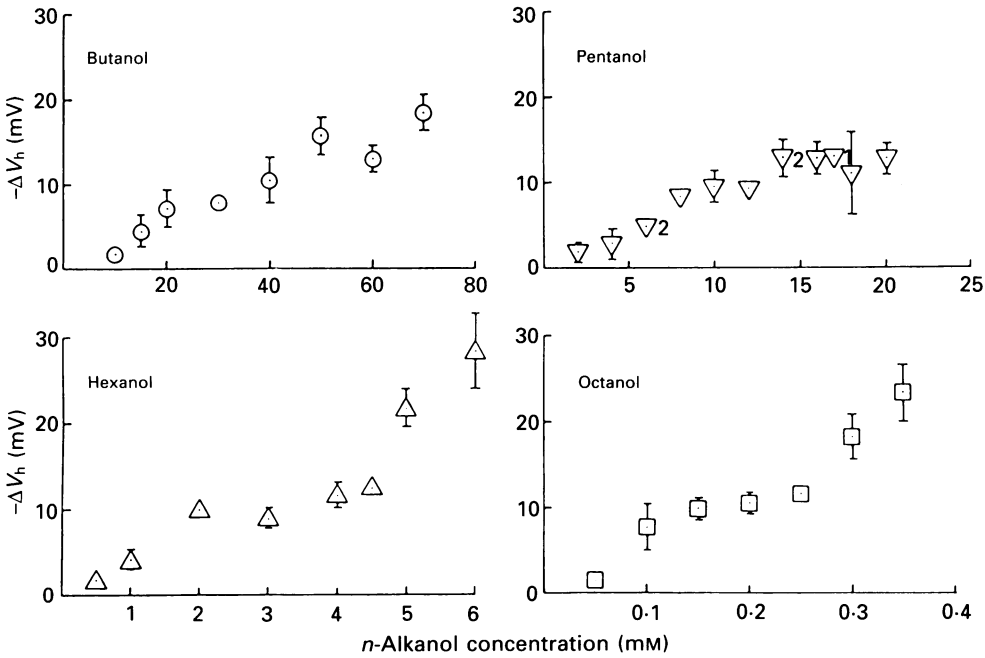

Fig. 4. The n-alkanol-induced shift in the mid-point of the h_∞ curve (ΔV_h) as a function of the aqueous n-alkanol concentration for: ⊙, n-butanol; ▽, n-pentanol; △, n-hexanol; and ⊡, n-octanol. Each point is a mean value from at least three experiments except where indicated by a number beside the point. The error bars give the standard error of the mean ($n \geqslant 3$) or the range ($n = 2$).

solutions (Elliott & McElwee, 1987)); or C, to -40 mV. The results given in Fig. 6A should therefore derive from the simplest mechanism of block, since the effects of all steady-state voltage-dependent processes have been removed. The lines drawn through the data points of Fig. 6A were fitted by eye to the relationship:

$$y = \frac{ax^2}{b+x^2},$$

where y is the mean current reduction and x is the n-alkanol concentration. This has the form of a Hill plot with a coefficient of two (see e.g. Barlow, 1980) and was used to estimate ED_{50} concentrations for the data of Fig. 6A. It was not thought appropriate to apply this analysis to the data of Fig. 6B or C because in those cases the current reduction was caused by a combination of the inactivation shift and the voltage-independent block. ED_{50} concentrations for Fig. 6B and C were estimated by linear interpolation.

Effects on the inactivation system may still contribute to the measured current reduction even when the cell has been pre-pulsed to remove steady-state inactivation. A decrease in the time constant of inactivation (τ_h) at the test pulse potential will reduce the peak current achieved. The effects of *n*-alkanols on the time course of inactivation of rat DRG sodium currents appeared relatively poorly reproducible and small, compared with the values for ΔV_h and peak current reduction, and were not pursued.

