Journal of Physiology (1988), **402**, *pp.* 363–374
With 4 text-figures
Printed in Great Britain

THE POTASSIUM CONDUCTANCE OF THE RESTING SQUID AXON AND ITS BLOCKAGE BY CLINICAL CONCENTRATIONS OF GENERAL ANAESTHETICS

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(*Received 29 October 1987*)

SUMMARY

1. The effects of some neutral clinical and experimental general anaesthetics on the resting potential of normal squid axons and squid axons exposed to tetrodotoxin and 3,4-diaminopyridine have been studied.

2. Depolarizations of 1–4 mV were produced by all the anaesthetics at 'clinical' concentrations in the normal axon. Larger depolarizations (5–11 mV) were produced by the same anaesthetic concentrations in axons exposed to tetrodotoxin and 3,4-diaminopyridine.

3. The conductance of axons exposed to tetrodotoxin and either tetraethylammonium or 3,4-diaminopyridine in zero Na^+, 430 mM-K^+ artificial sea water was examined by voltage clamp and AC bridge techniques.

4. The evidence that this conductance is due predominantly to K^+ is discussed.

5. Pre-pulse protocols under voltage clamp have been used to show that part of this conductance arises from the incompletely blocked delayed rectifier.

6. Substantial reductions in this conductance are produced by anaesthetics at 'clinical' concentrations.

7. It is concluded that there is a component of the K^+ conductance of the resting squid axon other than the Hodgkin–Huxley delayed rectifier which is extremely sensitive to anaesthetics and which to an appreciable extent determines the resting potential.

INTRODUCTION

It is well known that, at concentrations used clinically, general anaesthetics do not normally block the propagation of an action potential along an axon. In fact, in the squid axon they have very little effect on either the amplitude or the form of the action potential and, in the steady state, even the threshold stimulus current is not greatly affected. But important changes in an axon can occur on exposure to general anaesthetics at clinical concentrations as demonstrated by reports of spontaneous firing of action potentials (e.g. Haydon, Hendry, Levinson & Requena, 1977; Sevcik,

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1980; Urban & Haydon, 1987). General anaesthetics also tend to depolarize the squid axon (Haydon, Elliott & Hendry, 1984; Urban & Haydon, 1987) and this effect is obviously a possible origin of the enhanced excitability. There is no clear correlation, however, between the extent of the depolarization and the tendency to spontaneous firing since the interrelationship is complicated by the effects of the anaesthetics on the voltage-gated Na^+ and K^+ currents.

Two general questions emerge from the above observations: (i) How do general anaesthetics at 'clinical' concentrations depolarize the axons? (ii) How is the axonal excitability in the transient and steady states related to this depolarization? This paper is concerned with the first question, while the following paper will deal with the second.

Before considering how anaesthetics affect the resting potential it is desirable to know how this potential arises. A survey of the literature shows that this is not at all well understood. It seems generally agreed that Cl^- is not a significant contributor (Russell, 1984) and, by elimination therefore, K^+ is likely to be the principal ion involved. Chang (1986) has drawn attention to what he calls a passive K^+ conductance in the squid axon, i.e. a K^+ conductance other than the delayed rectifier of Hodgkin & Huxley (1952). Chang's evidence is essentially that, when the Na^+ conductance is blocked with tetrodotoxin (TTX) and the conductance of the delayed rectifier is blocked with 4-aminopyridine or tetraethylammonium (TEA), the resting potential remains strongly negative. This result is (or should be) well known to axonologists but it has attracted remarkably little attention. Despite reservations about just how well blocked the delayed rectifier is by TEA or 4-aminopyridine at the normal resting potential, the results emphasize that there are some unanswered questions.

METHODS

The experiments were performed on the giant axons of the squid *Loligo forbesi*. The axons were dissected from freshly killed animals and were cleaned of surrounding fibres and connective tissue; their diameters were usually between 600 and 1000 μm.

