J. Membrane Biol. 95, 143–149 (1987)

The Journal of
Membrane Biology

Local Anesthetic Action of Carboxylic Esters: Evidence for the Significance of Molecular Volume and for the Number of Sites Involved

J.R. Elliott†, R.D. Murrell‡, and D.A. Haydon‡

‡Physiological Laboratory, Cambridge CB2 3EG, United Kingdom, †Department of Physiology, The University, Dundee DD1 4HN, United Kingdom, the Laboratory of the Marine Biological Association, Plymouth PL1 2PB, United Kingdom

Summary. The effects of the homologous series of carboxylic esters, methyl propionate to methyl decanoate, on the steady-state inactivation of the sodium current in squid axons have been studied. The esters moved the relationship between the inactivation parameter, h_∞, and the membrane potential in the hyperpolarizing direction, thus reducing the number of sodium channels available at the resting potential. The concentration dependence of the shift at the mid-point of the curve of h_∞ against potential has been measured for all esters except decanoate, which was almost inactive. Two aspects of these concentration dependences suggest that molecular volume is an important determinant of the effectiveness of each ester. Firstly, there is a sharp decline in activity above methyl hexanoate. This cut-off in activity resembles that for hydrocarbons where it has been suggested [e.g., Haydon, D.A., Urban, B.W. 1983) *J. Physiol. (London)* **341**:411–427] to a result from a decrease in uptake with increasing molecular volume. (Further data for the hydrocarbons *n*-butane to *n*-heptane are reported here.) Secondly, the smallest compounds, methyl propionate and methyl butyrate, are less effective than would be predicted if equal membrane concentrations of each ester produced the same shift. The aqueous concentration dependences for these esters indicate that below methyl hexanoate, as the series is descended, progressively higher membrane concentrations are required to produce a given shift. This would be expected if the volume of ester in the membrane, rather than the number of molecules, is important.

Differences between the effects of the ester series on steady-state inactivation and on the reduction of the peak sodium current suggest that, in the unclamped squid axon, excitability is influenced by at least two distinct mechanisms in which at least two sites of action are involved.

Key Words anesthetic mechanisms · local anesthesia · peripheral nerve · sodium current · inactivation · anesthetic esters

Introduction

A major factor in the local anesthetic action of carboxylic esters (which include the clinical local anesthetic benzocaine) is their ability to increase the level of inactivation in the resting axon (Elliott, Haydon & Hendry, 1984). This is of particular interest since, although the esters are strongly polar (or amphipathic) molecules, their influence on the steady-state inactivation (h_∞) is very similar to that of the entirely nonpolar hydrocarbons (Haydon & Urban, 1983*a*; Elliott et al., 1985). There is now considerable evidence that the hydrocarbons are absorbed into the interior of the axonal membranes and that the resulting increase in membrane thickness accounts for the negative shift in h_∞ (Haydon & Urban, 1983*a*; Haydon, Elliott & Hendry, 1984; Hendry, Elliott & Haydon, 1985).

It is obviously tempting to suppose that the esters act in a similar manner but, unlike the hydrocarbons, there is no direct evidence that these molecules increase the membrane thickness. Moreover, since the esters are amphipathic in character, it is not immediately clear why they should accumulate in the interior of membranes in the same way as hydrocarbons. The answer to this problem may lie in the properties of the carbonyl group, the presence of which has been shown to enhance greatly the capability of polar molecules to shift the h_∞ curve (Khodorov, 1980; Elliott et al., 1984; J.R. Elliott, D.A. Haydon & B.M. Hendry, *submitted*). Thus, through its exceptionally strong hydrogen-bonding capacity it is possible that the ester interacts with a group on the sodium-channel protein and that this increases its tendency to partition into an otherwise nonpolar or alien environment. The result could then be a local increase in membrane thickness and a concomitant shift in h_∞. The difficulty is to find direct evidence for such a mechanism since thickness changes strongly local to the sodium-channel protein would be too small to be detectable by the capacity techniques used for the more general changes apparently produced by the hydrocarbons. There are, however, other approaches to the problem. One of these is to compare the effects on the steady-state inactivation of homologous series of hydrocarbons and carboxylic esters. If the mechanism proposed for the esters is

