Biochimica et Biophysica Acta, 978 (1989) 337–340
Elsevier

BBA 70594

BBA Report

The effects of homologous series of anaesthetics on a resting potassium conductance of the squid giant axon

A.A. Elliott [1,3], J.R. Elliott [1,3] and D.A. Haydon [2,3]

[1] *Department of Anatomy and Physiology, The University, Dundee,* [2] *Physiological Laboratory, Cambridge and* [3] *Plymouth Marine Laboratory, Plymouth (U.K.)*

(Received 3 October 1988)

Key words: Anesthetic; Potassium conductance; (Squid giant axon)

The effects of *n*-alkanes (*n*-pentane to *n*-octane), *n*-alkanols (*n*-pentanol to *n*-undecanol) and two carboxylic esters (methyl pentanoate and methyl octanoate) on the conductance of squid giant axons in a high potassium, zero soddium bathing solution have been examined. Sodium and delayed rectifier potassium channels were as far as possible pharmacologically blocked. A substantial fraction of the measured conductance is attributed to a recently-described, voltage-independent, potassium channel. Anaesthetics block this channel but its sensitivity is markedly different from those of other squid axon ion channels.

The anaesthetic actions of members of the *n*-alkane and *n*-alkanol homologous series have been widely reported. The *n*-alkanes are active local and general anaesthetics up to a cut-off point at or around *n*-nonane [1–5] but the *n*-alkanols retain activity up to around *n*-dodecanol [6–10]. These observations of the occurrence and position of a decline in activity have been used in attempts to model the site or sites of action of anaesthetics [4,11,12]. Studies have also been made of the local [13,14] and general [14,15] anaesthetic actions of the methyl *n*-alkyl carboxylic ester series. Methyl octanoate is an effective local and general anaesthetic.

Many voltage clamp investigations of local anaesthetic activity have been carried out using the squid giant axon and have focussed on the best characterised ion conductances in that preparation, the voltage-dependent sodium and delayed rectifier potassium conductances [16]. Relatively little attention has been paid to non-voltage-gated conductances, although the possible contribution of effects on such channels to anaesthetic-induced changes in the resting potential has been considered [17]. Recently, however, attention has been drawn to the presence of an axonal potassium conductance separate from the delayed rectifier. This is not voltage-gated, appears to make a substantial contribution to the resting membrane potential and is appreciably reduced by clinical concentrations of general anaesthetics [18]. Inhibition of this conductance can lead to spontaneous firing of the axon [19]. It may be pertinent that hyperexcitability is sometimes encountered during the induction of general anaesthesia [20].

This paper is a report of work carried out to further characterise the sensitivity of this 'resting' potassium conductance to structurally simple anaesthetics and in particular, to investigate the occurrence of any cut-off or marked decline in activity of homologous series of *n*-alkanes, *n*-alkanols and carboxylic esters.

Experiments were carried out on finely cleaned axons of *Loligo forbesi*, at the Plymouth Marine Laboratory. Details of the axon chamber, the electrodes, the means of preparing and introducing test solutions and the a.c. bridge technique used to measure conductance have been described previously [18,21]. The signal applied to measure conductance was at a frequency of 200 Hz and an amplitude of 2 mV root mean square. The temperature of the bathing solution was maintained at $6 \pm 1°C$. The high potassium, zero sodium artificial sea water (asw) used as a bathing solution contained (concentrations in mM): KCl, 440; $CaCl_2$, 10; $MgCl_2$, 50; Trizma base, 10. The pH was adjusted to 7.6 (at 6°C) by addition of HCl. 0.3 μM tetrodotoxin (TTX) was added to block Na^+ currents and in most experiments 1 mM 3,4-diaminopyridine (3,4-DAP) was added to block the delayed rectifier channel. In some experiments, internal tetraethylammonium ions (TEA) at 30 mM were used to perform that function, with no difference in the actions of anaesthetics. Axons were dialysed internally with a solution containing (concentrations in mM): potassium

Correspondence: J.R. Elliott, Department of Anatomy and Physiology, The University, Dundee, DD1 4HN, U.K.