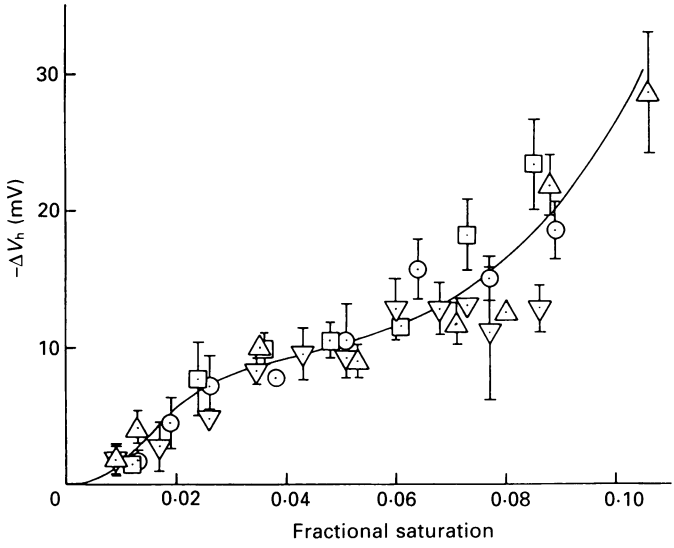

Fig. 5. The shift in the mid-point of the h_∞ curve (ΔV_h) as a function of the fractional saturation of *n*-alkanol. ⊙, *n*-butanol; ▽, *n*-pentanol; △, *n*-hexanol; ⊡, *n*-octanol. The line indicates the relationship:

$$y = \frac{9{\cdot}5x^3}{5{\cdot}5 \times 10^{-6} + x^3} + 1{\cdot}7 \times 10^5 x^4,$$

where y is the mean shift and x the alkanol fractional saturation. It has been fitted to the data points by eye.

As the pre-pulse potential was made more positive, the ED_{50} concentration for current suppression declined. Thus for *n*-butanol, the ED_{50} values were 40, 25 and 18 mM for pre-pulses $h_0 = 1$ (sufficient to remove resting inactivation), −60 mV and −40 mV, respectively. This is shown in Fig. 7 where the natural logarithm of the ED_{50} concentration is plotted against the number of methylene groups for each *n*-alkanol and pre-pulse. For a given pre-pulse the ED_{50} values decline by an approximately equal factor for each additional methylene group. The slope of the relationship between ln ED_{50} and chain length can be interpreted as indicating the standard free energy of transfer of methylene groups from the aqueous phase to a 'site of action'. This involves the assumptions that each component part of the *n*-alkanol molecule acts independently and that equal effect is produced by an equal number of *n*-alkanol molecules at the site of action (for a full discussion, see Haydon & Urban, 1983*b*). The free energies thus calculated are $-3{\cdot}05 \pm 0{\cdot}15$, $-2{\cdot}95 \pm 0{\cdot}18$ and $-2{\cdot}98 \pm 0{\cdot}05$ (S.E.M.) kJ/mol for the pre-pulses $h_0 = 1$, −60 mV and −40 mV, respectively. These are essentially identical and are very similar to the value of −3·2 kJ/mol calculated for the effects on steady-state inactivation.

DISCUSSION

Shifts in the voltage dependence of h_∞

The main finding of this investigation is that *n*-alkanols do produce large hyperpolarizing shifts in the relationship between h_∞ and membrane potential for the

