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EXCITATION OF THE SQUID GIANT AXON BY GENERAL ANAESTHETICS

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SUMMARY

1. The effects of 'clinical' concentrations of some general anaesthetics on the minimum stimulus required to produce an action potential in the squid giant axon have been examined as a function of time from exposure to the anaesthetic. The resting potential in these experiments was also monitored.

2. The minimum stimulus varied with time in different ways for different anaesthetics. For chloroform, diethyl ether, *n*-pentanol, halothane and cyclopropane the stimulus initially declined, reached a minimum after about 3 min and then recovered to near-normal values at 10–15 min. For *n*-pentane, cyclopentane and, to a lesser extent methoxyflurane, the stimulus often declined to such low values that the axon exhibited spontaneous action potentials which persisted until the anaesthetic was removed. For one substance, the experimental local anaesthetic diheptanoyl phosphatidylcholine, the stimulus increased considerably over the 10–15 min required to reach the steady state. In all instances the axons reverted to normal behaviour after removal of the anaesthetic although the time course by which they did so was more variable than for the initial exposure.

3. For all anaesthetics the resting potential changed in the positive direction monotonically by *ca.* 1–5 mV and reached a steady state in approximately 3 min. On removal of the anaesthetic the resting potential returned to normal, also monotonically.

4. The voltage-gated Na^+ and K^+ currents were significantly affected even at the low anaesthetic concentrations used. Estimates of the changes in the Hodgkin–Huxley parameters were obtained partly by direct experiment and partly from results previously obtained for higher anaesthetic concentrations.

5. The time dependencies of the minimum stimuli have been accounted for semi-quantitatively in terms of the resting potential changes and the voltage shifts in the Na^+ current steady-state activation, and the time dependencies respectively of these two parameters.

6. Quantitative calculations of the resting potential changes for comparison with experiment have been made based on the changes in K^+ conductance determined in the preceding paper (Haydon, Requena & Simon, 1988) and changes in the Hodgkin–Huxley parameters of the Na^+ and delayed-rectifier K^+ currents.

7. Calculations of the minimum stimulus in the steady state have been made from the experimental resting potential changes and from the anaesthetic-affected Hodgkin–Huxley parameters. Good agreement with the experimental stimuli was found, especially in the prediction of high and low values.

INTRODUCTION

It is well known that, during the induction of anaesthesia by a number of general anaesthetics, convulsions may occur (Pinson, 1927; Di Giovanni & Dripps, 1956; Pittinger, Mitchell, Alen & Page, 1961; Eger, 1974). The origins of the convulsions are not well understood but it seems very likely that a depolarization of an excitable membrane of nerve or muscle by the anaesthetic is responsible. Clinical and experimental general anaesthetics are known to depolarize nerve and, in some instances, muscle cells (Seeman, 1972; Kendig & Bunker, 1972; Sevcik, 1980; Haydon, Elliott & Hendry, 1984). In the squid giant axon these depolarizations sometimes lead to spontaneous firing of the axon. This has been reported by Sevcik (1980) for thiopental, by Haydon, Hendry, Levinson & Requena (1977) for *n*-pentane and by Urban & Haydon (1987) for methoxyflurane and isoflurane. However, the incidence of spontaneous firing is not clearly linked to the depolarization. Thus, a number of anaesthetics produce depolarizations similar to those for the anaesthetics mentioned above and yet show little or no tendency to cause spontaneous firing (Urban & Haydon, 1987; Haydon *et al.* 1988). This, it will be shown, arises from small but important effects of the anaesthetics on the voltage-gated Na^+ and K^+ currents.

The fact that convulsions tend to occur during the induction of anaesthesia, rather than during the steady state, suggests that it could be instructive to follow the excitability of an axon with time after exposure to an anaesthetic. This has been attempted for a number of clinical and experimental anaesthetics. The results show interesting biphasic effects rather than the monotonic progression that might have been expected. An explanation for the transient phenomena is offered and their possible relevance to the clinical observations is briefly discussed.

METHODS

All experiments were carried out with giant axons from freshly killed specimens of the squid *Loligo forbesi*. The axons were cleaned of surrounding fibres and connective tissue and had diameters between 500 and 1000 μm. The apparatus, the experimental details and the numerical procedures have been described previously (Haydon, Requena & Urban, 1980; Haydon & Kimura, 1981; Haydon & Urban, 1983*a*, *b*, *c*). Compensation for the series resistance was applied in all voltage clamp experiments. Experiments were carried out at 6 ± 1 °C.

Only intact axons were studied. The external bathing solution usually contained 430 mM-NaCl, 10 mM-KCl, 10 mM-$CaCl_2$, 50 mM-$MgCl_2$ and 10 mM-Trizma base. HCl was added to bring the pH to 7·6. In order to examine Na^+ currents, voltage-gated K^+ currents were suppressed with 1 mM-3,4-diaminopyridine and 215 mM-choline chloride was substituted for 215 mM-NaCl in the bathing solution. Tetrodotoxin (TTX) at 0·3 μM was added usually after test records had been taken and the TTX-insensitive currents were subtracted from the control and test currents before analysis. Strength–duration and resting potential experiments, as well as voltage clamp experiments, were carried out using piggy-back electrodes.

The sources of the anaesthetics, which are listed in Table 1, were as described in Haydon & Urban (1983*a*, *b*, *c*). In each instance the anaesthetic was added to the outside of the axon.