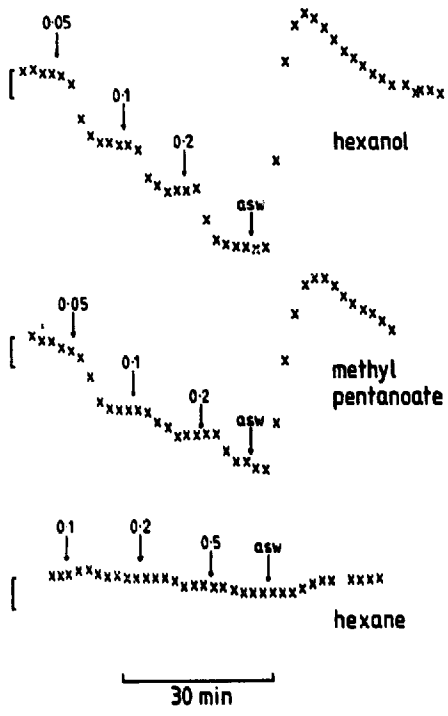

Fig. 1. The time course of effect of anaesthetics on the conductance of squid giant axons bathed in 440 mM K asw and with blockers for the sodium and delayed rectifier potassium channels. Arrows indicate the introduction of anaesthetic at the fractional saturation shown or a change to asw. The bars to the left of each record represent 0.5 $mS \cdot cm^{-2}$. The conductances at the start of the records were (in $mS \cdot cm^{-2}$): hexanol, 6.27; methyl pentanoate, 4.89; hexane, 4.44.

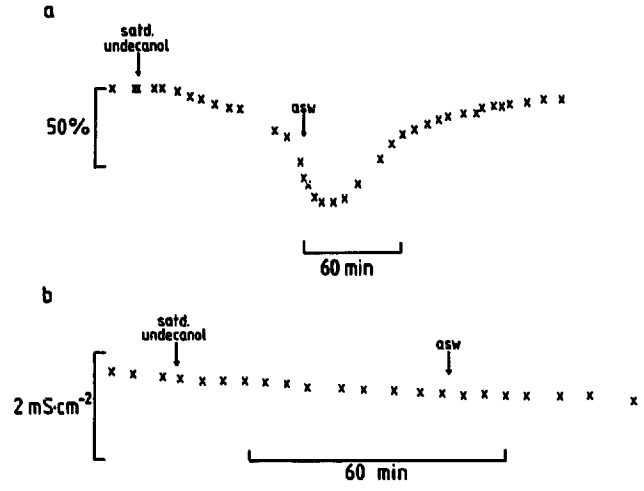

Fig. 2. (a) The effect of *n*-undecanol on action potential amplitude in a squid axon bathed in 430 mM Na asw with no additional ion channel blockers. The bar to the left indicates 50% reduction of action potential height relative to the average value before introduction of undecanol. Arrows indicate the switch from control asw to asw saturated with *n*-undecanol and then the return to control asw. (b) The effect of *n*-undecanol on the conductance of the squid giant axon under the conditions described in the legend to Fig. 1. The starting conductance was 3.70 $mS \cdot cm^{-2}$.

aspartate, 400; NaCl, 20; $MgCl_2$, 4; EGTA, 4; EDTA, 0.1; Hepes-Tris, 10; glycine, 100 (pH 7.3). The suppression of action potential height by anaesthetics was measured by standard techniques [5].

In the high potassium, zero sodium asw, and with sodium and delayed rectifier potassium channels blocked, the unclamped axon had a resting membrane potential of around 10 mV. The conductance (g) at 200 Hz declined over a period of minutes to a virtually steady level. Test solution containing anaesthetic was usually introduced after a steady conductance level was achieved. In some cases inspection of the control, test and reversal segments of the experimental records revealed a slow downwards drift of conductance which was not attributed to the anaesthetic. This has been allowed for in calculating the effect of the test substance.

