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THE ACTIONS OF SOME GENERAL ANAESTHETICS ON THE POTASSIUM CURRENT OF THE SQUID GIANT AXON

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SUMMARY

1. A number of small organic molecules with general anaesthetic action have been examined for their effects on the voltage-dependent potassium current of the squid giant axon. They include representatives of the three classes of anaesthetics examined in previous studies on the sodium current (Haydon & Urban, 1983*a*, *b*, *c*), i.e. the non-polar molecules *n*-pentane, cyclopentane and CCl_4, several *n*-alkanols and the inhalation anaesthetics chloroform, halothane, diethyl ether and methoxyflurane.

2. Potassium currents under voltage clamp were recorded in intact and in intracellularly perfused axons before, during and after exposure to the test substances, and the records were fitted with equations similar to those proposed by Hodgkin & Huxley (1952). Shifts in the curves of the steady-state activation against membrane potential and reductions in the potassium conductance at 60 or 70 mV membrane potential have been tabulated.

3. On the same intact axons, all the anaesthetics with the exception of methoxyflurane reduced potassium currents less than sodium currents by about a factor of two or more.

4. For the *n*-alkanols, butanol to decanol, the concentrations required to reduce the potassium current at 60 mV membrane potential by 50 % were determined. For *n*-butanol to *n*-heptanol, the standard free energy per CH_2 for adsorption to the site of action was estimated to be $-2{\cdot}91$ kJ mol^{-1} as compared with $-3{\cdot}04$ kJ mol^{-1} for reduction of the sodium current. The magnitude of the free energy decreased for alkanols with longer chain lengths.

5. At anaesthetic concentrations that reduce the sodium current by 50 %, the hydrophobic substances *n*-pentane and cyclopentane reduced the maximal sodium conductance, $\bar{g}_{Na}$, and the potassium conductance at 70 mV, g_K^{70}, equally by about a third, while the *n*-alkanols reduced both parameters by less than 10 %. By contrast, diethyl ether and methoxyflurane were more effective in reducing the maximal potassium conductance.

6. All of the test substances examined, except *n*-pentane and *n*-hexane, shifted the voltage dependence of the potassium steady-state activation in the depolarizing

direction. A broad qualitative correlation was found between the shifts in the activation curves for sodium and potassium currents but, quantitatively, the agreement between the two shifts was poor.

7. In *n*-decanol and methoxyflurane solutions, the voltage-clamped potassium currents exhibited pronounced inactivation-like behaviour. These currents can be fitted by the Hodgkin–Huxley formalism if an inactivation term analogous to the sodium current inactivation is added. Most of the other anaesthetics studied also produced some inactivation (or droop) in the potassium currents.

8. The effects of the various anaesthetics on the parameters of the Hodgkin–Huxley equations are consistent with the idea that, in the squid giant axon, the reduction of potassium currents, like the reduction of sodium currents, originates from more than one type of interaction. The comparison of the two current systems suggests also that, as well as interacting with the adjacent lipid, certain anaesthetics may have an additional site of action in or on a membrane channel itself.

INTRODUCTION

Inhalation anaesthetics such as diethyl ether, methoxyflurane, chloroform and halothane are but a few examples of a wide range of small organic molecules which act as both local and general anaesthetics. The spectrum includes non-polar molecules such as hydrocarbons and polar surface active molecules such as alcohols. To consider these two types of molecule as opposites, with the inhalation anaesthetics falling in between, proved a useful concept in recent studies of anaesthetic effects on the sodium current of the squid giant axon (Haydon & Urban, 1983*a*, *b*, *c*; Haydon, Elliott & Hendry, 1984). The observations could be rationalized in terms of the physico-chemical properties of the anaesthetic molecules. With the exception of esters and ketones (Haydon & Urban, 1983*a*; Elliott, Haydon & Hendry, 1984*a*, *b*), no strong reason emerged to invoke specific interactions with either the lipids or the proteins of the membrane, but several factors normally contributed to the changes in the sodium current.

The rather non-specific manner by which the above-mentioned anaesthetics affect the sodium currents leads quite naturally to the expectation that other membrane proteins might be affected in a similar fashion. There are already examples in the literature that this is so. Johannsson, Keightley, Smith, Richards, Hesketh & Metcalfe (1981) and Johannsson, Smith & Metcalfe (1981) described the influence of *n*-alkanes on the activity of ATPases. It is also known that the potassium currents in the squid giant axon can be reduced by general anaesthetics (Armstrong & Binstock, 1964; Moore, Ulbricht & Takata, 1964; Haydon & Kimura, 1981; Paternostre, Pichon & Dupeyrat, 1983). Studies of interactions of general anaesthetics with the potassium currents are scarce, probably because the sodium current is usually the more sensitive. However, one of the reasons for this extra sensitivity is that the sodium current, unlike the potassium current, has an inactivation process which is affected by anaesthetics. Thus, a reduction in inactivation time constant can by itself produce a substantial suppression of the sodium current. To compare more satisfactorily non-specific anaesthetic actions on the sodium and potassium currents, the effects on the sodium inactivation mechanism have therefore to be separated out.

It will be shown in this paper that when such an analysis is attempted, close parallels between anaesthetic effects on the sodium and potassium currents become apparent, as well as similarities between the responses to anaesthetics of these biological channels and the channel-forming antibiotic gramicidin A in artificial bilayer membranes (Hendry, Urban & Haydon, 1978; Pope, Urban & Haydon, 1982). There are also differences, however. Thus, the quantitative correlation between the shifts in the steady-state activation curves for sodium and potassium currents is poor. This suggests that the lipid environment of the two types of ion channel may be different or that there is, at least in some instances, an interaction of the anaesthetics with the channel protein.

METHODS

The experiments were performed on the giant axon of the squid *Loligo forbesi*. 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*). Intact axons were checked for acceptable resting potentials (-55 to -60 mV) and leakage currents. Both intact and perfused axons were studied. The external bathing solution, unless stated otherwise, was 430 mM-choline chloride, 10 mM-KCl, 10 mM-$CaCl_2$, 50 mM-$MgCl_2$, 10 mM-Trizma base, and 0·3 μM-tetrodotoxin (TTX). HCl was added until the solution was at pH 7·6. Axons were internally perfused by a modification of the Tasaki technique; the perfusate consisted of 150 mM-KF, 780 mM-sucrose, and 10 mM-Trizma base. HCl was added to give pH 7·4.

Intact and perfused axons were voltage clamped at -55 to -60 mV, and series resistance compensation was applied. Care was taken to separate successive voltage-clamp pulses by time intervals sufficiently long (typically 5 s) to prevent each voltage-clamp current from being influenced by its predecessor. Experiments were carried out 6 ± 1 °C unless otherwise indicated.