RESULTS

Action potentials and excitability

The test substances, with one exception, are clinical or experimental general anaesthetics and the concentrations selected for the experiments are, for the clinical

TABLE 1. Excitability and resting potential changes produced by anaesthetics in the squid giant axon

Anaesthetic	Concentration (mM)	$I_{c,tr}/I_c$	$I_{c,ss}/I_c$	$I_{c,r}/I_c$	No. of axons	Spontaneous action potentials	$\Delta V_{R,ss}$(mV)
Chloroform	1·0	0·81	1·01	0·97	3	2*/5	2·9 (8)
Cyclopentane	0·19	0·18	0·21	0·93	3	2/3	3·5 (5)
Methoxyflurane	0·55	0·42	0·71	0·93	5	5/16	4·0 (13)
Diethylether	13·5	0·85	0·94	0·96	3	1/14	1·1 (12)
n-Pentanol	6·0	0·73	0·94	0·94	3	1*/6	4·4 (4)
Halothane	0·5	0·84	0·95	0·99	2	0/6	2·1 (4)
Cyclopropane	1·4	0·78	0·87	0·93	3	0/2	2·6 (3)
n-Pentane	0·061	0·15	0·18	1·0	5	4/5	1·8 (3)
Diheptanoyl phosphatidylcholine	0·2	0·96	2·27	0·96	2	0/4	0·9 (2)

* Only when axon had been exposed previously to an anaesthetic. I_c is the minimum current over 100 μs required to stimulate an action potential; the subscript 'tr' indicates the lowest value of I_c recorded during the introduction of the anaesthetic; 'ss' indicates the value of I_c after the anaesthetic had equilibrated with axon; 'r' indicates the I_c after removal of the anaesthetic; I_c denotes control, i.e. before anaesthetic was introduced. The ratios are means for the numbers of axons shown. The incidence of spontaneous action potentials is shown in the seventh column as a ratio to the number of axons examined. $\Delta V_{R,ss}$ is the change in the resting potential produced by the anaesthetic in the steady state; it is the test value minus the average of the control and recovery values, and is a mean for the numbers of axons shown in parentheses. The mean resting potentials of these axons are given in Table 1 of the preceding paper (Haydon *et al.* 1988).

anaesthetics, within a factor of two of the minimum alveolar concentration for dogs (Eger, 1974). The diheptanoyl phosphatidylcholine at 0·52 mM reduces the maximum Na^+ current under voltage clamp by approximately 50% (Hendry, Elliott & Haydon, 1985). At the concentrations used in this work, none of the test substances induced significant stimulus dependence of the amplitude of the action potential nor

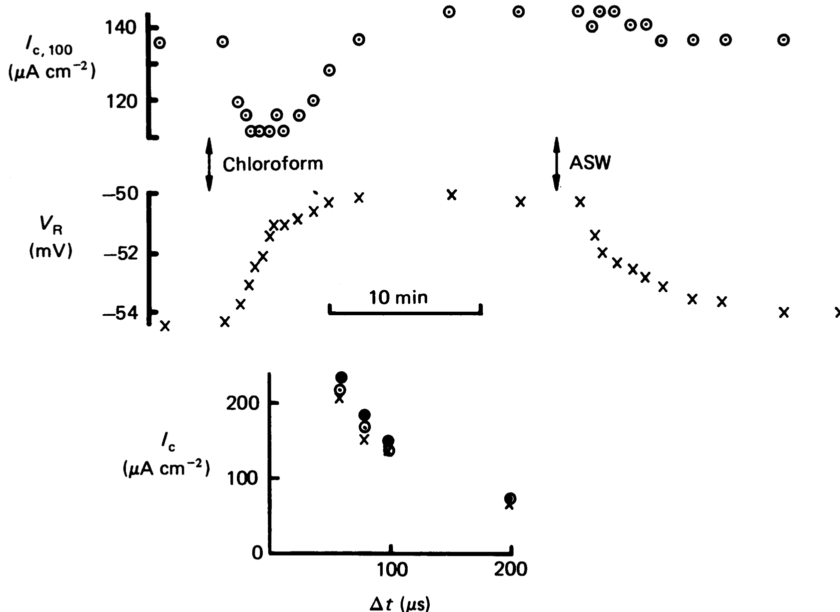

Fig. 1. The time courses of the excitability and of the resting potential after exposure of the axon to 1 mM-chloroform. The excitability (upper plot) is expressed as the minimum stimulus current applied over 100 μs ($I_{c,100}$) required to produce an action potential. The resting potential (V_R) is shown in the middle plot. The arrows indicate the times of introduction and removal of the anaesthetic. The lower plot shows the strength–duration curve for the stimulus current: ⊙, for the control; ●, for the steady state in the anaesthetic; and ×, after the anaesthetic had been removed. ASW, artificial sea water.

did they reduce the amplitude by more than about 15%. The minimum current pulse for 100 μs ($I_{c,100}$) required to evoke an action potential did, however, vary considerably during the introduction of the anaesthetics, to the extent that action potentials occurred spontaneously in some systems.

The excitability and resting potential changes are given in Table 1. Three distinct types of response were observed on exposure of the axons to the anaesthetics.