The chamber in which the axons were mounted, the electrodes, the means of introducing, and controlling the temperature of, the external bathing solution and the AC bridge technique, have been described by Haydon, Requena & Urban (1980). The voltage clamp and data acquisition procedures were as in Kimura & Meves (1979). Compensation for the series resistance was applied in all voltage clamp experiments. The perfusion method was essentially that introduced by Tasaki and is described in Haydon & Kimura (1981). The perfusate consisted of 150 mM-KF, 780 mM-sucrose and 10 mM-Trizma base. HCl was added to give pH 7·4. Some axons were internally dialysed using regenerated cellulose acetate tubing kindly supplied by Dr John M. Russell, Department of Physiology and Biophysics, University of Texas. The technique was similar to that described by Boron & Russell (1983). The dialysing solution consisted of (concentrations in mM): potassium aspartate, 400; NaCl, 20; $MgCl_2$, 4; EGTA, 4; EDTA, 0·1; HEPES Tris, 10; tetraethylammonium chloride (TEA), 30; and glycine, 100. The pH was adjusted to 7·3. Approximately 20 min were required from the commencement of the dialysis for the equilibrium, or steady state, to be established. The effects of the anaesthetics were independent of whether or not the perfusate or dialysis solution was flowing through the axon.

The artificial sea water consisted of (concentrations in mM): NaCl, 430; KCl, 10; $CaCl_2$, 10; $MgCl_2$, 50; Trizma base, 10. The pH was adjusted to 7·6 by addition of HCl. External K^+ concentrations were varied by substitution of KCl for NaCl. Tetrodotoxin (TTX) was used at 0·3 μM to block Na^+ currents. 3,4-Diaminopyridine (Kirsch & Narahashi, 1978) was preferred to 4-

aminopyridine to block delayed rectifier currents since it was easier to wash out of the apparatus. Normally 1 mM-3,4-diaminopyridine was used but concentrations up to 10 mM were tried without obtaining different results.

The sources of the anaesthetics were as described in Haydon & Urban (1983*a*, *b*, *c*). When dealing with the volatile anaesthetics, precautions were taken during delivery to the axon to avoid loss of vapour into air spaces.

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

RESULTS

K^+ conductance and the resting potential of the squid axon

Hodgkin & Huxley (1952) gave a general equation for the membrane potential, V, of the squid axon in the form

$$V = \frac{g_{Na}E_{Na} + g_K E_K + g_L E_L}{g_{Na} + g_K + g_L}, \tag{1}$$

where g_{Na} is the voltage-gated, TTX-sensitive Na^+ conductance, g_K is the delayed rectifier (K^+) conductance and g_L is the leakage conductance which comprises all remaining conductances. E_{Na} and E_K are the equilibrium potentials for these two ions. They have been assigned the values used by Hodgkin and Huxley, i.e. 55 and -72 mV respectively. With an E_L of $-49{\cdot}4$ mV, $g_L = 0{\cdot}3$ mS cm^{-2} and g_{Na} and g_K as calculated from Hodgkin and Huxley's empirical equations, the excitability of the squid axon is well described.

When the Na^+ and K^+ conductances are blocked, e.g. by TTX and 3,4-diaminopyridine and $g_{Na} = g_K = 0$, eqn (1) requires the resting potential to be $-49{\cdot}4$ mV. This is close to the mean value of $-47{\cdot}3 \pm 0{\cdot}93$ mV found for all the experiments reported in Table 1. The potential of $-47{\cdot}3$ mV apparently arises from the K^+ concentration gradient. This was confirmed by five experiments in which the removal of all K^+ from the system by perfusion of the axon with CsF + sucrose resulted in an average resting potential of $-0{\cdot}5$ mV. This suggests that, when the voltage-gated Na^+ and K^+ conductances are blocked, the membrane potential might be expressed as:

$$V = E_L = \frac{g'_K E_K + g'_L E'_L}{g'_K + g'_L}, \tag{2}$$

where g'_K is a 'residual' K^+ conductance (i.e. what is left after the delayed rectifier has been blocked), g'_L is a non-specific leak and E'_L is effectively its reversal potential. Since the removal of K^+ from the system yields only small and variable membrane potentials, E'_L could, to a first approximation, be taken as zero and eqn (2) would then become

