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THE ACTION OF HYDROCARBONS AND CARBON TETRACHLORIDE ON THE SODIUM CURRENT OF THE SQUID GIANT AXON

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SUMMARY

1. The effects of the *n*-alkanes propane to hexane, cyclopropane, cyclopentane and cyclohexane and carbon tetrachloride on the ionic currents and electrical capacity of the squid giant axon membrane have been examined.

2. Both the peak inward and steady-state outward currents were reduced reversibly by each substance, though propane at 1 atm had very little effect.

3. The membrane capacity at 100 kHz was reduced by all substances except propane at 1 atm.

4. Na currents were recorded in intracellularly perfused axons before and during exposure to the hydrocarbons and the records were fitted with equations similar to those proposed by Hodgkin & Huxley (1952).

5. Shifts in the curves of the steady-state activation and inactivation parameters (m_∞ and h_∞) against membrane potential, changes in the peak heights of the activation and inactivation time constants (τ_m and τ_h) and reductions in the maximum Na conductance ($\bar{g}_{Na}$) have been tabulated.

6. The effects of the various hydrocarbons and carbon tetrachloride on the parameters of the Hodgkin–Huxley equations suggest that the suppression of the Na current by these substances originates from several different phenomena. The underlying physico-chemical events are considered in the light of the observed capacity changes and of information on artificial pore-containing membranes.

INTRODUCTION

Many simple lipophilic or surface active substances suppress nervous impulse propagation (Seeman, 1972) but few studies have been made of the detailed mechanism of this suppression. Usually investigations have been concerned with one or two substances only and have not been carried out in any great depth. Thus, while a number of facts are available, no very general conclusions can be drawn from them as to the molecular origins of the impulse suppression.

It seems probable from the wide diversity of molecules which evoke similar responses, that no highly specific interactions can be dominant and that the

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mechanisms by which these molecules perturb the Na channel (particularly) must be based upon a small number of physico-chemical principles. In order to be clear about the common features of the mechanisms, however, it is necessary to examine in detail a large range of structurally related substances. This has now been attempted. The results are voluminous and it is hoped to present them in several papers. The first is concerned primarily with hydrocarbons.

For ease of interpretation the hydrocarbons, and other substances (such as carbon tetrachloride) which have a dielectric constant similar to that of the membrane lipid (i.e. ~ 2) are of particular interest. How such substances interact with lipid bilayers (Haydon, Hendry, Levinson & Requena, 1977 and references therein; Gruen & Haydon, 1981) and how they suppress conduction by a well-characterized pore-forming polypeptide (Haydon, 1975; Hendry, Urban & Haydon, 1978) are relatively well understood. In earlier papers the influence of some *n*-alkanes on the action potential, electrical capacity and ionic currents of the squid giant axon membrane were examined (Haydon *et al.* 1977; Haydon, Requena & Urban, 1980; Haydon & Kimura, 1981; Haydon & Hendry, 1982). It was found that *n*-pentane affected the Na current in at least three different ways: (*a*), it shifted the voltage dependence of both the steady-state activation and inactivation in the hyperpolarizing direction; (*b*), it suppressed the maximum membrane conductance, and (*c*), it reduced the time constants for activation and inactivation. Each of these effects was tentatively explained in terms of the adsorption of the alkanes into the lipid regions of the membrane and the consequent perturbations of the channel proteins. Both the shifts in the steady-state parameters and the loss of maximum conductance seemed to be accounted for by a thickening of the bilayer parts of the membrane, the former through the influence of the thickening on the internal electric field, and the latter through a thickness–tension mechanism somewhat analogous to that thought to apply to gramicidin A channels in lipid bilayers. The reduction in the time constants (after allowance for voltage shifts) was accounted for in terms of the increased 'fluidity' which the *n*-pentane was expected to have produced in the chain region of the membrane lipid.

The K current was also shown to be suppressed by the *n*-pentane and the comparable Hodgkin–Huxley parameters were affected in much the same way as for the Na current. In this respect the alkane appeared to act in a non-specific manner.

The results of the above investigation were considered sufficiently promising to justify a systematic examination of a range of hydrocarbons and other substances. In the present paper experiments have been carried out with several straight chain hydrocarbons, cyclic hydrocarbons, including cyclopropane and, because it is almost as non-polar as a hydrocarbon, carbon tetrachloride. Particular attention has been given to the potencies in suppressing Na current, and the extent of the correlation between capacity decrease and the shifts in the steady-state activation and inactivation curves. The correlation in time between the shift in steady-state inactivation and changes in the inactivation time constant on the one hand and the relative suppression of the maximum Na and K conductances on the other, has also been examined.

METHODS

Giant axons were dissected from the mantles of freshly killed *Loligo forbesi*. The axons were thoroughly cleaned of surrounding fibres and connective tissue and had diameters in the range 600–1000 μm.

The chamber in which the axons were mounted, the electrodes, and the means of introducing and controlling the temperature and flow rate of the bathing solutions were as described previously (Haydon *et al.* 1980). Axons were internally perfused by a variation of the Tasaki technique. In order to study the Na currents the perfusate usually consisted of 340 mM-CsF, 400 mM-sucrose, 10 mM-NaCl and 10 mM-HEPES buffer (pH 7·3). The external bathing solution for perfusion experiments consisted of 107·5 mM-NaCl, 322·5 mM-choline chloride, 10 mM-KCl, 10 mM-$CaCl_2$, 50 mM-$MgCl_2$ and 10 mM-Trizma base. The pH was adjusted to 7·6 by addition of HCl. Na currents were suppressed when necessary by the addition of 0·3 μM-tetrodotoxin. For capacity measurements and in most experiments with intact axons, the bathing solution was as above, except that it contained 430 mM-NaCl and no choline chloride.