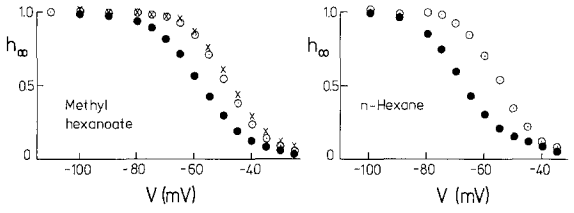

Fig. 1. The effects of 2.06 mM methyl hexanoate and 61 μM (0.95 saturated) *n*-hexane on the steady-state inactivation (h_∞) parameter of the sodium current of the squid giant axon. ⊙, control; ●, test; ×, recovery. Voltage-gated potassium currents were suppressed with 1 mM 3,4-diaminopyridine. Tetrodotoxin-insensitive currents have been subtracted

correct, the results should show the following features: (i) for the shorter chain homologues at comparable membrane uptakes (i.e., similar thermodynamic activities) the shift in the steady-state inactivation curve should increase with increasing chain length because the shift is believed to depend on the *volume* increase per unit area (or thickness) of the membrane, not on the number of anesthetic molecules, and (ii) towards the upper end of the chain-length range the inactivation shift should decline to zero since, by analogy with the hydrocarbons, the uptake should fall to zero as the molecular size of the anesthetic increases. Furthermore, a comparison of the steady-state inactivation shifts as a function of ester chain length, with the corresponding reductions in the peak sodium current where inactivation has been removed by hyperpolarizing prepulses, should provide evidence as to whether or not the same site of action is likely to be involved in the two processes.

In order to interpret the data for the carboxylic esters, results already published for hydrocarbons have had to be supplemented by further determinations of shifts in the steady-state inactivation for the alkanes *n*-butane to *n*-heptane.

Materials and Methods

Methods

Giant axons were dissected from freshly killed specimens of *Loligo forbesi*. The axons had diameters in the range 500–800 μm and were cleaned of connective tissue and surrounding small fibers. The chamber in which the axons were mounted, the arrangement of the electrodes, the means of regulating the temperature and of introducing the control and test solutions have been described in detail previously (Haydon, Requena & Urban, 1980; Haydon & Kimura, 1981; Haydon & Urban, 1983*a–c*). Only intact axons were used. For experiments in the presence of full potassium currents the external bathing solution contained (in mM): 430, NaCl; 10, KCl; 50, $MgCl_2$; 10, $CaCl_2$; and 10, Trizma base plus HCl to give pH 7.6. In some experiments the voltage-gated potassium currents were blocked by external addition of 1 mM 3,4 diamino-pyridine (3,4-DAP), the NaCl concentration reduced to 215 mM and 215 mM choline chloride added. Leakage currents were determined in axons treated with 1 mM 3,4-DAP and 0.3 μM tetrodotoxin (TTX) to suppress sodium currents. The voltage-clamp and data-acquisition procedures were essentially as described by Kimura and Meves (1979). Compensation for the series resistance was >95% in all cases; analysis of the effects of TTX indicate that the maximum error in assessing voltage shifts was ~1 mV. All experiments were carried out at 6 ± 1°C.

Materials

The electrolytes used were of A.R. grade. The liquid hydrocarbons were supplied by Koch-Light Laboratories (Colnbrook, U.K.) and the esters obtained from the same source or from Fluorochem (Glossop, U.K.). These materials were purum or puriss grade. Butane was supplied by Cambrian Gases (Croydon, U.K.) and was of research grade.

Results

The resting potentials of the axons used were between −50 and −60 mV. The steady-state inactivation parameter (h_∞) was determined by applying to the axon a range of 50 msec conditioning potential prepulses followed by a test pulse sufficient to give approximately the maximum inward current. The h_∞ parameter was then calculated as the ratio of the current achieved following a given prepulse potential to that following the most negative prepulse (i.e., that which effectively removed inactivation). Most experiments were carried out in the presence of full potassium currents, which distorted the h_∞ curves at large depolarizations. Comparison of results achieved under such conditions with those in experiments where K currents were suppressed and leakage currents subtracted show the error introduced by maintaining physiological conditions as far as possible was negligible (*see* Fig. 3).