$$V = E_L = \frac{g'_K E_K}{g'_K + g'_L}. \tag{3}$$

For a normal intact axon Hodgkin & Huxley (1952) give

$$g_L = g'_K + g'_L = 0{\cdot}3 \text{ mS cm}^{-2}, \tag{4}$$

and, for $V = E_L = -49{\cdot}4$ mV, it is found from eqns (3) and (4) that

$$g'_K = 0{\cdot}206 \text{ mS cm}^{-2}, \quad (5)$$

$$g'_L = 0{\cdot}094 \text{ mS cm}^{-2}. \quad (6)$$

(E_K has been taken as the value used by Hodgkin & Huxley (1952), i.e. -72 mV.)

According to eqn (3), if the external K^+ concentration is increased by substitution for Na^+, the slope of the curve of membrane potential *versus* the logarithm of the K^+ concentration should be somewhat less than that expected from the Nernst equation. This is found experimentally. In Fig. 1 the maximum slope of the plot is $-51{\cdot}5$ mV compared with the value of $-55{\cdot}3$ mV for a K^+ electrode at 6 °C. Changes in activity coefficients may contribute to this discrepancy but, apart from this, the result tends to confirm the conclusion from eqn (6) that the leak g'_L is not negligible.

TABLE 1. The effects of anaesthetics on the resting potential (V_R) of the squid axon

		Normal axon		TTX + 3,4-DAP	
Anaesthetic	Concentration (mM)	V_R (mV)	ΔV_R (mV)	V_R (mV)	ΔV_R (mV)
Chloroform	1·0	−56·5	2·9 (8)	−42·0	6·6 (1)
Cyclopentane	0·19	−57·9	3·5 (5)	−47·4	7·0 (2)
Methoxyflurane	0·55	−56·2	4·0 (13)	−45·6	7·8 (2)
Diethylether	13·5	−55·8	1·1 (12)	−48·6	6·3 (4)
n-Pentanol	6·0	−57·2	4·4 (4)	−47·4	6·3 (3)
Halothane	0·5	−56·8	2·1 (4)	−49·1	7·2 (5)
Cyclopropane	1·4	−59·6	2·6 (3)	−45·7	4·4 (2)
n-Pentane	0·061	−55·7	1·8 (3)	−52·2	3·4 (3)
Diheptanoyl phosphatidylcholine	0·2	−57·4	0·9 (2)	−47·5	2·4 (1)

'Normal axon' indicates an intact axon in ASW. 'TTX + 3,4-DAP' indicates an intact axon in ASW containing 0·3 μM-TTX and 1 mM-3,4-diaminopyridine. V_R is the mean of the resting potentials before and after exposure to anaesthetic. The depolarizations ΔV_R are the mean of changes on introduction and removal of the anaesthetic. The figures in parentheses indicate the numbers of axons examined.

In order to clarify the effects of anaesthetics on the resting potential it was necessary to find conditions under which the perturbation of the residual K^+ conductance g'_K could be measured. For an axon in artificial seawater (ASW) where the voltage-gated Na^+ and K^+ currents have been blocked respectively with TTX and 3,4-diaminopyridine, the conductance is not only too small for convincing quantitative studies, it is also the sum of g'_K and g'_L. As the external K^+ concentration is raised (by substitution for Na^+), however, the conductance rises several-fold. At 430 mM-external K^+ the conductance is in the region of 4 mS cm^{-2} and it seems reasonable to assume that, under these conditions, $g'_K \gg g'_L$.