The voltage-clamp and data acquisition procedures were essentially as described by Kimura & Meves (1979). Compensation for the series resistance was applied in all experiments. Analysis of the Na currents (after subtraction of the tetrodotoxin-insensitive currents) was as described in Haydon & Kimura (1981).

Impedance measurements were carried out at the resting potential as reported in Haydon *et al.* (1980), with the Wayne Kerr Universal (B221) and Radio Frequency (B601) bridges. An inductance which becomes obvious above *ca.* 300 kHz was corrected for in the present analysis of the axon impedances; its effect at 100 kHz was, however, small.

Hydrocarbon and carbon tetrachloride solutions in artificial sea water were prepared at room temperature. The gases propane, cyclopropane and *n*-butane at 1 atm were equilibrated with the artificial sea water for several days in a continuously stirred vessel. The volumes of gas dissolved were estimated by standard volumetric procedures. The final solutions were delivered directly from the preparation vessel into the axon chamber via a heat exchanger. The liquid hydrocarbons and carbon tetrachloride were prepared as saturated solutions by gentle stirring for several days, and diluted for use when required during the experiment in closed delivery vessels above the axon chamber. Care was taken during these procedures to avoid loss of vapour into air spaces. While the concentrations of the gases were directly determined, this could not so readily be done for the liquids. The molar concentrations given in Tables 1–3 for the liquid hydrocarbons have been calculated from the literature values for saturated solutions in water, together with a correction for the influence of 0·5 M-NaCl (see Tanford, 1980). The solubility of carbon tetrachloride in 0·5 M-NaCl was determined gravimetrically. The concentrations quoted are all for room temperature (20 ± 1 °C). The cooling of the solutions prior to their introduction to the axon chamber should not have affected these values since the temperature coefficients of solubility, where they are available (Tanford, 1980), are small and negative. The main uncertainty in the molar concentration should have arisen from the correction for 0·5 M-NaCl, which is 30–40 % in some instances. The fractional saturations should be relatively accurate.

All experiments were carried out at 6 ± 1 °C.

RESULTS

Current suppression

As a preliminary to more detailed measurements, and in order to assess their potency, the hydrocarbons and carbon tetrachloride were introduced in various concentrations to voltage-clamped axons and the amplitudes of the currents observed. The effects on the peak inward and steady-state outward current are shown in Table 1. Superscripts have been used to indicate currents under test (t) and recovery (r) conditions. No superscript has been used for controls. The peak inward currents were obtained from experiments with axons perfused with CsF, as described in Methods, and in bathing solutions containing either 215 mM-NaCl + 215 mM-choline

chloride, or 107·5 mM-NaCl + 322·5 mM-choline chloride. Neither on CsF-perfused, nor on intact axons was any effect of NaCl concentration on the potency of the hydrocarbon discernible. The holding potential in all CsF-perfused axons was −70 mV and the pre-pulse −20 mV for 50 ms. Experiments at higher holding potentials did not yield significantly different results. Determinations of the influence of the hydrocarbons on the peak inward current of intact axons suggested that, at comparable holding potentials, such axons might be a little more sensitive than CsF-perfused axons.

TABLE 1. The effects of hydrocarbons and carbon tetrachloride on the peak inward current I_p^t as determined from CsF-perfused axons, and the steady-state outward current I_{ss}^t in intact axons. I_p and I_{ss} are the corresponding control currents for zero time of exposure to the hydrocarbon. I_p^r/I_p and I_{ss}^r/I_{ss} indicate the extent of recovery after treatment with hydrocarbon. The values quoted are the average for the number of axons shown in parenthesis. I_{ss} and I_{ss}^t were taken after 15 ms test pulses

	Concentration in artificial sea water					
Hydrocarbon	μmol l^{-1}	Fractional saturation	I_p^t/I_p	I_p^r/I_p	I_{ss}^t/I_{ss}	I_{ss}^r/I_{ss}
Propane	933 (1 atm)	0·12	0·92* (1)	0·96 (1)	0·99 (1)	1·2 (1)
n-Butane	675 (1 atm)	0·48	0·97 (2)	0·97 (4)	0·88 (4)	1·07 (4)
n-Pentane	306	1·0	0·65 (4)	0·89 (9)	0·66 (8)	0·94 (8)
	275	0·9	0·76 (5)	0·88 (2)	0·56 (2)	0·79 (2)
n-Hexane	64	1·0	—	—	0·67 (2)	> 0·8 (2)
	61	0·95	0·44 (1)	—	—	—
	58	0·91	1·04 (1)	—	—	—
Cyclopropane	7000 (1 atm)	0·18	0·73 (8)	0·90 (5)	0·89 (7)	1·05 (6)
Cyclopentane	1330	0·7	0·41 (4)	0·89 (3)	0·64 (3)	0·90 (3)
	760	0·4	—	—	0·88 (2)	1·08 (2)
Cyclohexane	379	0·7	0·59 (3)	0·81 (4)	0·76 (4)	0·84 (4)
	216	0·4	—	—	0·89 (2)	0·95 (2)
Carbon	4000	1·0	0·17 (2)	0·86 (6)	0·57 (4)	1·12 (4)
tetrachloride	3000	0·75	0·23 (1)	0·81 (1)	—	—
	1000	0·25	0·53 (5)	0·81 (4)	0·76 (3)	1·08 (3)

* Intact axon; holding potential −60 mV, pre-pulse −20 mV, 50 ms.

In hydrocarbon solutions the changes in the membrane currents tend to flatten off after a period consistent with that expected for the diffusion of the hydrocarbon throughout the axon. However, as mentioned previously (Haydon *et al.* 1980), axons deteriorate in hydrocarbon solutions and a completely time-independent state is not usually observed. To this extent the values given for I_p^t/I_p are somewhat arbitrary but their reliability can be checked by evidence of reversibility. For reasons associated with the determination of leakage currents, attempts were not usually made to reverse CsF-perfused axons, but reversal in similarly treated intact axons, held at −55 to −60 mV, was examined. The recovery in the peak inward current I_p^r/I_p is shown to be generally 80–90 %.