(i) The minimum stimulus decreased over the first 2–3 min though not to the extent that spontaneous firing occurred. Over the next 5–10 min the minimum stimulus increased and eventually reached a steady level fairly close to its control value. On switching the bathing solution back to artificial sea water the changes in the stimulus were less reproducible. The final state was not usually distinguishable from the control but the time course varied somewhat from one axon to another. The anaesthetics producing this response were chloroform, diethylether, halothane, cyclopropane and *n*-pentanol (Table 1; Fig. 1).

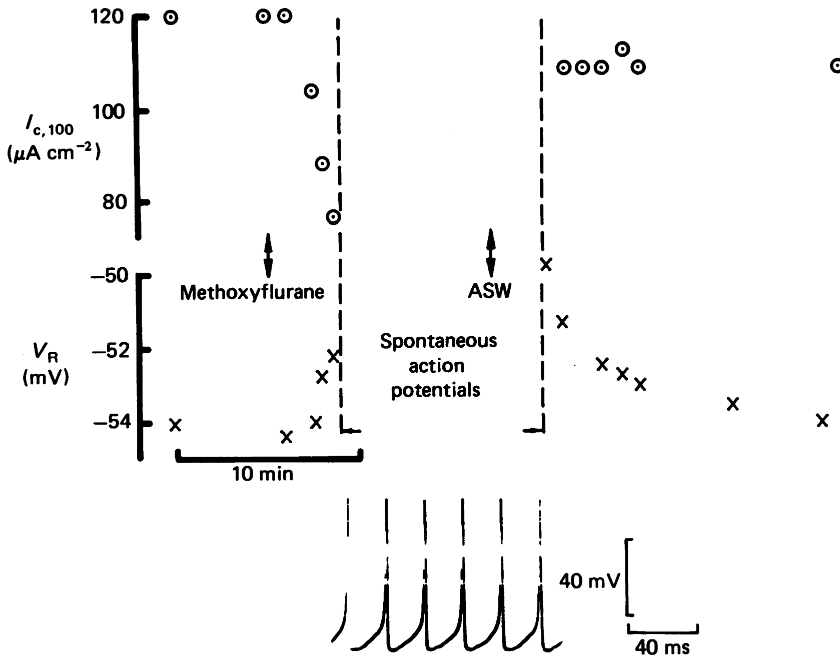

Fig. 2. The time courses of the excitability and of the resting potential after exposure of the axon to 0·55 mM-methoxyflurane. The details are as for Fig. 1. At the time indicated by the first vertical dashed line, this axon began to produce action potentials spontaneously, as shown in the photographic record. The action potentials ceased to be spontaneous at the second dashed line.

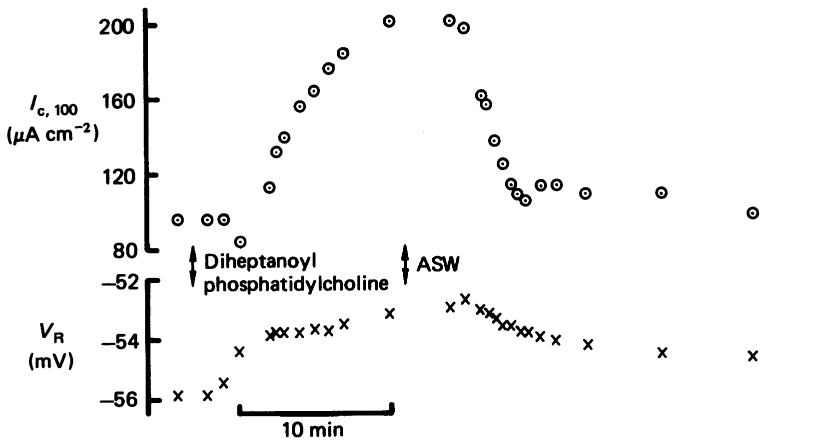

Fig. 3. The time courses of the excitability and the resting potential after exposure of the axon to 0·2 mM-diheptanoyl phosphatidylcholine. The details are as for Figs 1 and 2.

(ii) The minimum stimulus decreased sharply on introduction of the anaesthetic and within 2–3 min action potentials occurred spontaneously at intervals of approximately 30 ms. The firing persisted for 15–20 min (when the anaesthetic could be assumed to have reached an equilibrium distribution throughout the axon) and ceased only when artificial sea water was re-introduced. Complete recovery to the control state then usually occurred. The aliphatic hydrocarbons, *n*-pentane and cyclopentane, produced this response as did methoxyflurane in about one-third of the experiments (Table 1; Fig. 2).

(iii) The minimum stimulus *increased* over the first few minutes (though occasionally via a rapid transient in the opposite direction) until, after 10–15 min, it reached a steady high value. On removal of the anaesthetic the stimulus fell back, sometimes with small undulations, to its control value. Only diheptanoyl phosphatidylcholine produced this response (Table 1; Fig. 3).

Minimum stimulus strength–duration curves were determined in each experiment for all three steady states (control, test and recovery). An example is shown for chloroform in Fig. 1.

Resting potential

All of the test substances produced positive (depolarizing) changes (ΔV_R) in the resting potential (V_R). Average values for ΔV_R are given in Table 1. The time courses for three experiments are plotted in Figs 1–3. The reversibility of the resting potential changes was normally $> 90\%$. The changes were invariably monotonic, in contrast to the variations in minimum stimulus. The origins of the resting potential changes have been considered in the preceding paper (Haydon *et al.* 1988).