For measurements of ionic current reduction the axons were voltage clamped at −60 mV and the 15-msec depolarizing stimulus pulse was preceded by a 50-msec hyperpolarizing prepulse sufficient to remove resting inactivation in both the control and test cases. The stimulus pulse for the measurement of peak inward current (which was equated with the sodium current) was that which gave the maximum current under control conditions. In all experiments on intact axons, the carboxylic esters were washed out of the axons after the test records had been taken, and further records were obtained some 20 min later, when the currents were again steady with time. This reversal procedure was not always adopted when 3,4-DAP was present since, in order

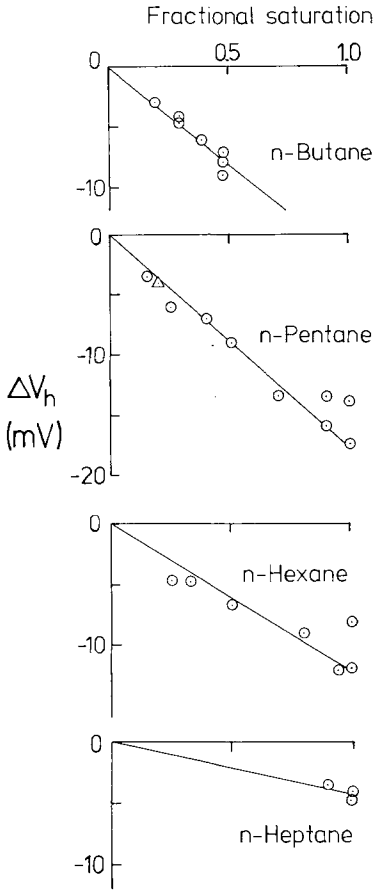

Fig. 2. The concentration dependence of the shift, ΔV_h in the midpoint of the steady-state inactivation curve produced by *n*-alkanes. ⊙, axons with full potassium currents; △, axons treated with 1 mM 3,4-diaminopyridine to suppress voltage-gated potassium currents

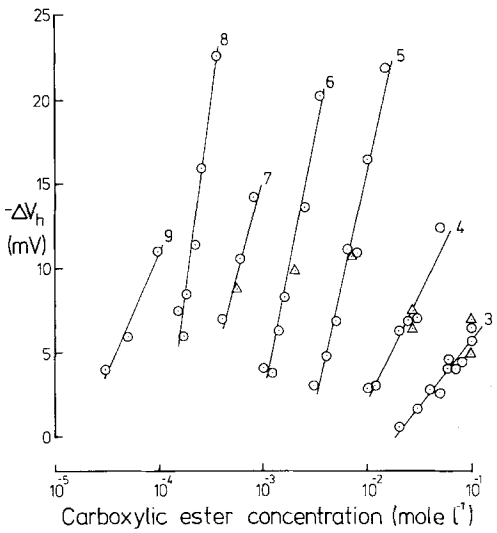

Fig. 3. The shift ΔV_h in the midpoint of the steady-state inactivation curve as a function of methyl ester concentration for methyl propionate (*3*) to methyl nonanoate (*9*). ⊙, axons with full potassium currents; △, axons treated with 1 mM 3,4-diaminopyridine to suppress voltage-gated potassium currents. A liner regression line is drawn through each set of points

to obtain accurate estimates of the leakage currents under test conditions, it was necessary to add TTX immediately after the test records had been taken.

In Fig. 1 are shown typical examples of the effects of hydrocarbons and esters on the steady-state inactivation curves. Somewhat similar results have been shown previously (Haydon & Urban, 1983*a*; Elliott et al., 1984). There is both a shift of the curve (relative to the control) in the hyperpolarizing direction and a decrease in the slope at $h_\infty = 0.5$. Figure 2 shows plots of the shift ΔV_h in the midpoint of the h_∞ curves produced by the various *n*-alkanes at different concentrations (expressed as fractional saturation in artificial seawater). The results for *n*-butane are limited in range by the experimental procedure, which did not permit the use of partial pressures greater than 1 atm. For *n*-heptane, the values of ΔV_h were so small that only results for near saturation were of worthwhile accuracy. N-octane gave still smaller shifts and was not examined in detail.