Detailed studies of the K currents in these systems are not yet complete, but the effects of the hydrocarbons on the steady-state outward current I_{ss}^t/I_{ss} are shown. Recoveries I_{ss}^r/I_{ss} are also shown. Values of greater than unity among the latter are often attributable to a rise in the outward leakage current. Holding potentials and

depolarizations in these experiments were usually *ca.* −60 mV and 120 mV respectively. As for *n*-pentane (Haydon & Kimura, 1981), the other hydrocarbons do not produce appreciable droop in the outward currents.

Na currents

Records of inward currents after exposure of an axon to *n*-pentane have been shown previously (Haydon & Kimura, 1981). Many, though not all, of the general features for the other hydrocarbons and carbon tetrachloride were similar. The currents were analysed by means of an equation derived from the relationships proposed by Hodgkin & Huxley (1952), i.e.

$$I_{Na} = I'_{Na}[1-\exp(-t/\tau_m)]^3 [h_\infty(1-\exp(-t/\tau_h))+\exp(-t/\tau_h)]. \quad (1)$$

Since the holding potential was −70 mV and a pre-pulse to −90 mV for 50 ms was applied immediately prior to depolarization, m_0 and h_0, the activation and inactivation parameters for $t = 0$ have been taken as zero and unity respectively. After recording the currents in the hydrocarbon solutions, tetrodotoxin (3×10^{-7} M) was added and the tetrodotoxin-insensitive currents determined. These were subtracted from the test currents and the remaining curves fitted to yield I'_{Na}, τ_m, h_∞ and τ_h. As for *n*-pentane, the tetrodotoxin-insensitive currents were usually quite small ($\lesssim 100$ μA cm^{-2}) in each instance. The determination of τ_h in the vicinity of its peak was achieved essentially as described in Gillespie & Meves (1981). From the holding potential of −70 mV a pulse of 10 ms to −10 mV was applied to inactivate the Na system. The axon was then held for periods Δt of 2, 3, 5, 7 or 20 ms at the membrane potential of interest to remove inactivation and a test pulse to −10 mV given. The peak inward current, I, for each potential reached a maximum value, I_∞, for $\Delta t \geqslant 20$ ms. Plots of ln $(I_\infty - I)$ *vs.* Δt are linear and have a slope of $-1/\tau_h$. It was checked that no significantly different results were obtained for initial pulses of > 10 ms or for values of $\Delta t > 20$ ms. Prior to the above analysis, the tetrodotoxin-insensitive currents were subtracted from the records. The steady-state inactivation parameter, h_∞, was determined by applying 50 ms pre-pulses followed by a test pulse sufficient to give approximately the maximum Na current. Results for selected axons are shown in Figs. 1 and 2 and data for all the experiments are given in Table 2.

The main qualitative features of the effects of the hydrocarbons on the Na currents are illustrated in Figs. 1 and 2. The steady-state inactivation curve is in all instances shifted to more negative potentials. The peak of the inactivation time constant, τ_h, is shifted to a corresponding extent and the peak height tends to be either unchanged or reduced. The steady-state activation curve for all the hydrocarbons except cyclopropane is shifted to more negative potentials, though never to the same extent as is the inactivation curve. Carbon tetrachloride produces no significant shift, while cyclopropane yields a shift to more positive potentials. The activation time constant, τ_m, shifts as does m_∞, and, as for τ_h, the peak height tends to be reduced. The maximum sodium conductance $\bar{g}_{Na}$ is reduced in most instances, the exceptions being *n*-butane and cyclopropane.

Quantitative comparisons of the various shifts and suppressions are difficult to make since the basis for such comparisons is arbitrary. As far as possible, experiments were carried out at about 50 % reduction of the maximum Na current. For *n*-butane