A typical voltage clamp record of an axon in 430 mM-K^+ ASW containing TTX and 3,4-diaminopyridine is shown in Fig. 2*A* and a corresponding current–voltage curve in Fig. 2*C*. The comparable Figures for when TEA was used as the blocking agent differ in some respects. The differences are evidently related to potential-dependent blocking or unblocking effects (Armstrong, 1971; Meves & Pichon, 1977;

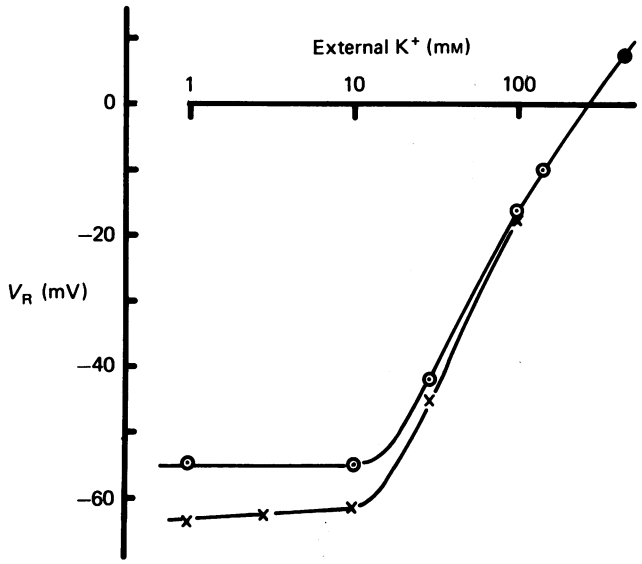

Fig. 1. The resting potential, V_R, of the squid giant axon as a function of external K^+ concentration on a logarithmic scale and in the presence of TTX and delayed-rectifier blockers TEA (30 mM inside) and 3,4-diaminopyridine (1 mM outside). The K^+ was substituted for Na^+ in the ASW leaving the composition otherwise unchanged. ×, TEA only; ⊙, TEA and 3,4-diaminopyridine; ● is the mean of nineteen experiments with either or both blockers in 430 mM-K^+ ASW.

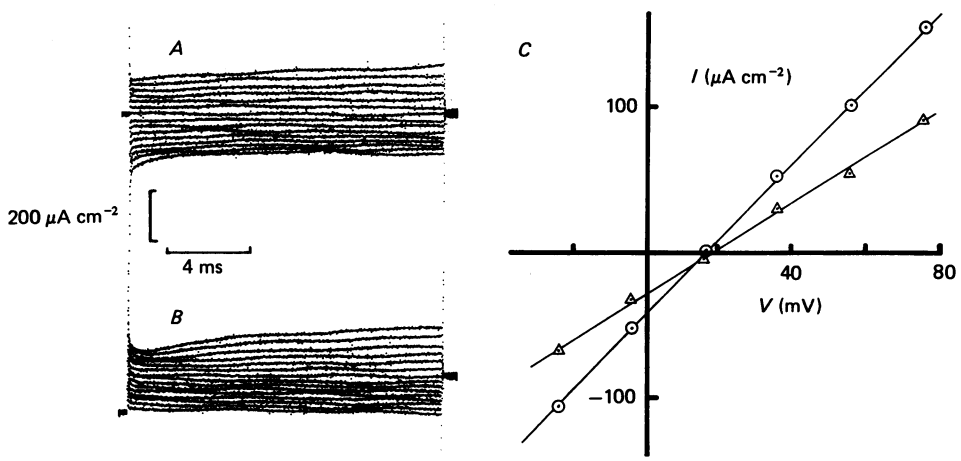

Fig. 2. Voltage clamp results for an intact axon in 430 mM-K^+, TTX and 1 mM-3,4-diaminopyridine. The axon was clamped at its resting potential of 16 mV. Pulses were in 10 mV steps. *A*, no pre-pulse. *B*, pre-pulse of −90 mV for 50 ms. *C*, current–voltage curves for the records of *A* and *B*. Currents were measured at approximately 0·7 ms after the beginning of the pulse or in *B* at the minimum in the trace if this was significantly different in time. ⊙, no pre-pulse; △, pre-pulse of −90 mV. Variation of the pre-pulse between −80 and −110 mV did not affect the current–voltage curve.

Kirsch & Narahashi, 1978) but occur sufficiently far from the reversal point to have no significant effect on the conductance at this potential. Table 2 shows good agreement between conductances measured at the reversal potential for axons exposed respectively to the two blocking agents.