The corresponding results for the carboxylic esters are shown in Fig. 3, ΔV_h being in this instance plotted against the logarithm of the molar concentration. Methyl decanoate was sufficiently inactive that no useful results were obtained. Methyl acetate was not examined owing to the very high concentrations, and hence nonphysiological osmolarities, involved.

The reduction of the peak Na (or inward) current by the carboxylic esters after steady-state inactivation had been removed by hyperpolarizing prepulses (described above) is shown in Figs. 4 and 5. The points for the Na (as opposed to the inward) current correspond to experiments in which no reversal records were obtained but, as for the h_∞ data, there is no significant discrepancy between the two types of result.

Discussion

It is common to consider the effects of homologous series of substances on biological activity in terms of the concentrations or activities which produce comparable responses. This approach often reveals that, for the lower homologous at least, the concentrations required for a given effect decrease by a constant factor for each additional methylene group in the hydrocarbon chain. The results of this type of analysis will be shown later, but it is immediately obvious from inspection of Fig. 3 that, for the shifts

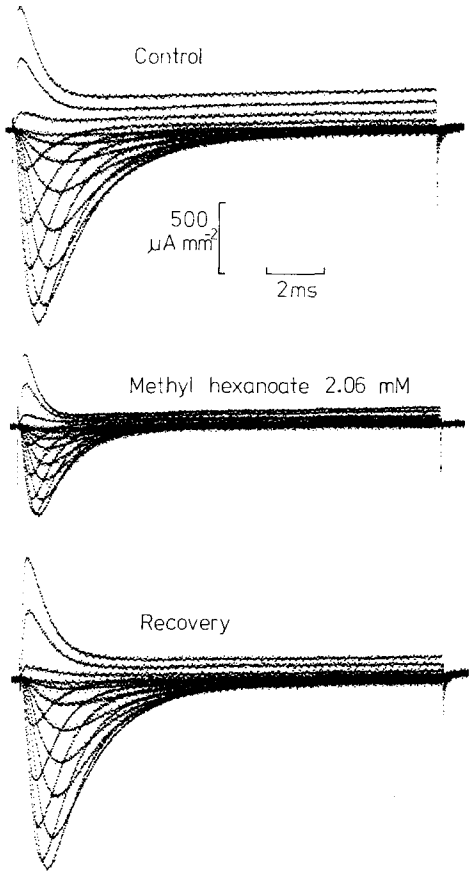

Fig. 4. Voltage-clamp records of sodium currents showing the effect of 2.06 mM methyl hexanoate. The axon was intact but with voltage-gated potassium currents suppressed with 1 mM 3,4-diaminopyridine. The NaCl concentration in the artificial seawater was 215 mM (*see* Methods). TTX-insensitive currents have been subtracted. The holding potential was −60 mV, with 50 msec prepulses to −90 mV. Test pulses were to −40 to −10 mV in 5-mV steps and thence to 70 mV in 10-mV steps

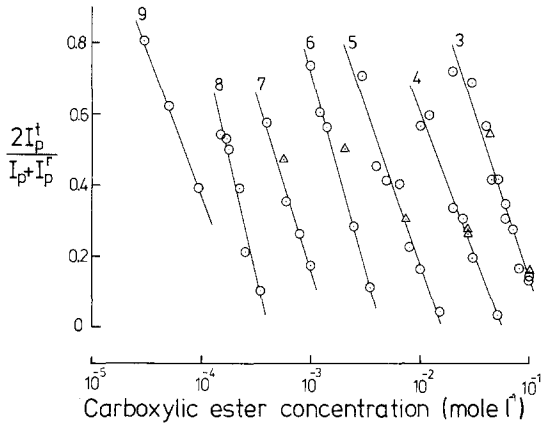

Fig. 5. The fractional reduction in the peak inward current, I_p in voltage-clamped intacts axons as a function of methyl ester concentration for methyl propionate (*3*) to methyl nonanoate (*9*). Superscripts *t* and *r* indicate test and recovery, respectively. No superscript indicates control. ⊙, axons with full potassium currents; △, axons with voltage-gated potassium currents suppressed with 1 mM 3,4-diaminopyridine. A linear regression line is drawn through each set of points

ΔV_h in steady-state inactivation, the plots for the various ester homologues do not form a set of parallel lines and are thus not interpretable in any simple way.