The sources for the anaesthetic compounds were as described in Haydon & Urban (1983*a*,*b*,*c*).

RESULTS

Potassium and sodium current suppression in the intact axon

Figs. 1 and 2 show records from voltage-clamped, intact axons that have been exposed to representatives of hydrophobic (cyclopentane, CCl_4), amphipathic (*n*-octanol, benzyl alcohol), and inhalation (chloroform, diethyl ether, methoxyflurane) anaesthetics.

The sodium currents are the most heavily reduced in all instances except that of methoxyflurane. These records are typical of the substances discussed in this paper. The unusual effect of methoxyflurane is shared by a number of other halogenated ethers and is the subject of another investigation (D. A. Haydon & B. W. Urban, unpublished observations). Halothane, apart from reducing potassium currents, also reversibly added to the instantaneous leak conductance. The effect was present both in intact and in potassium fluoride perfused axons, but has not been examined further.

From records such as in Figs. 1 and 2, the concentrations were determined at which the steady-state outward current for a given membrane potential (usually 60 or 70 mV) was reduced by 50 % (ED_{50}^{ss}). Four or five experiments at concentrations which varied by a factor of about two were usually sufficient for this purpose. Comparable data for the peak sodium current (ED_{50}^{p}) have been compiled previously (Haydon *et al.* 1984). Only intact axons, in artificial sea water of ionic composition

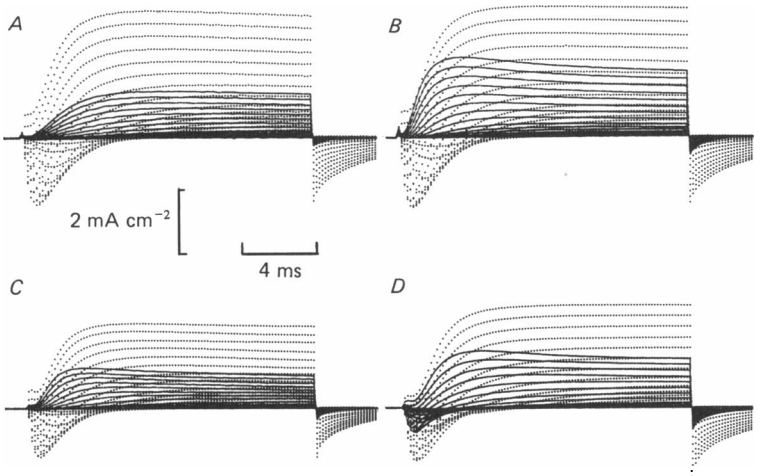

Fig. 1. Plot of voltage-clamp records for intact axons in artificial sea water at anaesthetic concentrations at which potassium currents are approximately half-suppressed (continuous traces) and the respective control records (dotted traces). With the exception of methoxyflurane, the sodium currents have all vanished. *A*, CCl_4 (4 mM), *B*, *n*-octanol (1·05 mM), *C*, chloroform (12 mM), and *D*, methoxyflurane (1·99 mM). Axons were held at −60 mV (−55 mV for axons *A* and *B*) and series resistance compensated. The depolarizations from the holding potentials were 20–50 mV in 5 mV steps, and 60–130 mV in 10 mV steps, preceded by a −30 mV (−15 mV for axons *A* and *B*) pre-pulse lasting 50 ms. For display purposes the base lines towards the end of the pre-pulse have been zeroed. The test data sets were recorded when sodium and potassium currents had ceased to decline. The sea water was as described in Methods except that 430 mM-NaCl replaced the choline chloride.

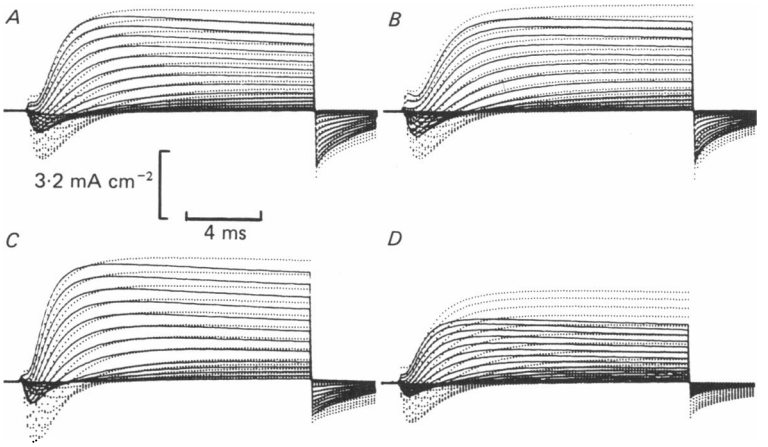

Fig. 2. Plot of voltage-clamp records for intact axons at anaesthetic concentrations at which sodium currents are approximately half-suppressed (continuous traces) and the respective control records (dotted traces). In relation to sodium currents, the potassium currents are reduced much less, and all four examples show an increased droop of potassium currents at large depolarizations. *A*, cyclopentane (0·76 mM), *B*, benzyl alcohol (8 mM), *C*, chloroform (3·75 mM), and *D*, diethyl ether (100 mM). Conditions as in Fig. 1. Axons *C* and *D* were held at −55 mV and preceded by a −15 mV pre-pulse. Axons *A* and *B* were held at −60 mV and preceded by a −20 mV pre-pulse. The artificial sea water was as for Fig. 1.

TABLE 1. Comparison of the anaesthetic concentrations which suppress reversibly by approximately 50 % inward or steady-state outward currents in voltage-clamped intact axons. Sodium current reduction is measured as the ratio of the peak inward currents (measured for a fixed depolarization in the control and test); potassium currents are measured similarly as the maximum of (steady-state) outward current at large depolarizations (membrane potentials of 60 or 70 mV). Numbers in parentheses give the number of axons examined. Data for suppression of sodium currents by alcohols are from Haydon & Urban (1983*b*)

Anaesthetic	ED^{p}_{50} (mM)	ED^{ss}_{50} (mM)	ED^{ss}_{50}/ED^{p}_{50}
n-Butanol	—	108 (5)	—
n-Pentanol	14·8 (7)	32·6 (4)	2·2
n-Hexanol	3·52 (3)	8·96 (3)	2·5
n-Heptanol	0·93 (3)	2·71 (3)	2·9
n-Octanol	0·29 (10)	1·04 (12)	3·6
n-Nonanol	0·068 (2)	0·355 (3)	5·2
n-Decanol	0·022 (2)	0·207 (2)	9·4
CCl_4	1·0 (8)	2·0 (8)	2·0
Chloroform	3·0 (9)	11·2 (7)	3·7
Halothane	2·0 (9)	6·4 (9)	3·2
Diethyl ether	75 (8)	150 (8)	2·0
Methoxyflurane	1·5 (6)	2·1 (7)	1·4

similar in each instance, have been considered. The results for the sodium and potassium currents have been combined in Table 1. With the exception of methoxyflurane, the potassium current half-suppression concentrations are all larger by a factor of at least two.