Voltage-gated Na^+ and K^+ currents

Na^+ currents. All of the substances listed in Table 1 reduce the Na^+ current at sufficiently high concentrations. Voltage clamp studies of the Na^+ current under these conditions have shown clearly the changes produced by the anaesthetics in the Hodgkin–Huxley parameters (Haydon & Urban, 1983*a, b, c*; Hendry *et al.* 1985). At the concentrations of interest in this paper, which are 3–5 times smaller than the nerve block concentrations, there are still significant effects on the Na^+ currents. To illustrate this the current–voltage curves for cyclopropane, which typify the size of effect, are shown in Fig. 4. In order to analyse fully the excitability data it is desirable to have the changes in the Hodgkin–Huxley parameters not only at the low concentrations but also under appropriate conditions (e.g. for intact axons). This presents difficulties since the elimination of K^+ currents by perfusion, especially with solutions containing fluoride, causes appreciable departures from normal behaviour (Meves, 1978). The least unsatisfactory procedure seemed to be to suppress the K^+ currents with 1 mM-3,4-diaminopyridine. This involved the risk of synergistic or antagonistic effects with the anaesthetics and careful checks of e.g. Na^+ current, reduction and peak shift, and steady-state inactivation shift were made against the corresponding changes in normal axons. These revealed that while the effects of the hydrocarbons, *n*-pentanol and diheptanoyl phosphatidylcholine, were apparently unaffected by the 3,4-diaminopyridine, some effects of the other anaesthetics were considerably reduced. The origins of this antagonism were not

investigated, but it completely precluded the direct determination at low anaesthetic concentrations of some Hodgkin–Huxley parameters for these systems.

The Na^+ currents were obtained and analysed as described in previous papers (e.g. Hendry *et al.* 1985). Axons in which the K^+ currents had been blocked with 3,4-diaminopyridine were voltage clamped at −70 mV. The 15 ms depolarizing pulse

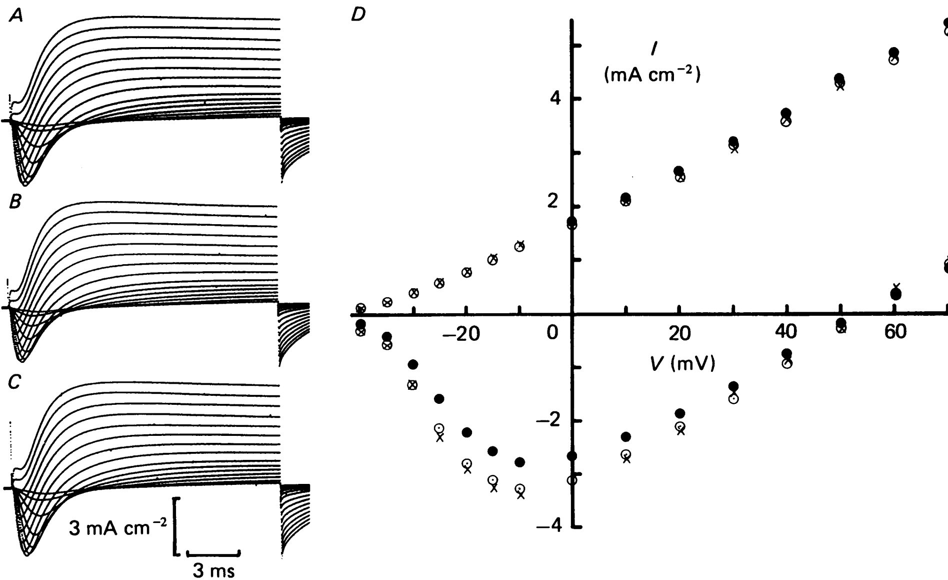

Fig. 4. Voltage clamp records and current–voltage plots for an intact axon exposed to 1·4 mM- (0·2 atm) cyclopropane in the steady state. The records *A*, *B* and *C* are for control, test and recovery conditions respectively. The artificial sea water contained 430 mM-Na^+. The holding potential was −60 mV but a pre-pulse to −80 mV was applied immediately prior to each test pulse. The test pulses were 20–50 mV in 5 mV steps and 50–130 mV in 10 mV steps. For the current–voltage curves (*D*) ⊙, ● and × represent respectively the control, test and recovery states. The lower plots are for the peak of the early transient (essentially Na^+) current while the upper plots are for the peak outward (essentially K^+) current.

was preceded by a pulse of −20 to −90 mV for 50 ms to remove fast inactivation. The steady-state inactivation parameter h_∞ was obtained by applying 50 ms pre-pulses at various potentials, followed by a test pulse to −10 or 0 mV. Some 15–20 min were usually required for the Na^+ current to become steady in the anaesthetic solutions, and a similar period was required for the recovery in artificial sea water. It was established that the anaesthetic effects were reversible in each system, though the reversal was not attempted in all experiments. Instead TTX (0·3 μM) was sometimes added after the test records had been obtained. The TTX-insensitive currents were subtracted from the control, test and, where relevant, recovery current records. The remaining currents were analysed by means of equations of Hodgkin & Huxley (1952)

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

$$I'_{Na} = \bar{g}_{Na}m^3_\infty h_0(V-V_{Na}), \quad (2)$$

to give the parameters I'_{Na}, τ_m, τ_h, h_∞, $\bar{g}_{Na}$ and m_∞. I_{Na} is the Na^+ current, t is the time from the beginning of the depolarizing pulse, V is the membrane potential and V_{Na} is the value of V at which $I_{Na} = 0$. It was assumed that at -90 mV $h_0 \approx 1$ and that for $0 < V < 20$ mV, $m_\infty \approx 1$. The value of τ_h in the vicinity of its peak was obtained essentially as described by Gillespie & Meves (1981).