For ΔV_h, therefore, it is proposed to adopt the opposite approach and show how the shifts vary when the concentrations are changed by a constant factor for each successive homologue. These data for both the hydrocarbons and the esters are shown in Fig. 6. Comparison of the two types of anesthetic is complicated by the facts that the hydrocarbons have two methyl groups while the ester chains have only one, and by the molecular volume of a methyl being twice that of a methylene (*see* e.g. Gruen, 1981). For this reason the ΔV_h has been plotted against the equivalent number of methylene groups in the hydrophobic chain, i.e., for *n*-pentane this number is 7 whereas for methyl pentanoate it is 5. These numbers are proportional to the volume of the hydrophobic chain. The shifts, ΔV_h, for the esters have been given for concentrations which decrease by ×4.3 for each additional methylene group. This factor has been chosen on the basis that the partitioning of *n*-alkanes between 0.5 M NaCl and pure alkane changes by ×4.46 per CH_2 (Haydon et al., 1977) and that the corresponding factor for the *n*-alkanols is approximately ×4.25 (Aveyard & Mitchell, 1969; Aveyard & Heselden, 1975). There are no comparable data for the esters, but there is no reason to suppose that the factor in question would be significantly different. It is also unimportant for present purposes as to what value precisely is adopted for this factor. (The absolute values of ΔV_h for the esters relative to the hydrocarbons in Fig. 6 are merely a consequence of the levels of concentration or thermodynamic activity, which it was convenient to select in the two systems; comparisons of the absolute values between the systems are thus not meaningful.)

Both the hydrocarbons and the esters exhibit maxima in ΔV_h and then decline, approaching zero for chain lengths of approximately 9 and 10 equivalent methylene groups, respectively. The hydrocarbons have been more thoroughly investigated than the esters, and it is convenient that they should be discussed first.

The decline in ΔV_h exhibited by the longer chain hydrocarbons appears to be another manifestation of the well-known "cut-off" effect in which, for both general and local anesthesia, the *n*-alkanes be-

come less potent with increasing chain length, and ultimately inactive for homologues with nine or more carbon atoms (Fuhner, 1921; Crisp, Christie & Ghobashy, 1968; Haydon et al., 1977). It has been shown that these cut-off effects are paralleled by the decline in the uptake of the *n*-alkanes by lipid bilayers (Haydon et al., 1977) and, in some respects, by the water-soluble protein luciferase (Franks & Lieb, 1985, 1986; Elliott & Haydon, 1986). A statistical mechanical analysis of alkyl chain energetics in lipid bilayers has been able to account for the cut-off in the uptake of the hydrocarbons by these structures (Gruen, 1981; Gruen & Haydon, 1981). The hydrocarbons, being entirely nonpolar, tend to accumulate preferentially in the hydrophobic interior of membranes and, in doing so, they increase the membrane thickness. For lipid bilayers this is now well established (Hanai, Haydon & Taylor, 1964; McIntosh, Simon & MacDonald, 1980; Dilger, Fisher & Haydon, 1982; Elliott et al., 1985) and for the squid giant axon there are strong indications that the average thickness does increase (Haydon & Urban, 1983*a*, 1985; Elliott et al., 1985). On the basis of these experiments a simple model has been proposed which accounts semi-quantitatively for the shifts ΔV_h produced by the hydrocarbons in the steady-state inactivation curve for the sodium current of the squid axon. Essentially, the increase in thickness at constant membrane potential reduces the internal field in the membrane and this produces the negative shift in the steady-state inactivation (Haydon & Urban, 1983*a*; Haydon et al., 1984). According to this hypothesis the shift ΔV_h should be a linear function of membrane thickness and hence of the volume of the adsorbed hydrocarbon since, in bilayers at least, the uptake of the hydrocarbon has little effect on the area per molecule of the phospholipid. The question then arises as to whether the data in Fig. 6 are consistent with these ideas.