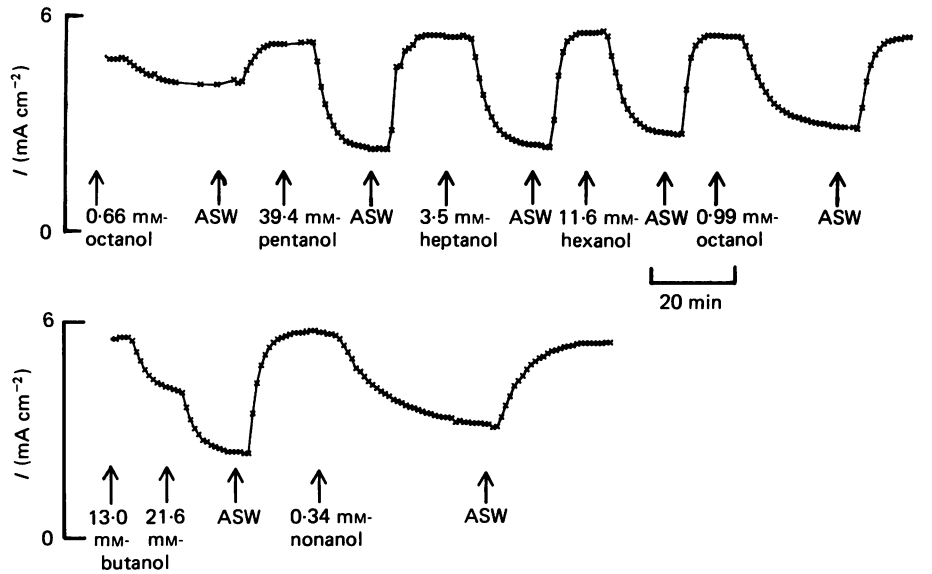

Fig. 3. A continuous record of the time course of action of different chain length *n*-alkanols on the potassium current of an intact axon (following a 120 mV depolarization from the membrane holding potential of −60 mV). At time zero, the axon was exposed to a 0·66 mM-*n*-octanol solution. Note the 'over-recovery' of the potassium current after the return to artificial sea water (ASW). This over-recovery is also evident in the next exposure to *n*-heptanol, but thereafter is less prominent.

n-Alkanol concentrations for 50 % suppression of potassium current

Members of the homologous series of *n*-alkanols were investigated in more detail. On changing from artificial sea water to the test solution the potassium current declined to a value which remained approximately constant (Fig. 3). Whereas for substances with seven (or less) carbon atoms 5–15 min were required to reach the steady state, for *n*-octanol, *n*-nonanol, and *n*-decanol progressively longer times were

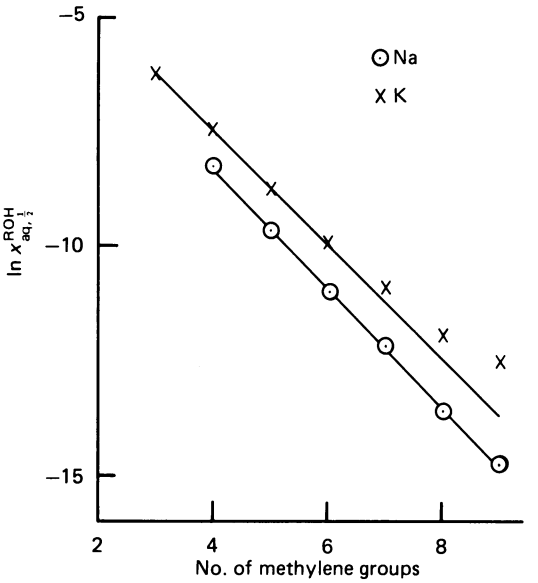

Fig. 4. The chain length dependence of the mole fractions $x^{ROH}_{aq,\frac{1}{2}}$ ($x \approx$ mol l^{-1} alkanol/mol l^{-1} water) of *n*-alkanols in the aqueous phases required to suppress potassium currents (at 130 mV depolarizations from the holding potential) by 50 %. The abscissa is the number of methylene groups in the molecule. For comparison the corresponding mole fractions for peak sodium current suppression are also shown (Haydon & Urban, 1983*b*). A linear regression analysis of the first four potassium current data points yields a straight line almost parallel to the linear regression fit for all the sodium current data points.

required (see Fig. 3). Reduction of the sodium current followed a similar pattern (Haydon & Urban, 1983*b*). Another typical observation is the initial over-recovery of potassium currents after the alkanol has been washed out. Thus the potassium current for a given membrane depolarization in alkanol-free sea water is larger after than before the exposure to alkanol. This effect is most noticeable with the first alkanol application, after which successive recoveries are more uniform. Axons usually lasted for several hours and allowed repeated application of alkanol without showing the irreversible effects found with non-polar substances such as *n*-pentane (Haydon *et al.* 1980). Over-recovery was found for all anaesthetics examined and, even in the absence of anaesthetic, the potassium current (I_K) drifted upwards with time for as long as an hour. The over-recovery was somewhat variable and has been observed (though not discussed) by others (Moore *et al.* 1964). It is as though anaesthetics accelerate the natural upward drift and cause the current to level off rather sooner.