In order to monitor effects of anaesthetics on the conductance at the reversal potential it was convenient to use an AC admittance bridge (see Methods) at a frequency of 200 Hz and with an applied signal of 2 mV root mean square. The AC data for the present systems, corrected for the series resistance, are also shown in Table 2. The conductances were not frequency dependent in the range 200–500 Hz and, for thirteen axons, were not significantly different from those obtained from the voltage clamp technique.

TABLE 2. Conductances (mean ± S.E.M.) at the resting potential (8–15 mV) of axons in zero Na^+, 430 mM-K^+ ASW + TTX

Delayed-rectifier blocker	Total conductance (mS cm^{-2})		Residual conductance (mS cm^{-2})
	g_{vc} (no pre-pulse)	g_{ac}	g_{vc} (pre-pulse to −90 mV)
TEA	3·75 ± 0·06 (5)	4·01 ± 0·11 (5)	—
3,4-Diaminopyridine	3·89 ± 0·35 (7)	4·24 ± 0·30 (7)	1·33 ± 0·11 (6)
TEA + 3,4-diamino-pyridine	4·08 (1)	4·76 (1)	—

Axons were either dialysed with 30 mM-TEA (see Methods) or 1 mM-3,4-diaminopyridine was added to the ASW. g_{vc} are the conductances obtained from voltage clamp data and g_{ac} the conductances from an AC admittance bridge. For further details see text. Figures in parentheses indicate the number of axons examined.

Despite the presence of TEA or 3,4-diaminopyridine it was considered desirable to try to check whether the residual conductance could be wholly identified with g'_K. Even a few per cent inefficiency in the blocking could mean that as much of the observed conductance originated from unblocked delayed rectifier channels as from g'_K itself. As a consequence, voltage clamp experiments were carried out in which pre-pulses to negative potentials were applied. By holding the membrane at *ca.* −80 mV for 50 ms prior to the test pulse, it was reasoned that delayed rectifier channels would close and would open sufficiently slowly during the test pulse to reveal the relative contributions of the two types of K^+ conductance. Current records, with pre-pulses to *ca.* −80 mV, are shown in Fig. 2*B*. This protocol evoked currents with a time course characteristic of the delayed rectifier, but which did not commence from zero on the current scale. There appears to be a very fast-rising current which represents about one-third of the total and which forms, in effect, a pedestal beneath the delayed rectifier current. At ~ 0·7 ms some 5% only of the delayed rectifier current has developed and at this time, therefore, it is possible to segregate almost completely the two contributions to the conductance. The current–voltage curves for the pre-pulsed and non-pre-pulsed membrane are shown in Fig. 2*C*. The mean conductance after pre-pulsing is given for six axons in Table 2. The value of 1·33 ± 0·11 mS cm^{-2} is evidently the experimental value of g'_K under conditions of high external K^+. The fact that the delayed rectifier is not wholly

blocked by TEA or 3,4-diaminopyridine suggests that, with these blocking agents, eqns (3), (5) and (6) may not be strictly valid. Furthermore, correction of eqn (3) for unblocked delayed rectifier, based on data for 430 mM-external K^+, is unlikely to be a reliable procedure since, under the different conditions of voltage and electrolyte, the degree of blocking would not be similar.

The blockage of the residual K^+ conductance (g'_K) by anaesthetics

As mentioned in the Introduction, many, if not all, neutral anaesthetics produce, at 'clinical' concentrations, a small but significant depolarization of the squid axon. This effect is in magnitude usually 1–4 mV (Table 1) and is at first sight surprising in view of the fact that, at the concentrations involved, the anaesthetics have a very small effect on the delayed rectifier conductance (Haydon & Urban, 1986). These experiments were repeated in the presence of TTX with results that were not significantly different.