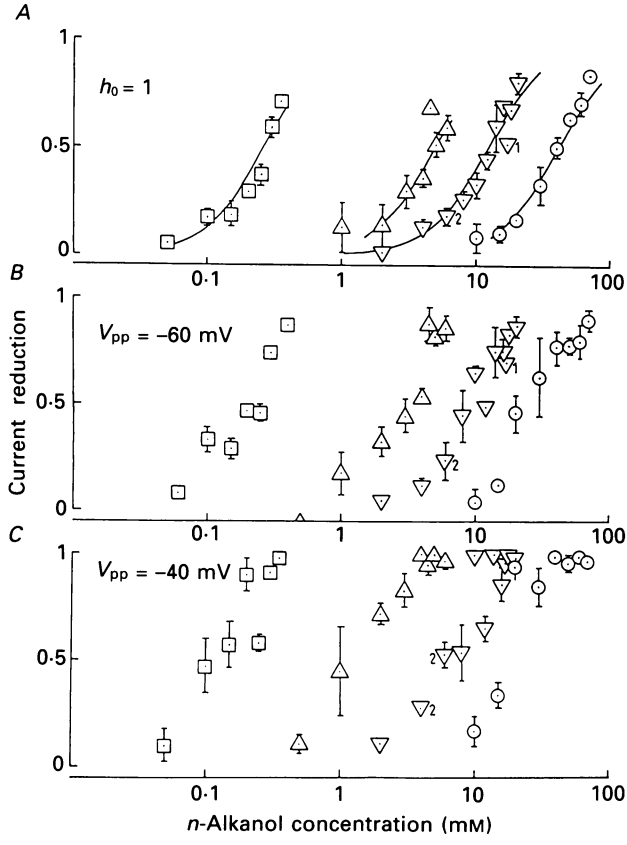

Fig. 6. The reduction of the sodium current by *n*-alkanols in cells pre-pulsed sufficiently to remove resting inactivation (*A*, $h_0 = 1$), to −60 mV (*B*) and to −40 mV (*C*). The symbols, error bars and numbers have been explained in Fig. 4. The lines shown in *A* indicate the function:

$$y = \frac{ax^2}{b + x^2},$$

where y is the mean current reduction (1 indicates total suppression) and x is the concentration of *n*-alkanol. These have been fitted to the data points by eye.

rat DRG cell sodium current. In that respect the response of these mammalian sodium channels to simple anaesthetics is distinctly different from that of squid axon sodium channels (Armstrong & Binstock, 1964; Oxford & Swenson, 1979; Haydon & Urban, 1983*b*) but resembles that of frog (Venitz & Schwarz, 1980; Hirche, 1985) and crayfish (Swenson & Narahashi, 1980) channels.

It seems improbable that *n*-alkanols affect the voltage dependence of h_∞ by

thickening the neuronal membrane. *n*-Alkanols do not increase the thickness of artificial pure lipid bilayers (Elliott & Haydon, 1979, 1984) and while one could propose a local thickening of the protein-containing nerve membrane, driven by hydrogen bond formation between the alkanol hydroxyl and some group on the

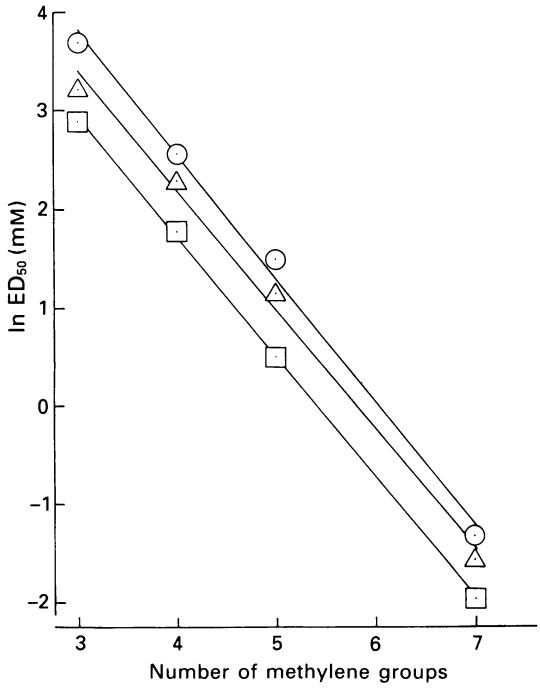

Fig. 7. The relationship between the natural logarithm of the ED_{50} concentration for current reduction interpolated from the data in Fig. 6 and the number of methylene groups in the *n*-alkanol. ⊙, $h_0 = 1$; △, pre-pulse potential = −60 mV; ⊡, pre-pulse potential = −40 mV. The lines shown are linear regression lines ($y = a + bx$) through the data points and have the following characteristics: ⊙, $a = 7{\cdot}56$, $b = -1{\cdot}25$, $r^2 = 0{\cdot}995$; △, $a = 7{\cdot}03$, $b = -1{\cdot}21$, $r^2 = 0{\cdot}993$; ⊡, $a = 6{\cdot}62$, $b = -1{\cdot}22$, $r^2 = 0{\cdot}999$.