Fig. 4 compares, as a function of *n*-alkanol chain length, the concentrations of

TABLE 2. Summary of the results of the Hodgkin–Huxley analysis of axons exposed to a range of general anaesthetics (column 1), at the concentrations given in column 2. Column 3 records the shift in the mid-point of the n_∞ parameter as a function of membrane potential. The superscript 't' refers to the quantity in the presence of the anaesthetic, 'cr' (control/recovery) denotes the average of the respective quantities before and after wash-out of the anaesthetic. The ratios of the steady-state potassium conductances (g_K) and time constants (τ_n) are given for a membrane potential of 70 mV unless denoted otherwise. i, intact, p, perfused axon; 1, no recovery recorded; 2, $V = 60$ mV; 3, corrected for inactivation; 4, $V = 50$ mV; 5, current ratio taken at the peak (as function of time) of the potassium current

	Concentration (mM)	$V_n^t - V_n^{cr}$ (mV)	g_K^t/g_K^{cr}	τ_n^t/τ_n^{cr}	Remarks
n-Pentane	0·275	−6·1	0·62	0·61	i
	0·275	−3·1	0·70	0·60	i
	0·275	−7·7	0·68	0·56	i
n-Hexane	0·064	−11·8	0·57	0·64	i[1]
Cyclopentane	1·33	6·9	0·66	0·65	i
	1·33	5·7	0·59	0·63	i
	1·33	11·3	0·62	0·76	i
CCl_4	1·0	13·4	0·56	1·21	i
	1·0	15·4	0·62	1·27	i
	2·0	18·0	0·36	1·38	p[2]
n-Pentanol	19·7	9·8	0·94	0·89	i
	19·7	8·7	0·88	0·90	i
	19·7	10·6	0·91	0·84	i
	45·4	17·6	0·76	0·83	p[2]
n-Octanol	0·33	1·9	0·97	0·89	i
	0·33	1·8	1·00	0·87	i[4]
	0·33	2·8	0·92	0·87	i[2]
	1·05	13·7	0·72	0·73	p[2]
	1·05	8·3	0·69	0·73	p[2]
	1·32	15·3	0·44	0·68	p[2]
n-Decanol	0·22	23·9	0·27	0·68	p[1,2,3,5]
	0·22	17·1	0·45	0·74	p[1,3,5]
Chloroform	5·0	13·9	0·98	0·77	i
	5·0	11·8	0·90	0·82	i
	7·5	7·2	0·92	0·87	p[2]
Halothane	5·0	27·7	0·48	0·87	i
	5·0	24·9	0·80	0·85	i
	7·5	26·3	0·32	1·04	p[2,3]
	7·5	22·6	0·55	1·19	p[3,4]
Diethyl ether	100	1·6	0·70	0·67	i
	100	1·6	0·65	0·66	i
	130	0	0·71	0·68	p[2]
Methoxyflurane	3·0	18·9	0·62	0·75	p[3,5]
	3·0	13·3	0·61	0·73	p[2,3,5]
	3·0	21·2	0·32	0·51	i[2,5]
	5·0	28·3	0·36	0·67	p[3,5]

alkanol (ED_{50}^{ss}) needed to reduce by 50 % the potassium currents measured at their maximum at large depolarizations. Included for comparison are the corresponding data for the peak sodium current as given in Haydon & Urban (1983*b*) where a standard free energy of adsorption per methylene group was calculated. The plot for the potassium currents deviates from linearity at higher chain lengths, but between *n*-butanol and *n*-heptanol the data can be fitted reasonably well by a straight line,

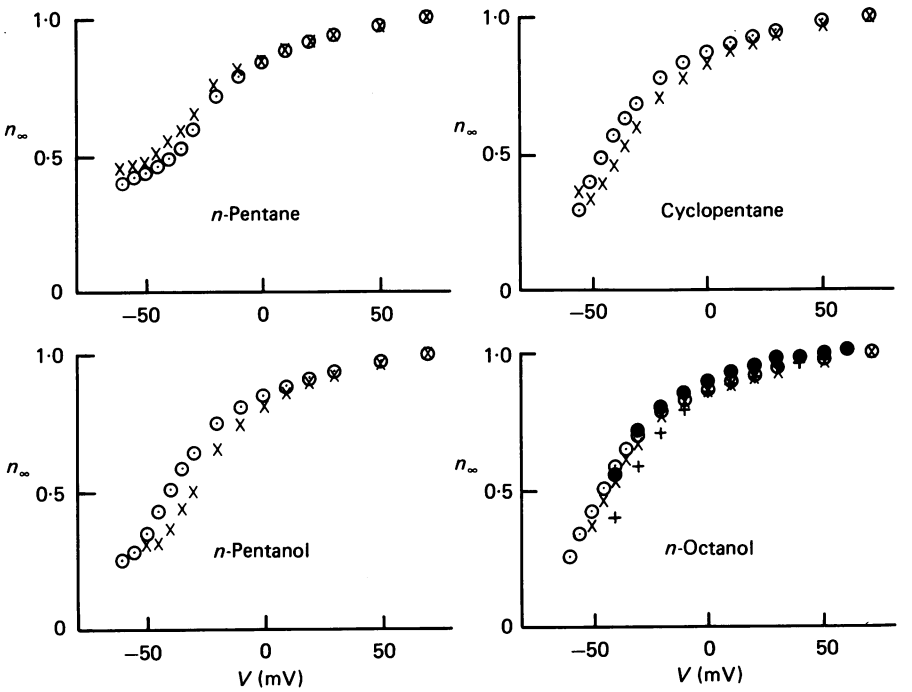

Fig. 5. The steady-state activation parameter n_∞ for the potassium current as a function of membrane potential, V, at an anaesthetic concentration at which the peak sodium current is half-suppressed. The results for the intact axons presented in this Figure approach most closely the average response of three axons (see Table 2). ⊙, average of control and recovery; x, test. *n*-Pentane (0·275 mM), cyclopentane (1·33 mM), *n*-pentanol (19·7 mM), *n*-octanol (0·33 mM). A higher *n*-octanol concentration is also shown (1·05 mM); ●, average of control and recovery; +, test. See Table 2 for further details.

yielding a free energy of $-2{\cdot}91$ kJ mol^{-1}. The free energy for the sodium system was $-3{\cdot}04$ kJ mol^{-1}.

Hodgkin–Huxley analysis of potassium currents

Records of potassium currents before, during and after exposure of intact or perfused axons in the presence of 0·3 μM-tetrodotoxin (TTX) and after subtraction of leakage currents (as in Haydon & Kimura, 1981) were fitted with the equation of Hodgkin & Huxley (1952):

$$I_K = I_{K_\infty}(1-\exp(-t/\tau_n))^4. \quad (1)$$

This equation fitted reasonably well for all anaesthetics studied for the first few milliseconds of the current records. Where pronounced maxima occur (e.g. for *n*-decanol and methoxyflurane), eqn. (1) is obviously unsatisfactory and a different approach was used (see below). The parameters of eqn. (1) for each experiment are given in Table 2. The steady-state activation parameter n_∞ and the time constant τ_n are plotted in Figs. 5 and 6.