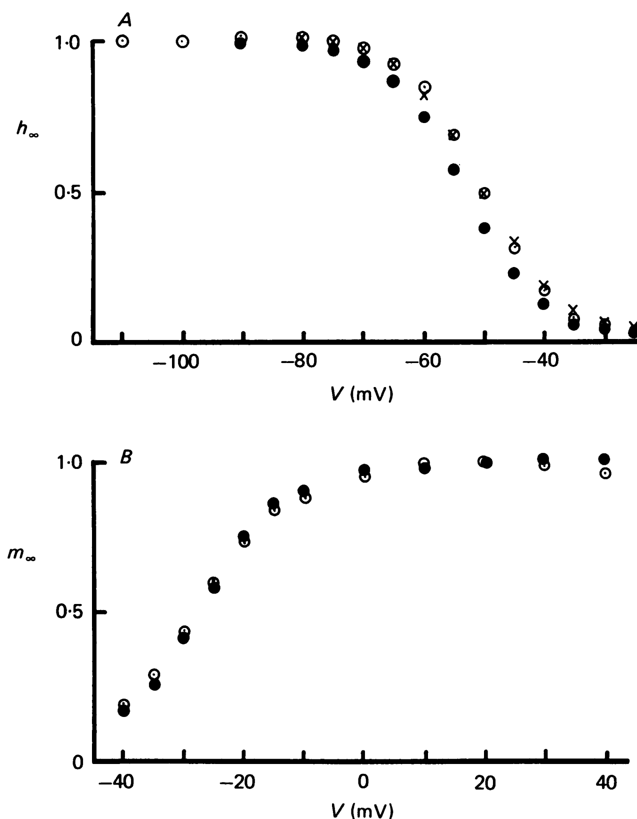

Fig. 5. The effects of cyclopropane (1·4 mM) on A the steady-state inactivation (h_∞), and B the steady-state activation (m_∞) curves for the Na^+ current. ⊙, control; ●, test; and ×, recovery. The test data were obtained approximately 20 min after exposure of the axon to cyclopropane.

At the low anaesthetic concentrations used, the only clearly detectable changes in the Hodgkin–Huxley parameters were in the voltage dependence of m_∞ and h_∞. Examples of these parameters for cyclopropane are shown in Fig. 5. The maximum values of τ_m and τ_h, and the value of $\bar{g}_{Na}$ are not so accurately measurable as are the voltage shifts in m_∞ and h_∞. Probably for this reason no effects of the anaesthetics on these parameters could be discerned in the relatively small numbers of experiments which could be carried out. The results generally, however, are much as expected from linear interpolation between zero and those obtained at higher concentrations (Haydon & Urban, 1983*a*, *b*, *c*; Hendry *et al.* 1985). Average values for the shifts ΔV_m and ΔV_h in the mid-points of the m_∞ and h_∞ curves respectively are given in Table 2. For the systems in which the K^+ currents could not be suppressed

TABLE 2. Changes in voltage-gated Na^+ and K^+ current parameters produced by anaesthetics at 'clinical' concentrations

Anaesthetic	Concentration (mM)	3,4-Diaminopyridine ΔV_m (mV)	3,4-Diaminopyridine ΔV_p (mV)	Intact ΔV_p (mV)	ΔV_h(mV) 3,4-Diaminopyridine	ΔV_h(mV) Intact	ΔV_n (mV)
Chloroform	1·0	—	—	4·0 (2)	−1·5 (2)	−1·5 (2)	2·6
Cyclopentane	0·19	−0·4	0·5 (2)	−3·0 (1)	−2·1 (2)	−3·0 (1)	1·0
Methoxyflurane	0·55	—	—	2·4 (4)	−1·9 (1)	−1·5 (4)	3·3
Diethylether	13·5	—	—	1·3 (3)	−0·5 (2)	−1·3 (1)	0·1
n-Pentanol	6·0	5·0	5·0 (2)	5·0 (1)	−0·7 (2)	−0·9 (1)	3·0
Halothane	0·5	—	—	1·5 (4)	1·0 (1)	−0·4 (2)	2·1
Cyclopropane	1·4	1·3	1·0 (2)	1·0 (2)	−3·0 (3)	−2·4 (2)	1·0
n-Pentane	0·061	−3·8	−3·0 (2)	−4·0 (2)	−4·0 (3)	−2·9 (1)	−1·3
Diheptanoyl phosphatidylcholine	0·2	6·4	6·3 (2)	7·0 (1)	2·8 (2)	2·4 (1)	0·0

ΔV_m, ΔV_h and ΔV_n are respectively the shifts in the mid-points of the Hodgkin–Huxley steady-state Na^+ activation and inactivation, and K^+ activation curves. ΔV_p is the shift in the maximum of the curve of peak Na^+ current *versus* membrane potential. ΔV_m values were obtained for axons in which the K^+ current was suppressed with 3,4-diaminopyridine. In these instances ΔV_p, which was interpolated by eye, is also given. ΔV_p and ΔV_h were obtained for intact axons as well as for axons treated with 3,4-diaminopyridine. ΔV_n was obtained by linear interpolation of the data of Haydon & Urban (1986). The numbers in parentheses indicate the numbers of axons examined.

by addition of 3,4-diaminopyridine owing to antagonistic effects, the shift, ΔV_p, in the maximum of the current–voltage curve for a normal intact axon (as in Fig. 4) is given in place of ΔV_m.