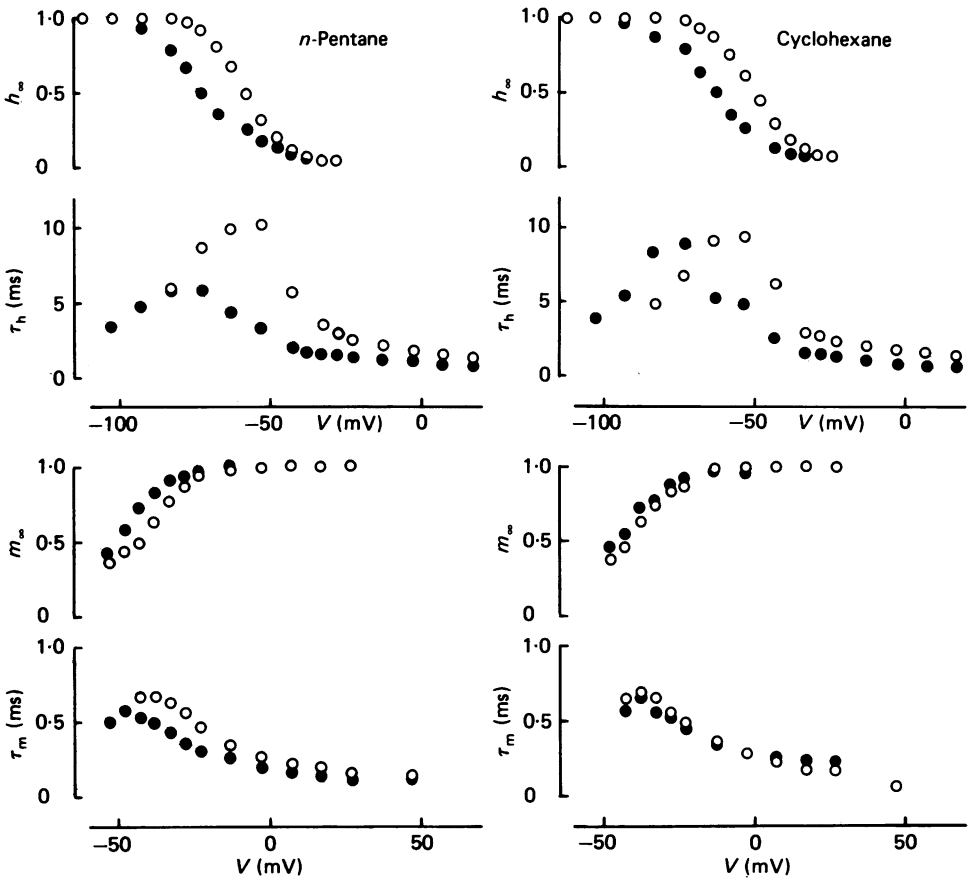

Fig. 1. The influence of 275 μM-*n*-pentane and 379 μM-cyclohexane on some parameters of the Hodgkin–Huxley equations for the Na current. The open and filled circles are for the control and hydrocarbon-affected axons respectively. The latter had been exposed to the hydrocarbon solution in artificial sea water, at a flow rate of approximately 12 ml/min, for about 20 min in each instance. V is the membrane potential. Axons 300 and 310 (see Table 2).

and cyclopropane, however, this level of reduction could not be achieved at the aqueous concentrations in equilibrium with these gases at 1 atm.

Fig. 3 illustrates a comparison of the time courses of the changes in h_∞, τ_h and τ_m for *n*-pentane. A simple normalization procedure, as described in the Figure legend, was used. Comparable experiments with other hydrocarbons yielded similar results.

Measurements of membrane impedance at high frequencies were carried out for most of the test substances. The capacity at 100 kHz was calculated essentially as described in Haydon *et al.* (1980); i.e. impedances were measured for frequencies between 50 and 300 kHz and the series resistance was obtained by extrapolating the impedance locus to infinite frequency (zero reactance). With this series resistance, the membrane capacity was calculated from the impedance data for 100 kHz. The assumptions involved in this procedure have been mentioned in Haydon *et al.* (1980) but some further remarks can now be made. It is clear that the hydrocarbons and carbon tetrachloride cause a membrane impedance change at high frequencies. It was

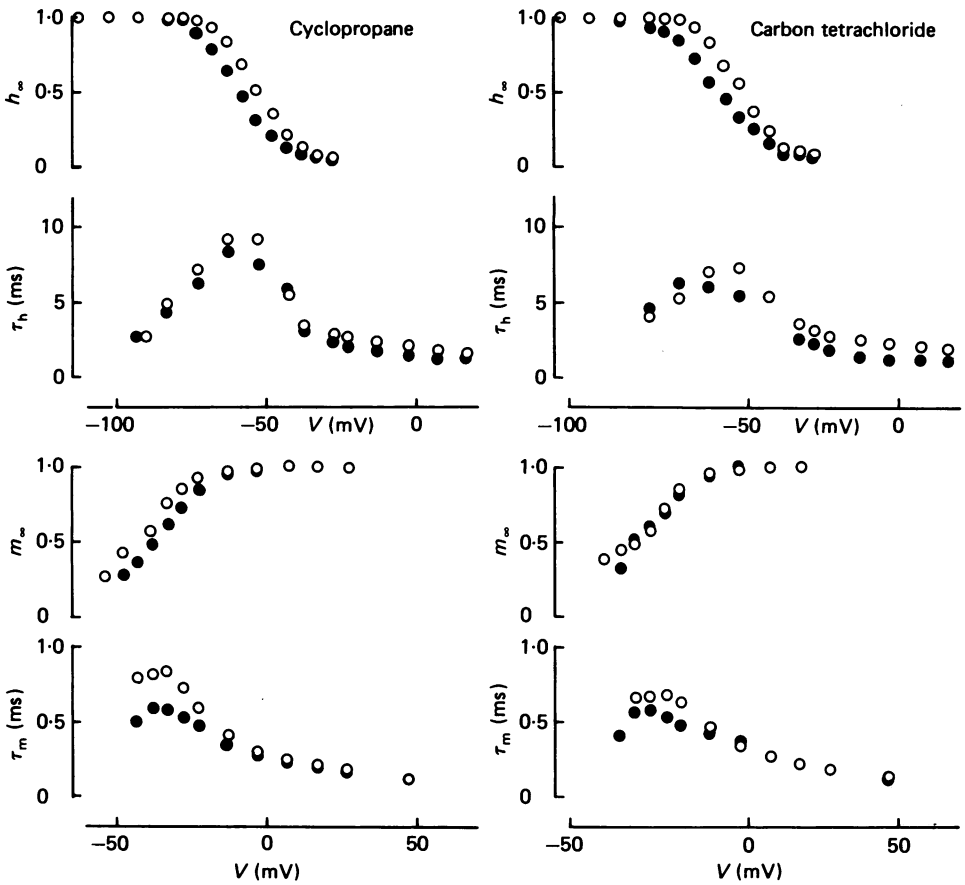

Fig. 2. The influence of 7 mM-cyclopropane and 1 mM-carbon tetrachloride on some parameters of the Hodgkin–Huxley equations for the Na current. The open and filled circles are for the control and hydrocarbon-affected axons respectively. The latter had been exposed to the hydrocarbon solutions, at a flow rate of 12 ml/min for 15–20 min. Axons 318 and 288 (see Table 2).

also found from the use of long hyperpolarizing pulses that the membrane resistance does not change significantly. The series resistance as determined either by extrapolation of the impedance locus (as described above) or as determined under voltage clamp also does not change significantly. The simplest interpretation of the data is therefore that the hydrocarbons produce a capacity change in the axon membrane similar to that observed in lipid bilayers.