If the membrane were effectively an ideal bulk hydrocarbon phase, for constant fractional saturation (as in Fig. 6), equal *numbers* of hydrocarbon molecules would be adsorbed. As discussed, however, ΔV_h should depend on the *volume* of the hydrocarbon adsorbed. Thus, as the chain length of the alkane increases, the volume of a constant number of molecules would increase linearly with the number of methylene groups and so also should ΔV_h. This tendency is indicated by the dashed line in Fig. 6 which has been constructed relative to *n*-butane. Beyond *n*-pentane, the adsorption cut-off mentioned above, and which arises from the fact that the bilayer does not behave like a bulk phase, appears to predominate and ultimately reduces ΔV_h to zero. The point of interest is that the introduction of the volume concept provides a possible explanation for the otherwise unexpected maximum in the curve.

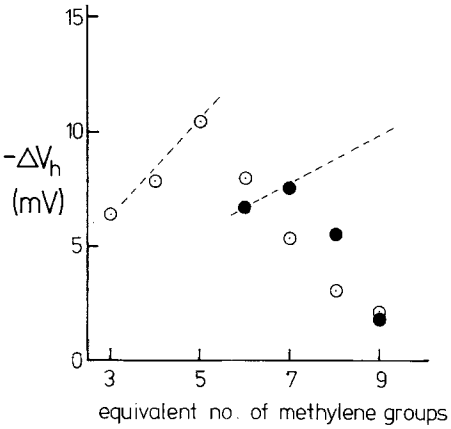

Fig. 6. Shifts ΔV_h in the steady-state inactivation curve produced by homologous series of methyl esters (⊙) and *n*-alkanes (●) at concentrations which decrease by a constant factor for each additional methylene group. For the esters a factor of ×4.3 and a methyl propionate concentration of 120 mM have been chosen. For the hydrocarbons, 0.4 saturation has been chosen, corresponding to a factor of ca. ×4.4. The abscissa gives the number of *equivalent* methylene groups in the hydrocarbon chains of the various substances. This involves counting two CH_2s for one CH_3, and facilitates comparison of the esters and hydrocarbons (*see* text). The two dashed lines indicate the ΔV_h change expected relative to methyl propionate and *n*-butane, respectively, if equal numbers of the different homologues were adsorbed and membrane volume (or thickness) was the significant parameter

The carboxylic esters, unlike the hydrocarbons but like the alcohols, are amphipathic molecules and adsorb preferentially at lipid/water interfaces. It is therefore somewhat surprising that the carboxylic esters not only differ from the alcohols in producing large negative shifts in the steady-state inactivation curve in the squid axon (Armstrong & Binstock, 1964; Haydon & Urban, 1983*b*; Elliott et al., 1984) but, as seen in Fig. 6, their effects closely resemble those of the hydrocarbons. A simple explanation would be that the carboxylic esters accumulate to a significant extent in the hydrophobic interior of the nerve membrane and that this arises from the possibility of strong hydrogen-bond formation with a proton-donor group on the sodium-channel protein (Elliott et al., 1984; Haydon et al., 1986). This effect would obviously be local, and, as mentioned in the Introduction, evidence for it cannot therefore be obtained by capacitance measurements as for the hydrocarbons. However, the similarity of the plots in Fig. 6 suggests that the mechanism of action of the esters has factors in common with that of the hydrocarbons. For example, the decline and cut-off in effectiveness as a function of size of the nonpolar part of the molecule is similar in the two

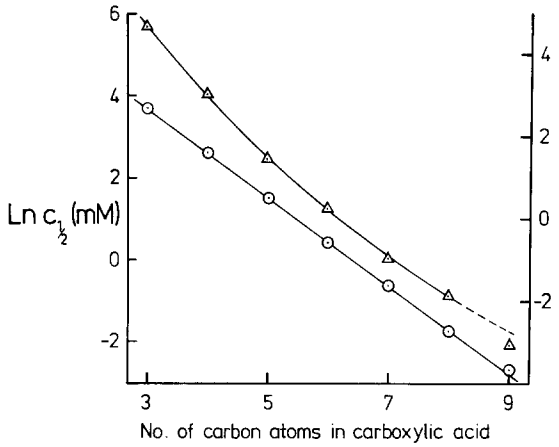

Fig. 7. The chain-length dependences of the concentrations of the methyl ester required to reduce the peak inward current by 50% (⊙, left hand ordinate) and to produce a shift ΔV_h in the midpoint of the steady-state inactivation curve of −6 mV (△, right hand ordinate). A linear regression line ($r^2 = 0.999$) for the former is shown. The curve through the latter points is merely to aid the eye; the point for methyl nonanoate is considered inaccurate

instances and, if the volume of the ester chain is considered, ΔV_h is predicted to increase relative to the propionate value as indicated by the second dashed line. The slope of this line for the esters is steeper than that for the *n*-alkanes, but this arises merely because the alkanes each contain two methyl groups while the esters contain only one. Thus the fractional increase in molecular volume caused by addition of a methylene group is larger for the esters than for the alkanes.