Except for the straight chain alkanes *n*-pentane and *n*-hexane, all anaesthetics shifted the steady-state activation parameter in the depolarizing direction. According

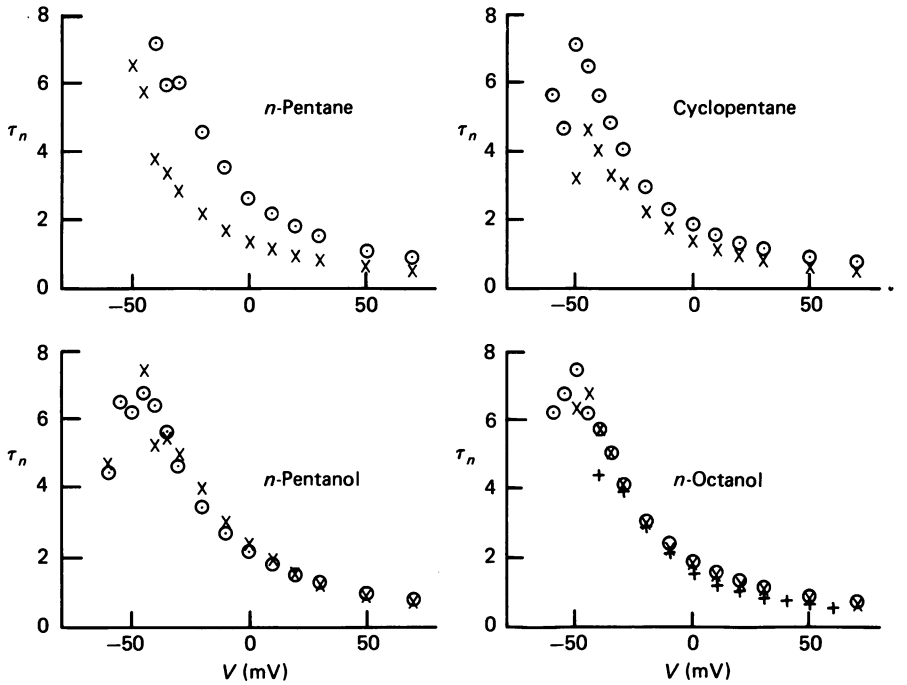

Fig. 6. Time constants τ_n of potassium current activation for the axons of Fig. 5. See Fig. 5 for explanation of symbols. + indicates data points at the higher *n*-octanol concentration. Except for the first two low depolarization values, the control values for this axon are almost identical to those for the lower concentration, and have therefore not been plotted.

to the Hodgkin–Huxley formulae, the peak in the curve of τ_n *versus* membrane potential should move with the mid-point of the n_∞ curve. Owing to the fact that the peak of τ_n occurs at membrane potentials where potassium currents are very small, the time constants in this region cannot be well resolved. Thus, it is possible neither to assess accurately how well shifts in the activation parameter and in the time constants correlate, nor whether the peak of the time constant has been depressed by the anaesthetic. However, where the shifts in the activation curves are large as, for example, for *n*-pentanol (Fig. 5) and CCl_4 and halothane (in Table 2), it appears that the peak of the time constant curve has also been moved in the depolarizing direction.

The potassium tail currents on repolarization to the membrane potential can be strikingly depressed (for example, Figs. 1 and 7), particularly for anaesthetics at concentrations where there is a large shift of the steady state in the depolarizing direction.

Inactivation-like behaviour of potassium currents

Normally potassium currents in the squid giant axon do not inactivate. However, in the presence of *n*-decanol (Fig. 7) and methoxyflurane potassium currents for larger membrane depolarizations exhibited a pronounced maximum. Although other *n*-alkanols and benzyl alcohol also cause some droop in potassium currents, the effect

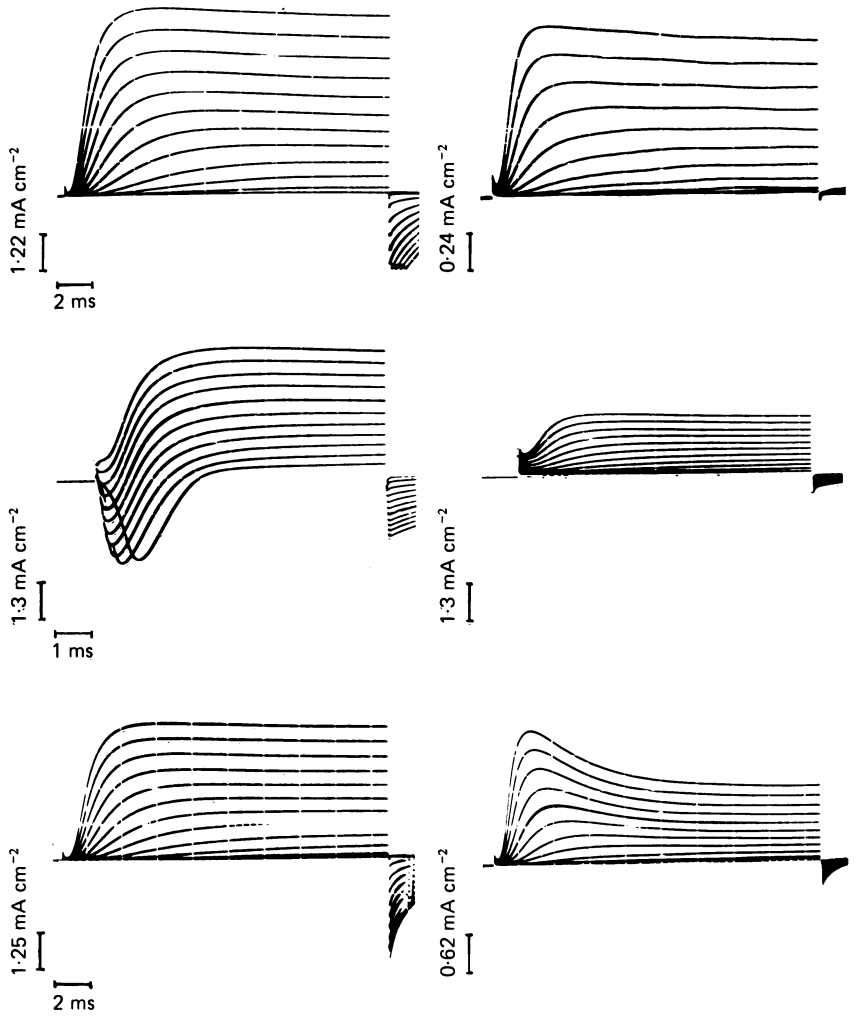

Fig. 7. Comparison of inactivation-like behaviour induced by different *n*-alkanols in voltage-clamped, intact axons. The records on the left have been recorded in the presence of TTX (except for *n*-octanol) before exposure to the alkanol. Top: 65·1 mM-*n*-pentanol, held at −60 mV, 50 ms pre-pulsed to −80 mV, depolarized by 10–120 mV in steps of 10 mV. Time scale as for control. Middle: 3·3 mM-*n*-octanol, held at −60 mV, depolarized by 30–130 mV in steps of 10 mV, no series resistance compensation applied. Bottom: 0·22 mM-*n*-decanol, held at −60 mV, depolarizations as in Top.

is most evident with *n*-nonanol (Fig. 8) and *n*-decanol. This not just a consequence of a high concentration as both the *n*-decanol and *n*-octanol (Fig. 7) are identical in terms of relative saturation. Moreover, *n*-octanol at 3·3 mM (Fig. 7) shows no more droop than at 1·05 mM (Fig. 1).