K^+ currents. Figure 4 shows the effect of cyclopropane on the late outward (essentially K^+) current. This is characteristic of most of the anaesthetics in that the deviations from the control are very small. Methoxyflurane and, to a lesser extent, *n*-pentanol were exceptions in that decreases in I_K were found for small depolarizations. These observations were expected from the shifts, ΔV_n, calculated for the steady-state activation of the K^+ current (n_∞) at higher anaesthetic concentrations (Haydon & Urban, 1986). In the present low concentrations such shifts were not satisfactorily measurable owing to the small magnitude of the K^+ currents between membrane potentials of −50 and −30 mV. As a consequence, estimates of ΔV_n have been made from the data of Haydon & Urban (1986) assuming a linear dependence on anaesthetic concentration (Table 2). In two instances (cyclopropane and diheptanoyl phosphatidylcholine) there is no such data, but inspection of the K^+ current–voltage curves (as in Fig. 4) suggests that for these substances ΔV_n is approximately 1·0 and zero respectively.

DISCUSSION

Variations in excitability: qualitative considerations

All of the anaesthetics depolarized the axons to some extent. It is not surprising, therefore, that initially there was in each instance a decrease in the minimum stimulus required to produce an action potential. In three instances (cyclopentane, *n*-pentane and methoxyflurane) the minimum stimulus often became sufficiently small and apparently remained so, that action potentials tended to occur spontaneously until the anaesthetic was removed. The trends in the minimum stimulus in these instances thus reflected the trends in the resting potential. For all the other anaesthetics this was true only for the first few minutes following the introduction of the anaesthetic. Thereafter, the minimum stimulus returned towards control values, while the resting potential changed monotonically to its new less-negative value. It thus appears that there is at least one other mechanism in these systems by which the anaesthetics influence the excitability.

Figure 4 shows that cyclopropane significantly reduces the peak Na^+ current at small depolarizations, and inspection of Table 2 reveals that, with the exception of cyclopentane and *n*-pentane, all the anaesthetics shift the voltage dependence of Na^+ current activation in the depolarizing direction. In the Hodgkin–Huxley formulation, shifts in the voltage dependence of activation simply subtract from changes in the resting potential. Depolarization of the axon and depolarizing shifts in activation thus tend to nullify each other. Other factors being equal therefore, when $\Delta V_R = \Delta V_m$ (or ΔV_p) (Tables 1 and 2) the excitability of the axon should be the same as its control value. Consideration of the data in Tables 1 and 2 shows that this is approximately as observed and, moreover, there is a correlation between the steady-state values of I_{ss}/I_c and $(\Delta V_R - \Delta V_m)$ (Fig. 6).

The temporary increase in excitability observed for short exposures to anaesthetic seems to arise because the resting potential tends to its steady-state value more rapidly than does the activation shift. Thus in Figs 1 and 3 the time for the resting

potential to reach half its final value is 1–2 min whereas the comparable time for the decline in the peak Na^+ current and the associated Hodgkin–Huxley parameters is at least twice as long at comparable or higher concentrations (Haydon *et al.* 1980; Haydon & Urban, 1983*a*, *b*, *c*). The reason for this is not necessarily that there is a

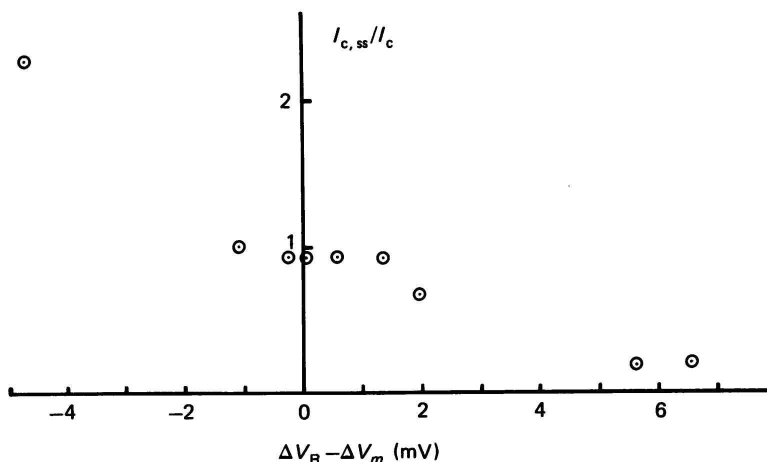

Fig. 6. The correlation in the steady state (after *ca.* 20 min exposure to anaesthetic) between the minimum stimulus ratio (test to control, $I_{c,ss}/I_c$) and the difference between the change in the resting potential and the voltage shift of the steady-state activation curve, $\Delta V_R - \Delta V_m$.

slow conformational change in the Na^+ channel protein. More likely it arises because, at the concentrations used, the effect on the resting potential is near saturation (see Fig. 3 of Haydon *et al.* 1988) whereas the effects on the Na^+ channel are small and the concentration dependence still in the linear region. This being so, it is easy to see that the resting potential will reach its half-final value before the Na^+ channel activation does.