The capacities at 100 kHz are shown in Table 3. The changes are usually superimposed on long-term drifts and partly for this reason the reversibility is variable. In each instance, however, there is a significant decrease in the capacity of the axon exposed to hydrocarbon as compared with the average of the capacities before and after exposure.

In earlier papers (Haydon & Kimura, 1981; Haydon *et al.* 1980) it was suggested that the shifts of Hodgkin–Huxley parameters (h_∞ in particular) in a hyperpolarizing direction by *n*-pentane could be accounted for in terms of a thickening of the lipid

TABLE 2. The influence of hydrocarbons and carbon tetrachloride on parameters of the Hodgkin–Huxley equations. I_p^t/I_p gives the reduction in the peak inward current and $\bar{g}_{Na}^t/\bar{g}_{Na}$ is the corresponding reduction in the maximum Na conductance. In columns 6 and 7 are shown the shifts ΔV_h and ΔV_m in the mid-points of the h_∞ and m_∞ curves respectively, while in columns 8 and 9 $[\tau_h^t/\tau_h]_p$ and $[\tau_m^t/\tau_m]_p$ give the changes in the peak height of τ_h and τ_m

Hydrocarbon	Concn. (μmol l^{-1})	Axon	I_p^t/I_p	$\bar{g}_{Na}^t/\bar{g}_{Na}$	ΔV_h (mV)	ΔV_m (mV)	$[\tau_h^t/\tau_h]_p$	$[\tau_m^t/\tau_m]_p$
n-Butane	675	303	0·98	1·0	−8	−6·5	0·81	1·0
	675	312	0·96	1·0	−9	−4·5	0·69	0·96
n-Pentane	306	290	0·60	0·57	−17·5	−6·0	0·50	0·71
	275	298	0·79	0·79	−13·5	−7·0	0·73	0·89
	275	300	0·77	0·71	−15·0	−7·5	0·61	0·85
n-Hexane	58	344	1·04	1·04	−13·0	−7·5	0·68	1·0
	61	349	0·44	0·42	−13·0	−5·0	0·47	0·79
Cyclopropane	7000	162	0·72	0·97	−10·7	2·0	—	—
	7000	225	0·77	1·00	−7·5	4·5	—	0·61
	7000	318	0·93	1·20	−7·0	6·0	0·89	0·73
	7000	319	0·83	1·09	−6·0	5·0	0·68	0·69
Cyclopentane	1330	335	0·43	0·57	−18·5	−7·0	0·81	0·84
	1330	350	0·49	0·61	−17·5	−2·0	0·74	0·55
	1330	419	0·34	0·55	−16·5	−6·5	1·07	0·75
	1330	422	0·36	0·83	−13·5	−3·0	0·78	0·79
Cyclohexane	379	308	0·65	0·79	−14·5	−7·0	1·08	0·82
	379	310	0·51	0·66	−13·0	−3·0	0·93	0·93
	379	321	0·63	0·86	−13·5	−6·5	1·03	0·91
Carbon tetrachloride	1000	287	0·44	0·67	−8·0	0·0	0·71	0·69
	1000	288	0·51	0·83	−8·5	0·0	0·83	0·86
	1000	301	0·47	0·65	−4·5	0·0	0·79	0·75

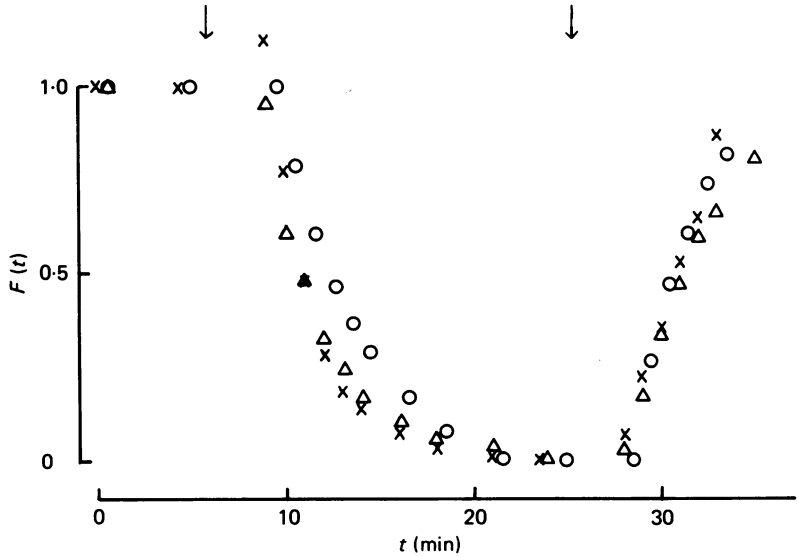

Fig. 3. A comparison of the time courses of the changes in the mid-point of the steady-state inactivation (h_∞) curve (○), the time constant (τ_h) for inactivation (△) and the time constant) (τ_m) for activation (×), both at a membrane potential of −10 mV, under exposure to 306 μM-*n*-pentane. The changes in the three parameters have been normalized by means of the expression of the form $F(t) = \{f(t)-f(ss)\}/\{f(O)-f(ss)\}$ where $f(O)$ and $f(ss)$ are the initial and steady-state values of the parameter respectively. The two arrows indicate the introduction and removal of the *n*-pentane solution. The flow rate was approx. 16 ml/min.

regions of the axon membrane and it was demonstrated that, consistent with such a thickening, the membrane capacity at 100 kHz decreased. The capacity measurements on the additional hydrocarbons studied here provide further data with which to test this hypothesis. In Haydon & Kimura (1981) the shift along the voltage axis in the mid-point of the h_∞ curve was related to the thicknesses d and d^t (in the present notation) of the lipid chain region of the membrane before and during exposure to hydrocarbon by the equation:

$$\Delta V_h = V_h^t - V_h = V_h(d^t/d-1), \tag{2}$$

where V_h is the membrane potential at which $h_\infty = 0{\cdot}5$ for the axon under normal conditions. If the membrane may be regarded as a parallel-plate geometrical capacitor and if the adsorption of the hydrocarbons and carbon tetrachloride, which all have dielectric constants between approximately 2·0 and 2·2, is assumed not to change the membrane dielectric constant significantly, then eqn. (2) may be rewritten:

$$\Delta V_h = V_h \frac{\Delta C}{C^t}, \tag{3}$$

where ΔC is the change in membrane capacity per unit area produced by the hydrocarbon and C^t is the membrane capacity per unit area in the hydrocarbon solution. This relationship was tested for a number of intact axons; capacity and h_∞ measurements being carried out within a space of a few minutes on axons with stable currents.