A much larger effect of the anaesthetics on the resting potential is found if the axon is first exposed to TTX and 3,4-diaminopyridine. As described above, the resting potential is then ~ -47 mV and, in presence of the anaesthetic, may decrease (in magnitude) by at least 10 mV (Fig. 3; Table 1). Halothane and diethylether were particularly effective even at concentrations comparable to the minimum alveolar concentrations (MACs) which have been reported for e.g. dogs (Eger, 1974). Equation (3) suggests the basis of an explanation for the results of Fig. 3. The literature contains ample evidence that anaesthetics do not, in general, affect equilibrium potentials. The effects of Fig. 3 can then only be accounted for if either g'_K decreases or g'_L increases.

These possibilities can be distinguished by measurements of the conductance with and without anaesthetic present. By use of the AC bridge at 200 Hz it was found that at low anaesthetic concentrations (in the presence of TTX and 3,4-diaminopyridine) the conductance decreased. The effects were small ($\sim 0{\cdot}1$ mS cm^{-2}) and not quantitatively reproducible but were sufficient to suggest that K^+ conductance was being reduced rather than leakage conductance increased. By using the data of eqns (5) and (6) and assuming g'_L is not changed, it can be calculated that in order to produce the result in Fig. 3 halothane at 0·4 mM (for example) would have to block 48% of the residual K^+ conductance g'_K.

In order to demonstrate the effects of the anaesthetics on g'_K, experiments were carried out in 430 mM-external K^+, as described in the preceding section. Approximately 1 h was allowed to elapse after changing the ASW to the 430 mM-K^+ ASW + TTX and introducing the TEA or 3,4-diaminopyridine. By this time the conductance was more or less steady and the time course of the action of the anaesthetic could be followed. Examples of the results are shown in Fig. 4. It is clear from these records that the anaesthetic reduces the conductance in a concentration-dependent manner and that the effects are reversible. On occasions a transient over-recovery was observed (see e.g. the records for chloroform, methoxyflurane and pentanol). This effect was found only for higher concentrations and may reflect the fact that at such concentrations some anaesthetics have been found to increase the leak (Haydon & Kimura, 1981; Haydon & Urban, 1986). Thus, if the leak recovered more slowly than the K^+ conductance, a maximum in the curve would occur. It

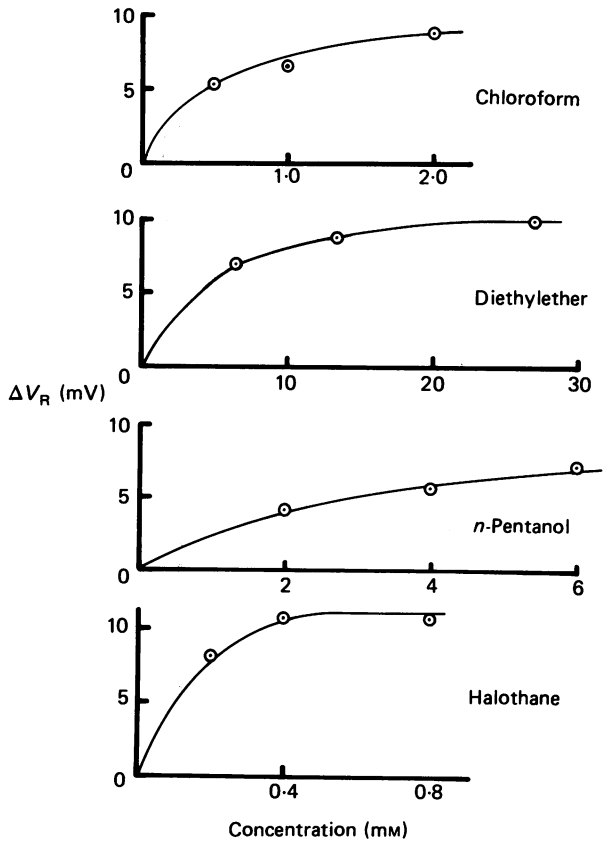

Fig. 3. The effects of anaesthetics on the resting potentials of axons in normal ASW+TTX and 3,4-diaminopyridine. ΔV_R is the depolarizing change relative to the resting potential. The mean resting potential in the absence of anaesthetic was −47·3 mV (for twenty-three axons) (Table 1). The continuous lines have no significance other than as guides to the eye. The anaesthetic concentrations are in the 'clinical' range. The resting potential changes are considerably larger than are produced by comparable anaesthetic concentrations in the normal axon (i.e. without TTX and 3,4-diaminopyridine; Table 1). The circle round each point gives an indication of the standard error.

would follow that at high anaesthetic concentrations ($\sim$ MAC $\times$ 10) the interpretation of the conductances would be greatly complicated.