Fig. 8 compares the effects of *n*-butanol and *n*-nonanol on the same axon at concentrations which produce comparable potassium current reduction. Each voltage-clamp trace is taken at the same membrane depolarization, and at 1 min intervals

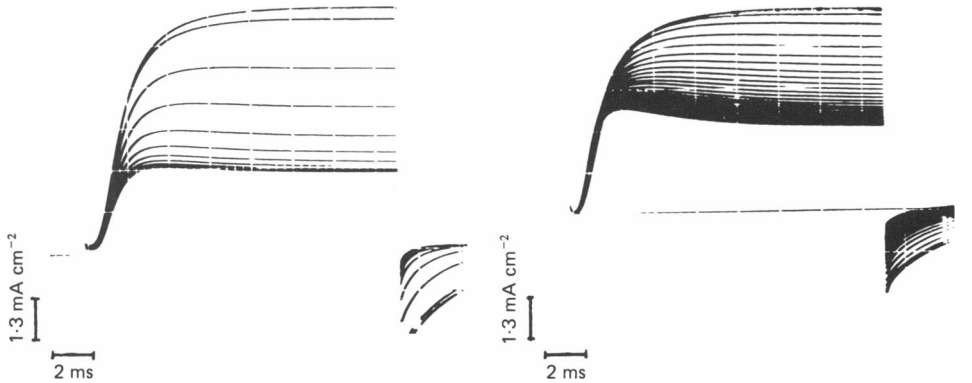

Fig. 8. Time course of potassium current supression in an intact axon (101) for a 120 mV depolarization from a holding potential of −60 mV, preceded by a 50 ms pre-pulse to −80 mV membrane potential. The traces are separated by 1 min intervals and have been taken following the exposure to 17·3 mM-*n*-butanol (left-hand record) and 0·34 mM-*n*-nonanol (right-hand record). In each case, the first three traces overlap (lag in the delivery system).

during the introduction of the alkanol. Potassium currents in the presence of *n*-nonanol droop significantly more than in the presence of *n*-butanol. As noted earlier, the current reduction is accomplished more rapidly by the short-chain alkanol.

The Hodgkin–Huxley description of potassium currents in the squid giant axon does not provide for inactivation-like behaviour. However, the Hodgkin–Huxley equations for the potassium currents can easily be modified to allow for inactivation by adopting the mathematical description used for sodium currents, i.e.

$$I_K = I_{K_\infty}(1-\exp(-t/\tau_n))^4(k_\infty(1-\exp(-t/\tau_k))+\exp(-t/\tau_k)). \quad (2)$$

Analogous to the sodium current nomenclature, the inactivation time constant is called τ_k (corresponding to τ_h), and the steady-state inactivation parameter is k_∞ (corresponding to h_∞) (Ehrenstein & Gilbert, 1966).

The current traces in the presence of *n*-decanol and methoxyflurane could be fitted readily by means of eqn. (2). In Fig. 9 are shown the values of k_∞ and τ_k for *n*-octanol and *n*-decanol. If the difficulties of analysing the data at low membrane potentials (where, e.g. $\tau_n \sim \tau_k$) are allowed for, there is no strong dependence of the parameters on potential.

As can be seen in Figs. 1 and 2, other anaesthetics also produce droop in the potassium currents. However, if droop is quantified as the potassium current at the end of a 16 ms pulse to 70 mV membrane potential, divided by the maximum current during that pulse, then the only anaesthetics listed in Table 2 which give values of this ratio < 0·9 are halothane, *n*-decanol and methoxyflurane. At the concentrations given in Table 2, droop was not a strong function of concentration (cf. the chloroform records in Figs. 1 and 2).

DISCUSSION

Relative efficacy of current reduction

In earlier papers it was concluded that the substances investigated here perturbed sodium currents in the squid giant axon in several different and apparently independent ways. The same appears to hold for the potassium currents. For this reason the comparison of the anaesthetic concentrations which reduce sodium or potassium currents by 50% is of limited value since these concentrations do not correspond to the mid-points of simple dose–response curves. On the other hand, the construction of individual dose–response curves for each of the different effects for each of the anaesthetics would be extremely laborious. The data of Table 1 indicate that the effects of the majority of the anaesthetics on the potassium currents parallel broadly their effects on the sodium current. The longer chain alkanols are, however, one exception and the halogenated ethers, of which methoxyflurane is only partly representative, form another. The halogenated ethers will be discussed more fully elsewhere.

From Table 1 it is concluded that, relative to saturation, more *n*-alkanol is required to reduce potassium currents as the alkanol chain length increases. Between *n*-butanol and *n*-heptanol the free energy of adsorption per methylene group is $-2{\cdot}91$ kJ mol^{-1}. This value is close to the $-3{\cdot}04$ kJ mol^{-1} found for sodium current reduction and suggests a rather similar lipophilic environment for the adsorption site. For the potassium current, however, the magnitude of this free energy decreases for longer-chain alkanols as if they were progressively excluded from a lipophilic environment. This suggests that there may be differences in the lipid environments of these channels or differences in the hydrophobic sites associated with the two channel proteins. Thus it is possible that, with regard to potassium channels, alcohols have two sites of action with differing physicochemical properties. One site would be as lipophilic as a lipid bilayer but might exclude molecules beyond a certain size, as found for the absorption of *n*-alkanes into bilayers or for the interaction of *n*-alkyltrimethylammonium ions with sodium channels (Haydon, Hendry, Levinson & Requena, 1977; Elliott, Haydon & Hendry, 1985). The other site would have a weaker interaction energy and, for substances insoluble in lipid, be accessible only via the open channel. This site is thought likely to be the one involved in the inactivation-like behaviour and is discussed below.

Hodgkin–Huxley parameters

Relevant parameters from a Hodgkin–Huxley type of analysis of sodium and potassium currents are compared for the same anaesthetic concentration in Table 3.