TABLE 3. Membrane capacity changes at 100 kHz on introduction and removal of some hydrocarbons and carbon tetrachloride

Hydrocarbon	Concentration in artificial sea water (μmol l^{-1})	Axon	Membrane capacity (100 kHz) (μF cm^{-2}) Control	Test	Recovery
n-Butane	675	278	0·335	0·299	0·335
	675	280	0·380	0·361	0·385
	675	286	0·289	0·276	0·306
Cyclopropane	7000	122	0·403	0·345	0·376
	7000	125	0·303	0·281	0·311
	7000	128	0·410	0·392	0·406
Cyclopentane	760	334	0·349	0·299	0·344
	1140	331	0·490	0·388	0·453
Cyclohexane	189	296	0·342	0·325	0·355
	216	297	0·241	0·230	0·255
	324	295	0·361	0·303	0·341
	486	291	0·291	0·218	0·272
Carbon tetrachloride	1000	155	0·453	0·439	0·473
	2000	143	0·224	0·207	0·238
	2000	173	0·372	0·347	0·417

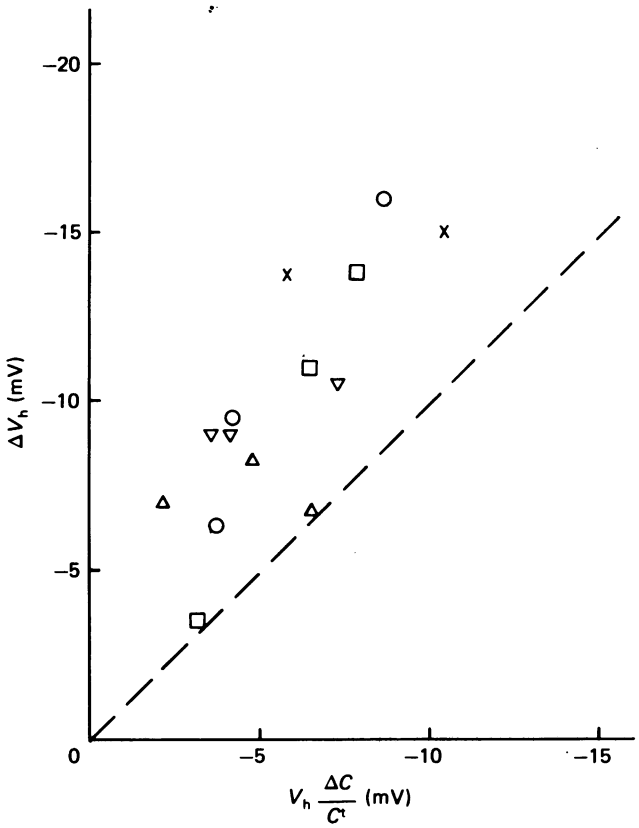

Fig. 4. A comparison of the shift ΔV_h in the mid-point of the steady-state inactivation curve with that expected from eqn. (3). Δ, cyclopropane; ×, cyclopentane; ○, cyclohexane; ▽, *n*-butane and □, carbon tetrachloride. The capacities are from Table 3. The dashed line represents eqn. (3).

The steady-state inactivation curve was determined for potentials between −140 mV and −40 mV applied for 50 ms; the test pulse was usually to −5 mV. The K current distorted the h_∞ curve at less negative internal potentials but comparison of the experimental results with the theoretical h_∞ curve, calculated from the approximate Hodgkin–Huxley expression for h_∞, showed that at $h_\infty = 0{\cdot}5$ this effect was not significant. The h_∞ shifts, V_h values and capacity changes used in Fig. 4 are averages for the uptake and wash-out of the hydrocarbon. Both the h_∞ shift and the capacity change were satisfactorily reversible. Eqn. 3 is represented in Fig. 4 by the dashed line; all the experimental points lie above the line, i.e. the inactivation curve shifts by more than would be expected from the capacity change.

DISCUSSION

Current suppression and relative potency

All of the hydrocarbons examined, and carbon tetrachloride suppressed the peak Na current to some extent (Table 1). The molar concentrations required to produce a suppression of 50 % varied widely but the fractional saturations varied far less. By expressing the concentrations as fractions of the saturation value the reference medium, with which the adsorption into the membrane is compared, becomes the pure liquid hydrocarbon (Ferguson, 1939; Tanford, 1980). The fact that the fractional saturations required to produce a given suppression of the inward current all lie within a factor of approximately four of each other, suggests that the sites of action are essentially lipophilic. The variations within the factor of four cannot readily be analysed in terms of interaction with the membrane components, since the reference medium differs somewhat in each instance, though it is notable that carbon tetrachloride seems more potent than the hydrocarbons. The lack of activity of propane, as compared with cyclopropane may be related to the fact that the latter has a bilayer/gas partition coefficient some four times larger than the former (Miller, Hammond & Porter, 1977).