Some experiments were carried out to check that the observed conductance changes did in fact originate from changes in K^+ conductance. After removal of all K^+ from the system, by perfusion of the axon with CsF (see Methods) and by replacing external K^+ by Na^+ in the ASW, the anaesthetics at low concentrations had no significant effect on the axonal membrane conductance. Replacement of the Cl^- in the K^+ ASW by methane sulphonate also had no effect on the conductance *change* produced by diethyl ether. It was concluded that the major part of the conductance change in presence of the anaesthetics was due to variations in K^+ conductance. In this connection it was established that the changes in conductance (and membrane

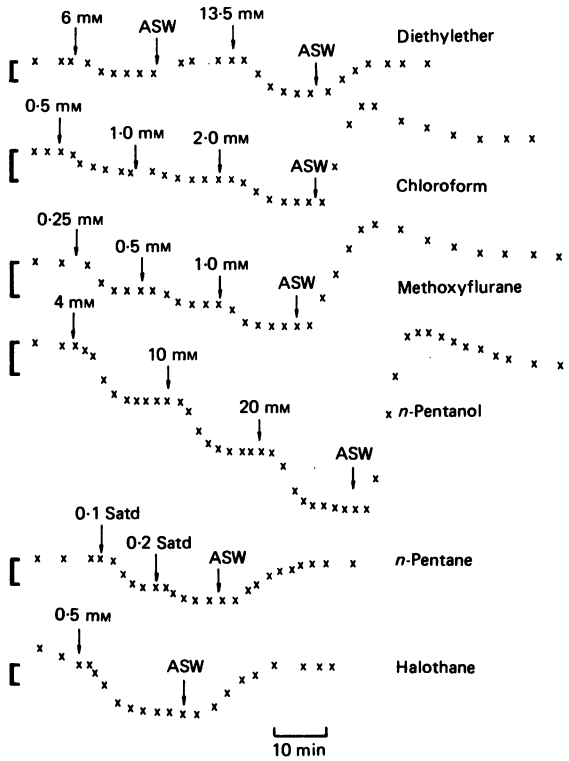

Fig. 4. The effects of anaesthetics on the conductance of axons bathed in 430 mM-K^+ ASW + TTX and either 3,4-diaminopyridine or TEA. The conductance was measured at 200 Hz by means of an AC bridge and corrected for the series resistance (see text). Arrows indicate the introduction of anaesthetic at the concentration shown or a change to ASW. The bars to the left of each record represent 0·5 mS cm^{-2}. The conductances at the start of the records were (in mS cm^{-2}): diethylether, 3·38; chloroform, 4·00; methoxyflurane, 3·40; *n*-pentanol, 3·86; *n*-pentane, 5·11; halothane, 4·08. The transient over-recoveries seen for chloroform, methoxyflurane and *n*-pentanol are discussed in the text. Satd, saturated.

potential in the normal axon) did not depend on the presence or absence of ATP in the dialysis fluid.