An alternative explanation for the reduced effectiveness of methyl propionate and butyrate relative to pentanoate and hexanoate is that the smaller esters fail to adsorb or have a reduced adsorption at the site of action. However, quite apart from the absence of a physical basis for such an explanation, inspection of Figs. 5 and 7 shows that the propionate and butyrate are as effective as the longer homologues in reducing the peak sodium current in axons where steady-state inactivation has been removed by hyperpolarizing prepulses. It is clear, therefore, that the lower chain-length esters do adsorb to some site in the axonal membrane.

The above evidence for the significance of molecular volume is of particular interest in view of the suggestion of Mullins (1954) that this factor could be important in anesthesia.

Comparison of the effects of the ester series on steady-state inactivation and on peak inward current reduction in prepulsed axons reveals significant differences. Unlike the inactivation results (Fig. 3), the data for the current reduction by the shorter chain homologues (Fig. 5) consists of a set of parallel, equally spaced lines. The concentrations of the various homologues which give 50% reduction in peak current are shown in Fig. 7. The linearity of the plot is consistent with a mechanism in which equal *numbers* of adsorbed molecules have equal effects (*see* e.g. Ferguson, 1939, or Haydon & Urban, 1983*b*). The comparable plot for equal effects on the steady-state inactivation is also shown. As was obvious from Fig. 3, this plot is not linear. The simplest explanation for these observations is that the reduction in excitability in the unclamped squid axon by the carboxylic esters occurs by at least two distinct mechanisms in which at least two sites of action are involved.

From the slope of the plot for peak current reduction in Fig. 7 and the *assumption* that equal numbers of molecules are adsorbed for each homologue, a standard free energy of adsorption per methylene group of approximately −2.4 kJ/mole may be calculated. This may be compared with the value of −3.04 kJ/mole per CH_2 for the effect of *n*-alkanols on peak current reduction in voltage-clamped squid axons (Haydon & Urban, 1983*b*). The difference is significant but the explanation is not known.

The authors wish to thank Mr. Eric Maskell and Mr. Peter Webb for valuable technical assistance. J.R.E. acknowledges support from the Medical Research Council and R.D.M. holds a Science and Engineering Research Council Studentship.

References

Armstrong, C.M., Binstock, L. 1964. The effects of several alcohols on the properties of the squid giant axon. *J. Gen. Physiol.* **48**:265–277

Aveyard, R., Heselden, R. 1975. Salting-out of alkanols by inorganic electrolytes. *J. Chem. Soc. Faraday I,* **71**:312–321

Aveyard, R., Mitchell, R.W. 1969. Distribution of *n*-alkanols between water and *n*-alkanes. *Trans. Faraday Soc.* **65**:2645–2653

Crisp, D.J., Christie, A.O., Ghobashy, A.F.A. 1968. Narcotic and toxic action of organic compounds on barnacle larvae. *Comp. Biochem. Physiol.* **22**:629–649

Dilger, J.P., Fisher, L.R., Haydon, D.A. 1982. A critical comparison of electrical and optical methods for bilayer thickness determination. *Chem. Phys. Lipids* **30**:159–176

Elliott, J.R., Haydon, D.A. 1986. Mapping of general anaesthetic target sites provides a molecular basis for cutoff effects. *Nature (London)* **319**:77–78

Elliott, J.R., Haydon, D.A., Hendry, B.M. 1984. Anaesthetic action of esters and ketones: Evidence for an interaction with the sodium channel protein in squid axons. *J. Physiol. (London)* **354**:407–418

Elliott, J.R., Haydon, D.A., Hendry, B.M., Needham, D. 1985. Inactivation of the sodium current in squid giant axons by hydrocarbons. *Biophys. J.* **48**:617–622