Membrane capacity

The changes produced by the hydrocarbons and carbon tetrachloride in the membrane impedance are comparable to those described for *n*-pentane by Haydon *et al.* (1980). The arguments that the impedance changes originate from changes in the membrane capacity have been given under Results. At present there seems no obvious alternative explanation, but the fact that, according to this interpretation, the membrane capacity decreases continuously with increasing frequency and has values at 100 kHz somewhat lower than would be expected for a lipid bilayer, suggests that, at a quantitative level, the results should be regarded with caution. Qualitatively, the capacities all decrease in the presence of hydrocarbon and, as discussed in earlier papers, this is consistent with a thickening of the membrane. Black film experiments with cyclohexane (Requena & Haydon, 1975), carbon tetrachloride (Andrews, Manev & Haydon, 1970) and cyclopentane (D. Needham & D. A. Haydon, unpublished results) show that, as for *n*-pentane and other small *n*-alkanes, these substances adsorb predominantly into the centre of the chain region of the bilayer and, at saturation, can increase the thickness of this region by ten or more Angström

units (Å). In a 0·4 saturated solution (corresponding to 760 mmol l^{-1} in artificial sea water), cyclopentane increased the thickness of a glycerol mono-oleate bilayer by approximately 1·5 Å. A saturated solution of carbon tetrachloride increased the thickness of a similar bilayer by about 8 Å. The axon membrane is believed to contain phospholipids and cholesterol (Camejo Villegas, Barnola & Villegas, 1969; Chacko Villegas, Barnola, Villegas & Goldman, 1976) and, while the adsorption of hydrocarbons into these structures will differ in some respects from that for monoglycerides, it is known that for the *n*-alkanes, appreciable thickness changes occur at nerve-blocking concentrations (Haydon *et al.* 1977). Franks & Lieb (1979) have examined the adsorption of cyclopropane into multilayered phospholipid structures by diffraction techniques and concluded that at 1 atm no structural changes occurred. On the other hand Miller *et al.* (1977) have measured the uptake of cyclopropane and *n*-butane into phospholipid and phospholipid–cholesterol multilayered liposomes. They found that the adsorption of these gases at 1 atm was readily measurable and, if the partial molar volumes of the adsorbed molecules are assumed to be similar to their values in the liquid hydrocarbon, volume increases of *ca.* 4% and 9% respectively would be expected in the lipid region of the bilayer. Evidence from black lipid film studies suggest that hydrocarbons accumulate predominantly in the centre of bilayers and that the area per lipid molecule is not appreciably affected. If this is so, then the volume changes calculated should be observed mainly as thickness changes. It is therefore of interest that the average changes in the reciprocals of the capacities recorded in Table 3 for cyclopropane and *n*-butane at 1 atm are 7·2% and 8·5% respectively. In so far as the nerve membrane capacities at 100 kHz are determined by the thickness of the bilayer regions of the membrane, the results are thus consistent with the data of Miller *et al.* (1977) but not with those of Franks & Lieb (1979).

The Hodgkin–Huxley parameters of the Na current

The maximum membrane conductance. With the exception of *n*-butane and cyclopropane (both of which were at low fractional saturations compared with the other hydrocarbons) all the hydrocarbons and carbon tetrachloride caused substantial reductions in $\bar{g}_{Na}$ (Table 2). For *n*-butane, *n*-pentane and *n*-hexane the decreases in $\bar{g}_{Na}$ are very similar to the corresponding decreases in the peak inward current. For the cyclic hydrocarbons and carbon tetrachloride, on the other hand, the reductions in the current are significantly greater. If the reductions in the steady-state outward current, I_{ss}, shown in Table 1 are taken as proportional to the maximum K conductance $\bar{g}_K$, it can be seen that $\bar{g}_{Na}$ and $\bar{g}_K$ are affected very similarly by the hydrocarbons. In this respect the non-polar molecules studied so far are not specific in their action on the Na and K channels and their mode of action could be similar to that by which they destabilize the polypeptide channel gramicidin, i.e. the channels may be disrupted by a combination of membrane thickening and tension increase. However, it has not yet been shown whether the decreases in $\bar{g}$ arise from a complete loss of conductance in some channels or a reduction in conductance of all of them.

The steady-state inactivation. Without exception, the h_∞ curves for the substances listed in Table 2 exhibit both a shift in the hyperpolarizing direction and a decrease

in slope at the mid-point (Table 4). A shift (without a slope change) would be produced by an asymmetric change in the ionic double layers at the membrane surfaces (Chandler, Hodgkin & Meves, 1965) or, if the voltage sensor for inactivation were within the membrane, by an asymmetric change in the surface dipole or χ potential (Haydon, 1975). A slope change corresponds to a change in the term $-1/4k$ in the Hodgkin–Huxley formalism (see eqn. (5) of Haydon & Kimura, 1981), where k contains the valency of the 'h particle' and the fraction of the membrane potential that it senses. An explanation for a variation in slope could therefore be that the anaesthetic either alters the valency or restricts the motion of the h particle. From this discussion it would seem necessary to invoke two unrelated effects to account for the changes in the h_∞ curve. However, if the hydrocarbon is assumed to thicken the membrane, as the capacity results suggest, both the shift and the slope change can be explained on the same basis (Haydon & Kimura, 1981). Thus, an increase in thickness of the non-polar part of the membrane would change the field and hence the apparent membrane potential experienced by a dipole in this region, even though the actual membrane potential remained constant. Furthermore, according to this model, the ratio of the slopes during and before hydrocarbon treatment is, from eqn. (7) of Haydon & Kimura (1981):

$$\left.\frac{\mathrm{d}h_\infty^{\mathrm{t}}}{\mathrm{d}V}\right|_{V_{\mathrm{h}}^{\mathrm{t}}} \Big/ \left.\frac{\mathrm{d}h_\infty}{\mathrm{d}V}\right|_{V_{\mathrm{h}}} = \frac{d}{d^{\mathrm{t}}} \tag{4}$$

Table 4 compares the observed slope ratios with those calculated from the shift by means of eqns. (2) and (4). Given that slopes cannot be determined very accurately from plots such as those in Figs. 1 and 2, the agreement is quite good. It does appear, however, that for cyclopentane and cyclohexane the actual slope decrease is less than would be expected from the shifts while, for carbon tetrachloride, the reverse is true. As pointed out in Haydon & Kimura (1981), eqn. (2) holds only if the internal electric field in the membrane is zero when the membrane potential is zero. Eqn. (4), however holds regardless of whether this is so. The general agreement between the observed and calculated slope ratios in Table 4 therefore suggests that the internal field experienced by the inactivation voltage sensor is close to that given by the membrane potential and that surface potential changes on the two sides are similar.