Quantitative considerations

The foregoing explanation of the variations in excitability may be examined more thoroughly by the use of the results of the preceding paper (Haydon *et al.* 1988) and by calculations based on the observed resting potentials and the changes in the Hodgkin–Huxley parameters.

The resting potential. It was concluded by Haydon *et al.* (1988) that the depolarizing changes produced by the anaesthetics in the resting potential originated from reductions in a non-voltage-gated component of the K^+ conductance of the axon. For six of the anaesthetics these reductions are listed in Table 3 of the above-mentioned paper and it is possible to estimate from them how large the changes in resting potential should be. The basic equation required is that proposed in Haydon *et al.* (1988), i.e.

$$V = \frac{g_{Na}E_{Na} + (g_K + g'_K)E_K + g'_L E'_L}{g_{Na} + g_K + g'_K + g'_L}$$

TABLE 3. A comparison of calculated and observed resting potential changes and minimum stimuli ratios

Anaesthetic	g'_K (mS cm^{-2})	ΔV_R(mV) Calculated	ΔV_R(mV) Observed	$I_{c,ss}/I_c$ Calculated	$I_{c,ss}/I_c$ Observed
Chloroform	0·071	2·26	2·9	1·41	1·01
Cyclopentane	—	—	3·5	0	0·21
Methoxyflurane	0·0	3·93	4·0	0·79	0·71
Diethyl ether	0·102	0·62	1·1	1·00	0·94
n-Pentanol	0·045	2·73	4·4	1·45	0·94
Halothane	0·070	2·51	2·1	0·72	0·95
Cyclopropane	—	—	2·6	0·93	0·87
n-Pentane	0·06	—	1·8	0	0·18
Dihepatonoyl phosphatidylcholine	—	—	0·9	2·38	2·27

The resting potential changes were deduced partly from the K$^+$ conductances g'_K determined in the preceding paper (Haydon *et al.* 1988) and given in the second column. The minimum stimuli were based on the observed resting potential values. Details of the calculations are given in the text.

where g_{Na} is the voltage-gated, TTX-sensitive Na$^+$ conductance, g_K is the delayed rectifier (K$^+$) conductance, g'_K is the residual K$^+$ conductance (i.e. the K$^+$ conductance other than the delayed rectifier) and g'_L is the leakage conductance. E_{Na}, E_K and E'_L are the corresponding equilibrium potentials for the different ions involved. g'_K is the conductance which appears to be especially sensitive to anaesthetics and was calculated to be normally 0·206 mS cm^{-2}. Similarly, g'_L was concluded to be 0·094 mS cm^{-2} and E'_L was, to a first approximation, taken to be zero. The values for g'_K for anaesthetics given in Table 3 of Haydon *et al.* (1988) cannot be used directly since they are for 430 mM-external K$^+$. It seems reasonable, however, to assume that the fractional changes in g'_K will be the same at 10 mM-K$^+$ and appropriately scaled values are shown in Table 3. The remaining terms in eqn (1) are standard Hodgkin–Huxley parameters and, for a normal axon, can be taken or calculated from the data given in Table 3 of Hodgkin & Huxley (1952). When anaesthetics are present the voltage shifts in the Hodgkin–Huxley parameters, given in Table 2, must be incorporated (the details of how this was done are described in the Appendix.)

The resting potential changes calculated are shown in the third column of Table 3 and, for ease of comparison, the observed values of Table 1 are given in the fourth column. The agreement between the two sets of results is probably as good as can be expected. The magnitudes are roughly correct and the trends observed are reasonably well reflected in the calculated values.

Action potentials and the minimum stimulus

Membrane action potentials were computed by means of a FORTRAN program based on that described by Palti (1971), but with various minor refinements introduced by J. E. Kimura, B. W. Urban and the present authors. For the normal axon, the input parameters are as in Table 3 of Hodgkin & Huxley (1952). These give a resting potential of $-60{\cdot}05$ mV, an action potential amplitude of 100 mV and a minimum stimulus applied over 100 μs of 58 μA cm^{-2}.

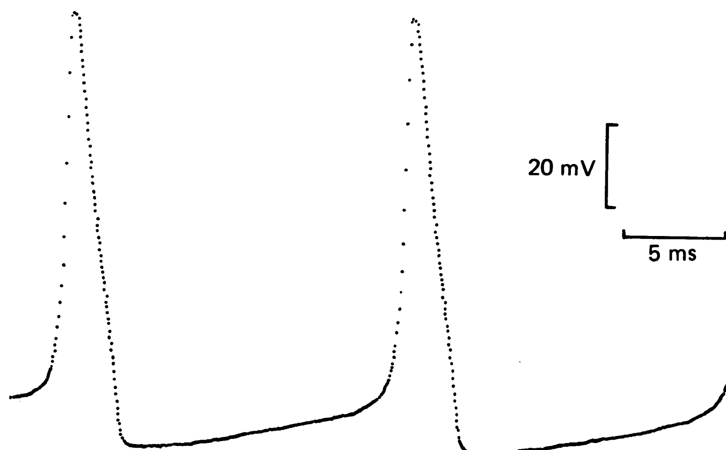

Fig. 7. Spontaneous action potentials, computed as described in the text, for an axon exposed to 0·2 saturated *n*-pentane for *ca.* 15 min.