Maximal potassium conductance. Owing to ion accumulation in the Frankenhaeuser–Hodgkin spaces there occur time-dependent changes in the electrochemical driving force for the potassium currents (Frankenheuser & Hodgkin, 1956; Adelman, Palti & Senft, 1973). This problem can be ameliorated by reducing the intracellular potassium in perfused axons, which has been done in some instances. But as ionic conditions can have a strong influence on the kinetics of voltage-gated currents, many experiments were carried out under 'physiological' conditions. The change in the electrochemical driving force due to accumulation leads to an underestimate of the

TABLE 3. Comparison of effects of general anaesthetics on the Hodgkin–Huxley parameters of the sodium and the potassium current system. The superscript 'c' indicates values obtained before, and 't' during, the application of anaesthetic; 'cr' indicates the average of control and recovery data. Data for the potassium system are taken from Table 2: data for the sodium system are from Haydon & Urban (1983 *a*, *b*, *c*). Mean values are followed by their standard deviations. The numbers of data points in each mean are as in the respective Tables

	Concentration (mM)	ΔV_n (mV)	ΔV_m (mV)	g_K^t/g_K^{cr}	$\bar{g}_{Na}^t/\bar{g}_{Na}^c$	τ_n^t/τ_n^{cr}	τ_m^t/τ_m^c
n-Pentane	0·275	−5·6±2·3	−7·3±0·4	0·67±0·04	0·75±0·06	0·59±0·03	0·87±0·03
n-Hexane	0·064	−11·8	−6·3±1·8	0·57	0·74±0·42	0·64	0·90±0·15
Cyclopentane	1·33	8·0±3·0	−4·6±2·5	0·62±0·04	0·64±0·13	0·68±0·07	0·73±0·13
CCl_4	1·0	14·4±1·4	0±0	0·59±0·04	0·72±0·10	1·24±0·04	0·77±0·09
n-Pentanol	19·7	9·7±1·0	16·0±3·6	0·91±0·03	0·96±0·06	0·88±0·03	0·57±0·13
n-Octanol	0·33	2·2±0·6	8·7±0·8	0·96±0·04	0·91±0·05	0·88±0·01	0·79±0·07
Chloroform	5·0	12·9±1·5	6·3±4·6	0·94±0·06	0·77±0·17	0·80±0·04	0·67±0·05
Halothane	5·0	26·3±2·0	8·0±1·4	0·64±0·23	0·86±0·04	0·86±0·01	0·69±0·03
Diethyl ether	100	1·6±0·1	8·0±1·1	0·68±0·04	0·90±0·10	0·67±0·01	0·62±0·09
Methoxyflurane	3·0	17·8±4·1	6·7±2·5	0·52±0·17	0·85±0·15	0·66±0·13	0·68±0·14

potassium conductance at large depolarizations. However, in the conductance *ratios*, which are here of more interest than the absolute values, accumulation effects to some extent cancel.

The potassium conductance ratios may be compared with the corresponding ratios ($\bar{g}_{Na}^t/\bar{g}_{Na}^c$) for the sodium system. To obtain the latter, some estimate of the rate of inactivation is needed and, for the values quoted, the Hodgkin–Huxley treatment was used. The accuracy of this procedure is still uncertain and, in the absence of any viable alternative, there must be some reservations over any quantitative comparison of the two sets of conductances. Subject to this difficulty, there are striking parallels between the sodium and potassium systems. Thus, the hydrophobic substances *n*-pentane, *n*-hexane and cyclopentane reduce the maximal potassium conductance and the maximal sodium conductance by a very similar amount, i.e. about one-third. The alkanols *n*-pentanol and *n*-octanol, on the other hand, produce decreases of $\lesssim 10\,\%$, again in both systems. Qualitatively, these findings parallel those made on another membrane channel, the antibiotic gramidicin A. There it was observed that alkanes reduced drastically the over-all membrane conductance of a lipid bilayer containing gramicidin A (Hendry *et al.* 1978) whereas the effect of *n*-alkanols under similar conditions was much smaller (Pope *et al.* 1982). As discussed previously, the thickness–tension hypothesis could provide an explanation for this parallel behaviour of three very different ionic membrane channels (Haydon *et al.* 1977). The agreement extends to the hydrophobic CCl_4 but it is not as good for other substances. Thus, methoxyflurane and diethyl ether reduce more efficiently the maximal potassium conductance. Chloroform appears to affect the sodium conductance more, but the scatter in the data is quite large and, as in the case of halothane, renders the comparison uncertain. At higher concentrations of the *n*-alkanols (Table 2) g_K^t decreases significantly. This effect was also found for $\bar{g}_{Na}$ (Haydon & Urban, 1983*b*) and was suggested to arise from a weaker interaction with another site, perhaps inside the channel.

Steady-state activation. All of the test substances, except *n*-pentane and *n*-hexane, shift the voltage dependence of the steady-state activation parameter n_∞ in the depolarizing direction. While there is a broad qualitative correlation between these shifts and those for the sodium current parameters m_∞, the quantitative correlation is rather poor. Thus, the hydrophobic substances cyclopentane and CCl_4 shift n_∞ in the depolarizing direction, contrary to their effect on m_∞. Moreover, *n*-pentanol, *n*-octanol and diethyl ether shift m_∞ substantially more than they shift n_∞, whereas chloroform, halothane and methoxyflurane have the opposite effect. It has been suggested previously (Haydon & Urban, 1983*b*; Haydon, Elliott & Hendry, 1984) that dipole potential changes may account for the m_∞ shift, and that this dipole potential might originate in the membrane lipids. If so, the lipid environment of sodium and potassium channels would have to be different. Alternatively, the dipoles could be situated on the membrane channel itself. Indeed, for substances such as the carboxylic esters (Elliott *et al.* 1984*b*) and the halogenated ethers (D. A. Haydon & B. W. Urban, unpublished observations) there is evidence for a direct interaction with the channel protein. Most of the above substances can form hydrogen bonds and, in addition, the halogen atoms in substituted substances may show specific interactions with chemical groups that possess electron donating properties (Martire, Sheridan, King & O'Donnell, 1976). In hydrogen bonds, diethyl ether interacts with electron acceptors, whereas chloroform, halothane and methoxyflurane interact with electron donors; the *n*-alkanols can do both (Joesten & Schaad, 1974). The observed differences in the shifts of sodium current and potassium current steady-state activation could then be rationalized as follows: n_∞ shifts exceed m_∞ shifts for substances like chloroform, halothane, methoxyflurane and CCl_4, which primarily interact with electron donors; n_∞ shifts are smaller than m_∞ shifts for the *n*-alkanols and diethyl ether, which are substances that can interact with electron acceptors.