channel protein, as suggested previously for the carboxylic esters (Haydon & Urban, 1983*b*; Elliott, Haydon & Hendry, 1984; Elliott, Murrell & Haydon, 1987), there are indications that such is not the case. A thickening hypothesis predicts that the shift produced should be proportional to the *volume* of anaesthetic adsorbed, not the *number* of molecules at the site. Thus for a constant number adsorbed, large molecules should be more effective than small ones, up to the maximum size the site accommodates when the effect declines or shows a cut-off. This increase then decrease in effectiveness with chain length is shown by methyl *n*-alkyl carboxylic esters in the chain length range methyl propionate to methyl nonanoate, with maximum effect at methyl pentanoate (Elliott *et al.* 1987). The data of Fig. 5, however, which show equal ΔV_h for equal fractional saturations of *n*-alkanols (with the possible exception of *n*-pentanol at fractional saturations greater than 0·07), suggest that the volume of *n*-alkanol adsorbed is not an important factor. Moreover, a simple quantitative theory of thickness-induced shifts predicts that the slope at the

mid-point of the h_∞ curve should be reduced by the anaesthetic (Haydon & Urban, 1983*a*; Haydon, Elliott & Hendry, 1984). The *n*-alkanols had no consistent effect on the slope of the rat sodium current h_∞ curve.

Hille (1977) proposed that anaesthetics increase resting inactivation by binding to a specific site on the sodium channel. The binding energy to the inactivated form was suggested to be much larger than to the resting state and so binding promoted the inactivated state. This basic model can be used in conjunction with the Law of Mass Action to predict the form of the relationship between ΔV_h and anaesthetic concentration. Bean, Cohen & Tsien (1983) analysed the effects of lidocaine on rat cardiac sodium channels and, by estimating dissociation constants for the resting and inactivated states, fitted a hyperbolic function to their data relating the shift in the mid-point of the h_∞ curve to the concentration of lidocaine. Meeder & Ulbricht (1987) used the same model to describe the effects of benzocaine on the h_∞ curve of frog sodium channels. However, the relationships shown in Figs 4 and 5 are clearly not hyperbolic. There is a plateau region (at *ca* −10 mV) for all four *n*-alkanols but the approach to that plateau is too shallow for a rectangular hyperbola. Furthermore, the data for *n*-hexanol and *n*-octanol at least show a very clear rise in effect after the plateau.

All four *n*-alkanol dose–response curves may be fitted by the type of function which is given in Fig. 5 (but see the Results section). In terms of this model, there are two distinct phases. The first is a sigmoidal phase, saturating at around −10 mV and (in terms of the fit) involving the binding of three alkanol molecules to a saturable site. This represents a high-sensitivity phase, with half-maximal effect at 0·018 saturated *n*-alkanol solution. The second, low-sensitivity phase has been modelled by a Freundlich isotherm (originally proposed to describe adsorption to a bulk phase such as charcoal, see e.g. Barlow (1980)), and perhaps represents a non-saturable, more diffuse site of action. The data are more scattered in this phase but there is reasonable agreement. The suggestion, therefore, is of two distinct sites of action, but both of them hydrophobic in nature.