DISCUSSION

It was shown in the Results that approximately two-thirds of the total conductance in the data of Fig. 4 arises from unblocked delayed rectifier channels. It is possible, therefore, that part at least of the conductance changes produced by the anaesthetics occur through an action on these channels. While there must certainly be some contribution from this effect, it is believed to be very small at low anaesthetic concentrations. The effects of the neutral anaesthetics on the delayed rectifier have been fairly thoroughly investigated (Haydon & Urban, 1986 and papers cited therein). As for the Na^+ conductance, anaesthetics tend to affect all the

Hodgkin–Huxley parameters of the delayed rectifier. However, the conditions of the present experiments are such that the ion channels are at a membrane potential of ~ 10 mV and in a steady state. Time-constant effects are not important therefore and, at 10 mV, the activation parameter n_∞ is on a plateau and the shifts that can occur in this quantity are also unimportant. The only parameter to consider is the maximum conductance g_K. The concentrations of anaesthetic which have a significant effect on g_K are an order of magnitude higher than those of interest here, and at lower concentrations the responses observed are very roughly linear. By interpolation, therefore, it is readily seen that, for present purposes, changes in the delayed rectifier conductance can be neglected for all but very high concentrations of anaesthetic.

TABLE 3. Conductance changes produced by anaesthetics in axons in zero Na^+, 430 mM-K^+ ASW + TTX and with TEA and/or 3,4-diaminopyridine to block the delayed rectifier.

Anaesthetic	Concentration (mM)	MAC (mM)	Conductance decrease (mS cm^{-2}; mean ± S.E.M.)
Diethylether	13·5	12·9	0·67 ± 0·13 (4)
Chloroform	0·5	1·0	0·87 (2)
	1·0		1·10 (2)
Methoxyflurane	0·25	0·34	1·09 (2)
	0·55		1·36 (2)
n-Pentanol	6·0	—	1·04 ± 0·25 (4)
n-Pentane	0·061 (0·2 satd)	—	0·94 ± 0·19 (6)
Halothane	0·5	0·23	0·88 ± 0·26 (3)

Some minimum alveolar concentrations (MAC) for dogs (Eger, 1974) are shown for purposes of comparison. The conductances should be considered in relation to the value of 1·33 mS cm^{-2} given for the residual K^+ conductance g'_K in Table 2. Figures in parentheses indicate the numbers of axons examined. Satd, saturated.

If changes in g'_K are mainly responsible for the observed effects of the anaesthetics on the conductance, then obviously the conductance decreases should not be larger than the value of g'_K deduced from the pre-pulse experiments in the Results. Ignoring concentrations > 4 mM in the pentanol experiment, all of the results in Fig. 4 and in Table 3 are less than or comparable to the 1·33 mS cm^{-2} given in Table 2. The conductance changes could therefore have arisen from effects on g'_K. Minimum alveolar concentrations are shown for comparison in one or two instances. These are for dogs (Eger, 1974) but, despite the temperature and species difference, they indicate that the general level of concentrations used here is the 'clinical' range. In one or two instances (e.g. methoxyflurane and *n*-pentanol) the conductance decrease clearly exceeds 1·33 mS cm^{-2} at higher concentrations. This is evidently because these substances do affect the delayed rectifier appreciably at higher concentrations. (Possibly, though not probably, they may reduce the leak.) Owing to the problem of the overlap of the effects on the two types of K^+ conductance no serious attempt was made to obtain dose–response curves. (The voltage clamp pre-pulse approach was not considered accurate enough to separate the effects for this purpose.)

It is possible, in principle, to calculate from the fraction of g'_K blocked by anaesthetic the membrane potential changes recorded in Fig. 3 and Table 2. However, it is necessary to use the Hodgkin–Huxley equations as well as the Goldman equation (Goldman, 1943) and, after various other assumptions, it is doubtful whether the results are quantitatively significant. However, resting potential changes in the normal axon (i.e. where the delayed rectifier conductance is not blocked) have been calculated in the following paper and fair agreement is found with the observations.

In conclusion, there is a component of the K^+ conductance in the resting squid axon which is extremely sensitive to a range of simple neutral organic molecules including several general anaesthetics in clinical use. Blockage of this conductance by the anaesthetics appears to result in small but significant depolarizations of the axon. The concomitant tendency to increase the excitability of the axon has implications for spontaneous action potential generation by anaesthetics. The extent to which this occurs will be the subject of the following paper.

J.R. was awarded a travel grant by the Fundacion Vollmer, Caracas, Venezuela, which made possible his visit to the Laboratory of the Marine Biological Association.

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