When anaesthetics are present, Hodgkin–Huxley parameters have to be adjusted as described for the calculation of the resting potential changes. For the stimulus calculation, unlike the resting potential, the experimental values of g'_K were not used since they were not available for all systems. Instead, the observed resting potential changes were used. These are known with reasonable accuracy for all systems and constitute a better starting point than the g'_K values. The basic model is nevertheless the same.

The minimum stimuli calculated for the steady state (I_{ss}) are shown as ratios to the control in Table 3. Beside these, for comparison, are shown the experimental values taken from Table 1. It is immediately obvious that for the very low values (cyclopentane and *n*-pentane) and for the very high value (diheptanoyl phosphatidylcholine) there is good agreement between calculated and observed results. (The closeness of the agreement is obviously limited by the fact that the experimental observations were for a relatively small number of axons which behaved variably, e.g. not all the axons in cyclopentane and *n*-pentane exhibited spontaneous action potentials.) For the remaining anaesthetics, with the possible exceptions of chloroform and *n*-pentanol, the agreement is also satisfactory. It is difficult to pinpoint the origin of such discrepancies as exist. It could be the model and the way in which g'_K is involved. On the other hand the results are quite sensitive to the values of the shifts in the Hodgkin–Huxley parameters. While ΔV_m and ΔV_h were directly measured and are reasonably certain, ΔV_n is more difficult to obtain and the linear interpolation used could have introduced errors of the order of 1 mV. This would be sufficient to account for the worst discrepancies.

Changes in the amplitude of the action potentials by the anaesthetics have not been emphasized because in all instances they were less than ~ 15%. An example of the computed spontaneous action potentials produced by *n*-pentane, based on the data in Tables 2 and 3, is given in Fig. 7. The frequency of 59 Hz may be compared with that of 39 Hz observed experimentally for one axon. The agreement may not

seem close, but the frequency is very sensitive to the voltage shifts ΔV_m and ΔV_n in the Na^+ and K^+ currents, especially as ΔV_m approaches zero. Considering the accuracy with which these shifts are known, therefore, the discrepancy is not actually very serious.

APPENDIX

In order to calculate resting potentials and minimum stimuli in the presence of anaesthetics, the Hodgkin–Huxley equations were modified in various, though relatively minor, ways. The equation for the membrane potential, V, has been given (eqn (1) above) and discussed at length in the preceding paper (Haydon *et al.* 1988). The other modifications involve the Hodgkin–Huxley rate constants.

As already mentioned, at the low concentrations of anaesthetics used, the only clearly detectable effects on the Hodgkin–Huxley parameters were voltage shifts although, in addition, changes in resting potential have to be included. The activation processes for both the Na^+ and K^+ currents as manifested by m_∞ and n_∞ parameters appear to undergo simple voltage shifts without obvious slope changes. These shifts, together with the resting potential changes ΔV_R, are additive to the membrane potential and are thus readily incorporated. The rate constants α_m and β_m for Na^+, and α_n and β_n for K^+ then become

$$\alpha_m = \frac{0{\cdot}1[-60-(V+\Delta V_R)+\Delta V_m+25]}{\exp\left[\frac{-60-(V+\Delta V_R)+\Delta V_m+25}{10}\right]-1}, \tag{A 1}$$

$$\beta_m = 4\exp\left[\frac{-60-(V+\Delta V_R)+\Delta V_m}{18}\right], \tag{A 2}$$

$$\alpha_n = \frac{0{\cdot}01[-60-(V+\Delta V_R)+\Delta V_n+10]}{\exp\left[\frac{-60-(V+\Delta V_R)+\Delta V_n+10}{10}\right]-1}, \tag{A 3}$$

$$\beta_n = 0{\cdot}125\exp\left[\frac{-60-(V+\Delta V_R)+\Delta V_n}{80}\right], \tag{A 4}$$

where ΔV_m and ΔV_n are respectively the shifts in m_∞ and n_∞ for the Na^+ and K^+ systems. Apart from the ΔV_R, ΔV_m and ΔV_n, eqns (A 1)–(A 4) are identical with those of Hodgkin & Huxley (1952) transformed to modern potential conventions.

The expressions for the inactivation rate constants α_h and β_h have been treated slightly differently because at higher anaesthetic concentrations, at least, it is quite clear that large shifts are accompanied by changes in slope at the mid-point of the h_∞ curve. As shown previously (Haydon & Urban, 1983*a*; Haydon *et al.* 1984), a combination of a shift and a slope change can be considered as arising from a change in membrane field. In the Hodgkin–Huxley equations this can be taken into account by multiplying the membrane potential by a factor F where $F = V_h/(\Delta V_h + V_h)$. V_h is the potential at the mid-point of the h_∞ curve under normal conditions and ΔV_h is the shift produced by the anaesthetic. The equations for α_h and β_h then become

$$\alpha_h = 0{\cdot}07\exp\left[\frac{-60-F(V+\Delta V_R)}{20}\right], \tag{A 5}$$

and
$$\beta_h = \frac{1}{\exp\left[\frac{-60-F(V+\Delta V_R)+30}{10}\right]+1}. \quad \text{(A 6)}$$

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