Ferguson, J. 1939. The use of chemical potentials as indices of toxicity. *Proc. R. Soc. London B* **127**:387–404

Franks, N.P., Lieb, W.R. 1985. Mapping of general anaesthetic target sites provides a molecular basis for cutoff effects. *Nature (London)* **316**:349–351

Franks, N.P., Lieb, W.R. 1986. Mapping of general anaesthetic target sites provides a molecular basis for cutoff effects. *Nature (London)* **319**:77–78

Fuhner, H. 1921. Die narkotische wirkung des benzins und seiner bestandteile (pentan, hexan, heptan, octan). *Biochem. Z.* **115**:235–261

Gruen, D.W.R. 1981. A mean-field model of the alkane-saturated lipid bilayer above its phase transition: I. Development of the model. *Biophys. J.* **33**:149–166

Gruen, D.W.R., Haydon, D.A. 1981. A mean-field model of the alkane-saturated lipid bilayer above its phase transition: II. Results and comparison with experiment. *Biophys. J.* **33**:167–188

Hanai, T., Haydon, D.A., Taylor, J. 1964. An investigation by electrical methods of lecithin-in-hydrocarbon films in aqueous solution. *Proc. Soc. London A* **281**:377–391

Haydon, D.A., Elliott, J.R., Hendry, B.M. 1984. Effects of anesthetics on the squid giant axon. *Curr. Topics Membr. Transp.* **22**:445–482

Haydon, D.A., Elliott, J.R., Hendry, B.M., Urban, B.W. 1986. The action of nonionic anesthetic substances on voltage-gated ion conductances in squid giant axons. *In:* Molecular and Cellular Mechanisms of Anesthetics. S.H. Roth and K.W. Miller, editors. pp. 267–277. Plenum, New York

Haydon, D.A., Hendry, B.M., Levinson, S.R., Requena, J. 1977. Anaesthesia by the *n*-alkanes. A comparative study of nerve impulse blockage and the properties of black lipid bilayer membranes. *Biochim. Biophys. Acta* **470**:17–34

Haydon, D.A., Kimura, J.E. 1981. Some effects of *n*-pentane on the sodium and potassium currents of the squid axon. *J. Physiol. (London)* **312**:57–70

Haydon, D.A., Requena, J., Urban, B.W. 1980. Some effects of aliphatic hydrocarbons on the electrical capacity and ionic currents of the squid giant axon membrane. *J. Physiol. (London)* **309**:229–245

Haydon, D.A., Urban, B.W. 1983*a*. The action of hydrocarbons and carbon tetrachloride on the sodium current of the squid giant axon. *J. Physiol. (London)* **338**:435–450

Haydon, D.A., Urban, B.W. 1983*b*. The action of alcohols and other nonionic surface active substances on the sodium current of the squid giant axon. *J. Physiol. (London)* **341**:411–427

Haydon, D.A., Urban, B.W. 1983*c*. The effects of some inhalation anaesthetics on the sodium current of the squid giant axon. *J. Physiol. (London)* **341**:429–439

Haydon, D.A., Urban, B.W. 1985. The admittance of the squid giant axon at radio frequencies and its relation to membrane structure. *J. Physiol. (London)* **360**:275–291

Hendry, B.M., Elliott, J.R., Haydon, D.A. 1985. Further evidence that membrane thickness influences voltage-gated sodium channels. *Biophys. J.* **47**:841–845

Khodorov, B. 1980. Drug-induced blockage of gating and sodium currents in myelinated nerve. *In:* Physiology of Excitable Membranes. J. Salanki, editor. Advances in Physiological Science. Vol. 4, pp. 89–99. Pergamon, Oxford

Kimura, J.E., Meves, H. 1979. The effect of temperature on the asymmetrical charge movement in squid axons. *J. Physiol. (London)* **289**:479–500

McIntosh, T.J., Simon, S.A., MacDonald, R.C. 1980. The organization of *n*-alkanes in lipid bilayers. *Biochim. Biophys. Acta* **597**:445–463

Mullins, L.J. 1954. Some physical mechanisms in narcosis. *Chem. Rev.* **54**:289–323

Received 3 September 1986; revised 29 October 1986