The depolarizing shifts in the steady-state activation result in a disproportionately larger effect on the potassium current at membrane potentials close to the resting potential. This may be important in determining the threshold for eliciting an action potential. The shifts also account for the large reduction in the tail currents (Figs. 1, 2, 7 and 8).

Time constants. Time constants for activation for the most part become smaller in the presence of anaesthetics but there are some exceptions, for example CCl_4 and halothane (Table 2). The time constants are affected by more than one action of the anaesthetic molecule, as was found for the sodium currents. An analysis of the time constant τ_n in terms of Hodgkin–Huxley rate constants shows that the effects of all the anaesthetics in this study can be understood as a combination of increased rate constants α_n and β_n (same multiplicative factor at all membrane potentials) together with a shift along the voltage axis. According to the magnitudes of these two effects τ_n may either increase or decrease.

Inactivation-like behaviour

The inactivation of the potassium current can be described mathematically, as illustrated in Fig. 9. However, it should be emphasized that the phenomenon is complicated (if only because the accumulation of potassium ions in the periaxonal spaces may contribute to the effect) and the physical origins of k_∞ and τ_k are obscure.

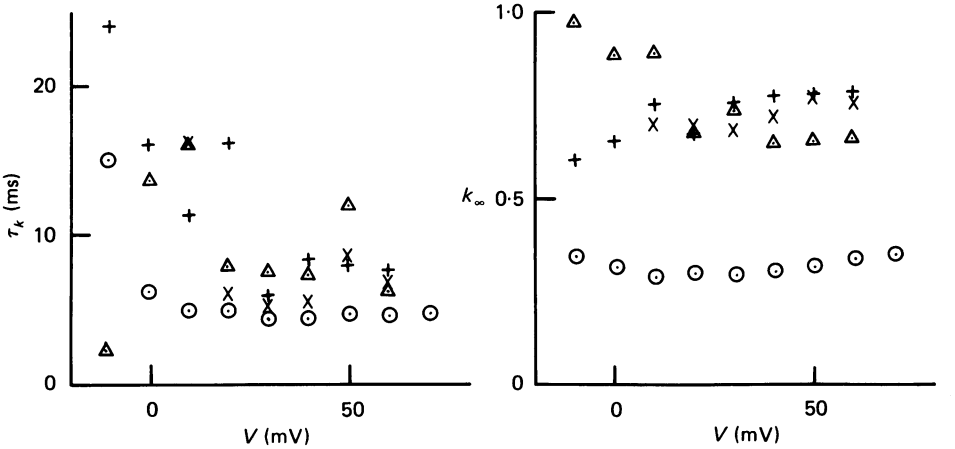

Fig. 9. Plot of potassium current inactivation parameters as a function of membrane potential during a test pulse for four different axons exposed to 1·02 mM-*n*-octanol (+, x), 1·32 mM-*n*-octanol (Δ), and 0·22 mM-*n*-decanol (○). Perfused axons were voltage clamped at −60 mV and compensated for series resistance. No pre-pulses preceded the 16 ms test pulses. Sodium currents were eliminated by addition of TTX.

Thus, although there is little to be gained at present from considering the inactivation quantitatively, the qualitative aspects merit some discussion.

The inactivation of potassium currents in the squid giant axon has been observed under special conditions (Ehrenstein & Gilbert, 1966; Inoue, 1981; Chabala, 1984) and in the presence of the longer chain tetraalkylammonium derivatives (Armstrong, 1975; French & Shoukimas, 1981; Swenson, 1981). Swenson also mentions inactivation caused by *n*-decanol, and Paternostre *et al.* (1983) report inactivation for *n*-octanol and lower-chain-length alcohols. There are similarities between the actions of quaternary ammonium compounds and the alkanols. For example, inactivation is incomplete, the most pronounced inactivation is observed at large depolarizations, and the inactivation produced by the quaternary ammonium ions also increases with increasing alkyl chain length. Armstrong accounts for his observations in terms of all-or-none block of the activated channel by the quaternary ammonium ions, acting from the intracellular side. The time constant of inactivation is considered to be the rate at which quaternary ammonium ions enter the potassium channel once it has been activated. Inactivation is observed for triethyl- and tripropyl- but not for trimethylammonium compounds, and Armstrong argues that the headgroup in the trimethylammonium compounds is small enough to pass through the potassium channel, and hence is no longer capable of blocking it. However, the fact that inactivation is caused by long-chain *n*-alkanols, which possess a much smaller headgroup than the alkyl trimethylammonium ions, demonstrates that a steric block is not an absolute requirement to produce this effect. Armstrong postulates that quaternary ammonium ions, by virtue of their hydrophobic groups, bind more tightly to the channel than, for example, a potassium ion. The *n*-alkanol would be capable of similar binding and so it is possible that such binding, rather than producing a direct block, results in conformational changes of the channel itself.

Although there are similarities, there are also clear differences between the action

of quaternary ammonium ions and *n*-alkanols. Among these are an increasing rate of inactivation with larger depolarizations for quaternary ammonium derivatives, a more complete inactivation than seen with alkanols, and a much smaller potency increase per $-CH_2$ group between heptyltriethylammonium to decyltriethylammonium than between *n*-heptanol to *n*-decanol. It is possible that for the quaternary ammonium compounds both a steric block and conformational changes have to be taken into consideration, whereas the *n*-alkanols may simply accelerate the processes that lead to the inactivation behaviour already observed in their absence (Ehrenstein & Gilbert, 1966; Inoue, 1981; Chabala, 1984). Consistent with this is the observation that the rate of inactivation is faster for *n*-decanol at 0·22 mM than it is for *n*-octanol at 1·32 mM (Fig. 9). The reverse might have been expected (on the grounds of diffusion rates) for the blockage of an open channel or activated state.

Inactivation-like behaviour has also been observed for some incompletely halogenated ethers (D. A. Haydon & B. W. Urban, unpublished observations). Those ethers that produce very pronounced inactivation of potassium currents share certain structural features with *n*-nonanol or *n*-decanol. In particular, they consist of a hydrophobic chain, terminated at one end by a polar group with a hydrogen bonding capability of comparable strength and the sizes of the molecules are similar (D. A. Haydon & B. W. Urban, unpublished observations). The sizes of the molecules relate to the strength of their hydrophobic interactions, which has been suggested to be an important factor in explaining variations in quaternary ammonium ion block (Armstrong, 1975; French & Shoukimas, 1981; Swenson, 1981). Again, therefore, it appears that both polar and hydrophobic interactions may be important in producing potassium current inactivation.

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