The plot in Fig. 4 is a further test of the thickening mechanism. A good correlation between capacity change and ΔV_{h} is obvious. If the shifts in the h_∞ curves resulted exclusively from a homogeneous membrane thickening, if as just discussed, the internal electric field in the membrane is zero when the membrane potential is zero, and if the capacity at 100 kHz reflected the changes in the thickness of the non-polar regions of the membrane, then the experimental points should lie on the dashed line of unit slope. The fact that they do not, might have interesting implications. The capacity measurement should give an average thickness change for the whole membrane whereas the response of the inactivation voltage sensors would presumably be determined by changes in only a very small fraction of the membrane which might well differ from the average in its lipid composition.

The steady-state activation. The shifts in the m_∞ curves are consistently less negative than in the h_∞ curves and for cyclopropane, where the h_∞ shift is small, the activation curve moves in a depolarizing direction. This suggests that, although the activation

process is influenced by the same factor (e.g. membrane thickening) as the inactivation process, there is another effect superimposed upon this which tends to move the m_∞ curve in the opposite direction and which predominates only when the first influence is small. From the data of Table 2 it appears that the second effect produces a shift in the m_∞ curve of about 8 mV. As will be shown in subsequent papers, all the substances examined, and not just the hydrocarbons, produce essentially the same type of shift. The physical origin of this effect is so far obscure. An obvious candidate is an asymmetric change in the surface potential, particularly in the dipole component of this potential, but protein conformations may be influenced equally by changes in the 'chemical' part of the membrane free energy. This question will be reconsidered in a later paper when more evidence of the generality of the effect has been presented.

TABLE 4. The effects of hydrocarbons on the slope at the mid-point of the steady-state inactivation curve. The slope ratio is that of the curve during, to that before exposure to hydrocarbon. The final column gives the slope ratio calculated from ΔV_h by means of eqns. (2) and (4). To aid comparison the averages of each column together with the S.E. of the means are shown

	Axon	Slope ratio (observed)	Slope ratio (calculated)
n-Butane	303	0·814	0·869
	312	0·821	0·855
n-Pentane	290	0·670	0·754
	298	0·896	0·807
	300	0·892	0·796
n-Hexane	344	0·857	0·814
	349	0·651	0·812
Cyclopropane	162	0·949	0·846
	225	0·860	0·883
	318	0·969	0·881
	319	0·864	0·898
Cyclopentane	335	0·866	0·752
	350	0·875	0·755
	419	0·794	0·776
	422	0·912	0·816
Cyclohexane	308	0·814	0·784
	310	0·899	0·807
	321	0·903	0·787
Carbon tetrachloride	287	0·782	0·882
	288	0·822	0·859
	301	0·876	0·924
Average ± S.E. of the mean		0·847 ± 0·017	0·827 ± 0·011

The time constants for activation and inactivation. As kinetic, rather than steady-state parameters, the time constants may be determined by factors such as membrane fluidity in addition to the factors discussed in connexion with h_∞ and m_∞. Since the membrane potential appears in the Hodgkin–Huxley expressions for τ_m and τ_h, the curves for these quantities (see Figs. 1 and 2) will be subject to the same shifts as for m_∞ and h_∞. Allowance must therefore be made for these shifts when considering changes in the magnitude of τ_m and τ_h. With one or two exceptions, the peak height of both τ_m and τ_h is reduced by the hydrocarbons. The largest effects are produced by the straight-chain hydrocarbons and carbon tetrachloride, τ_m and τ_h being very

similarly reduced. It is tempting to ascribe these changes to the increases in chain disorder that are presumably produced by the hydrocarbons in the membrane lipids. But the results for the cyclic hydrocarbons, particularly cyclohexane, where τ_h and τ_m exhibit zero and very little suppression respectively, do not seem obviously consistent with this explanation. On the other hand, it does appear that for both normal chain and cyclic molecules, the reduction in τ_m and τ_h is greater for the smaller molecules. It cannot be completely ruled out that part of the decrease in τ_h arises from a time-dependent blockage of open Na channels. However, the fact that τ_m is also reduced in almost every instance and that the time constant for activation of the K channel is reduced by *n*-pentane (Haydon & Kimura, 1981) makes it more likely that the hydrocarbons are accelerating the normal inactivation mechanism.

Several experiments of the type depicted in Fig. 3 were carried out to discover whether the time constants changed appreciably before the steady-state quantities (represented here by h_∞) and were therefore more sensitive to the hydrocarbons. From the plots there is some suggestion that this may be so, but the effect did not appear sufficiently striking to warrant further investigation.

A more detailed discussion of the time constants and of the Hodgkin–Huxley rate constants for activation and inactivation will be postponed until a later paper where data for all of the substances examined in the present series of experiments will be considered together.

In summary, when the axon is voltage clamped to potentials of −90 mV, or more negative potentials, there are several ways in which the hydrocarbons influence the Na current. The decreases in $\bar{g}_{Na}$ and in τ_h tend to reduce the current, whereas the shift in m_∞ and the decrease in τ_m have the opposite effect. At holding potentials less negative than −90 mV, a large additional factor is the shift in the h_∞ curve, leaving the axon heavily inactivated.

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