It is tempting to label the first a 'protein site' and the second a 'lipid site' but there are at present insufficient data available concerning the concentration dependence of *n*-alkanol effects on lipid phases to allow more than speculation as to the mechanism of the putative second effect. It has been suggested that alterations in surface tension or membrane dipole potential could affect steady-state gating parameters (Haydon & Urban, 1983*b*). Consistent with this, *n*-alkanols have been shown to increase the surface tension of lipid phases (Elliott & Haydon, 1979; Elliott, 1981) and to have the required effects on the dipole potential of lipid monolayers (Bangham & Mason, 1979; Haydon & Elliott, 1986). However, the effects of octanol on surface tension (Elliott & Haydon, 1979) and butanol on dipole potential (Bangham & Mason, 1979) do not show a sharply upwards-curving concentration dependence and the actions of the other alkanols are not well documented. In fact, the only well-documented action of simple anaesthetics on lipid phases which does give an upwards-concave concentration dependence is the thickening of lipid bilayers by hydrocarbons (Haydon, Hendry, Levinson & Requena, 1977; Elliott, Haydon, Hendry & Needham, 1985). But the arguments against a thickness hypothesis for the actions of *n*-alkanols have been given above.

Current reduction

The ED_{50} values for peak current inhibition interpolated from the data in Fig. 6*A* (i.e. where $h_0 = 1$) are very similar to those reported by Haydon & Urban (1983*b*) for the squid axon sodium current (also for $h_0 = 1$). Thus for pentanol the rat value was 13 mM and the squid 14·8 mM, for hexanol the rat gave 4·5 mM and the squid 3·5 mM and for octanol the corresponding values were 0·27 and 0·29 mM. Naturally, the calculated values for the standard free energy per methylene group for transfer from the aqueous phase to a site of action are also very similar, being −3·05 kJ/mol for the rat (at *ca* 23 °C) and −3·04 kJ/mol for the squid (at *ca* 6 °C). A virtually identical standard free energy (−3·03 kJ/mol at *ca* 21 °C) has recently been reported for the inhibition by *n*-alkanols of sodium flux through veratridine-stimulated lobster sodium channels in a vesicle preparation (Rodriguez, Villegas & Requena, 1988).

The current reduction seen when $h_0 = 1$ may still result from more than one action on the sodium channel (see Results) but it is mechanistically less complex than that achieved with more positive pre-pulse potentials, where the h_x effect contributes. The concentration dependence of the current reduction in Fig. 6*A* is not satisfactorily fitted by a model assuming 1:1 binding of *n*-alkanol to a site on the channel, as has been suggested to account for the effects of the neutral local anaesthetic benzocaine on amphibian sodium current (Århem & Frankenhaeuser, 1974; Khodorov, Shishkova, Peganov & Revenko, 1976; but see Meeder & Ulbricht, 1987, for an alternative view), but rather indicates the involvement of more than one molecule of *n*-alkanol, possibly two. Paternostre, Pichon & Dupeyrat (1983) have suggested that the concentration dependence of the reduction of squid sodium current by *n*-alkanols is consistent with a mechanism requiring the binding of two molecules of alkanol per channel. The effects of another class of surface-active compound, the carboxylic esters, on the squid axon sodium current may also suggest the participation of two molecules of anaesthetic (calculations performed on the data of Elliott *et al.* 1987). It has even been proposed that the action of TTX on the fast sodium current of frog nerve fibres involves two molecules (Benoit & Dubois, 1985).

Concluding remarks

This investigation has shown that the effects of *n*-alkanols on rat sodium channels are in some respects comparable to those seen in the squid (similar ED_{50} values for current suppression when $h_0 = 1$ and an almost identical relationship between ED_{50} and chain length) but that there is one very important difference. *n*-Alkanols can produce a large increase in the resting inactivation of the rat (and frog) sodium current, but this is not seen in the squid. However, the concentration dependence of the shift in V_h for the rat sodium current (Fig. 5) suggests that the origin of this species differences is not simply that the rat sodium channel has a single *n*-alkanol binding site which is absent in the squid. There are apparently two sites of action, one showing saturation and the other not, which may reflect a difference in both the protein and the lipid portions of the cell membrane.

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