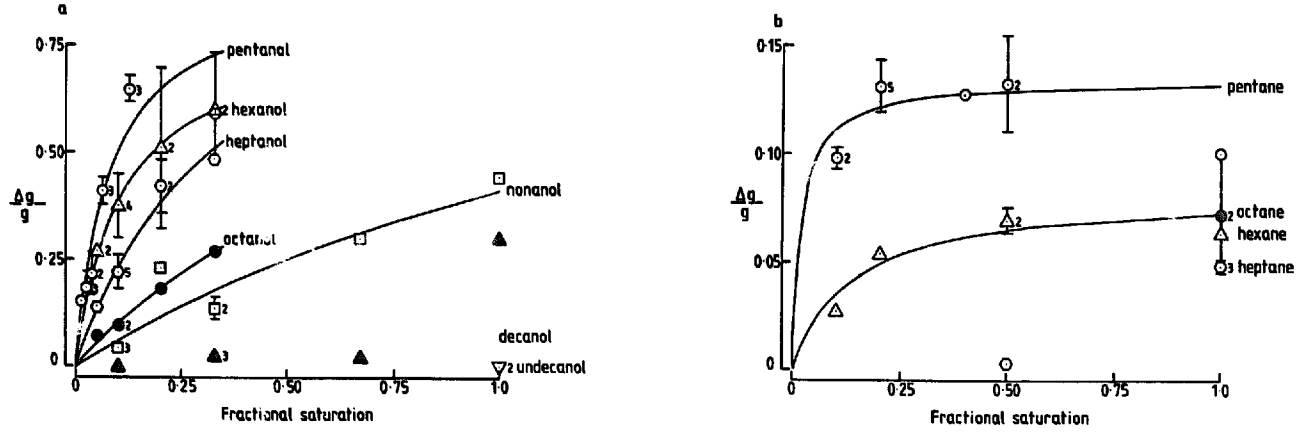

Fig. 3. (a) The effects of members of the *n*-alkanol homologous series on axonal conductance. The ordinate gives the fractional reduction in conductance caused by the fractional saturation of *n*-alkanol indicated on the abscissa. Numbers by the various symbols indicate the number of measurements made at that concentration. Error bars indicate the range ($n = 2$) or standard error of the mean ($n > 2$). The lines through points for *n*-alkanols pentanol to nonanol follow a Langmuir or Michaelis-Menten function ($y = ax/(b + x)$). The characteristics of the lines are: *n*-pentanol, $a = 0.89$, $b = 0.077$; *n*-hexanol, $a = 0.78$, $b = 0.10$; *n*-heptanol, $a = 1.0$, $b = 0.34$; *n*-octanol, $a = 0.91$, $b = 0.8$; *n*-nonanol, $a = 1.1$, $b = 1.7$. (b) The effects of members of the *n*-alkane homologous series on axonal conductance. ⊙, *n*-pentane; △, *n*-hexane; ○, *n*-heptane; ●, *n*-octane. The significance of the numbers and error bars is given above. The lines through the *n*-pentane and *n*-hexane points indicate Langmuir functions with the following characteristics: *n*-pentane, $a = 0.13$, $b = 0.019$; *n*-hexane, $a = 0.081$, $b = 0.13$.

Fig. 1 gives examples of the time course of the effects of alkanol, alkane and ester anaesthetics on the conductance of axons in the high external potassium medium described above. The transient over-recovery exhibited by hexanol and methyl pentanoate has been reported for other agents [18]. The relatively large effect of the polar anaesthetics compared to the non-polar *n*-hexane is obvious. Fig. 2 compares the effects of saturated *n*-undecanol on the height of the propagated action potential in an intact axon (in asw containing 430 mM NaCl, 10 mM KCl and no TTX or 3,4-DAP) and on the a.c. conductance measured under the conditions described previously. Undecanol has at most a very small effect on the 'resting' potassium conductance but is an effective local anaesthetic.

Fig. 3 presents the collected results of this study for (a) *n*-alkanols and (b) *n*-alkanes. The fractional change in conductance ($\Delta g/g$) is plotted against fractional saturation for each compound. Rather fewer experiments were carried out on the esters. Methyl pentanoate at 0.05 saturation produced a fractional decrease in conductance of 0.15 ± 0.05 ($n = 2$), 0.1 saturated methyl pentanoate a decrease of 0.29 ($n = 1$) but 0.1 saturated methyl octanoate produced a decrease of only 0.055 ± 0.044 ($n = 2$).

There are several notable features of these data. The first is that the *n*-alkanols (up to *n*-nonanol) and methyl pentanoate are much more effective in reducing g than are the *n*-alkanes. The second is that as the *n*-alkanol series is ascended the effect on g declines towards higher chainlengths and is very small around undecanol. This may be contrasted with the effects of alkanols on action potential height (Fig. 2) and the voltage-gated sodium channel [9], where decanol is as effective (on a fractional saturation scale) as *n*-pentanol. (The effects of *n*-decanol on the delayed rectifier channel are complex and not readily compared with those of *n*-pentanol but *n*-decanol does produce a substantial inhibition of the delayed rectifier current [22].) Third, the decline in activity of methyl octanoate relative to methyl pentanoate is also special to the 'resting' potassium channel and is not seen with sodium or delayed rectifier channels [14]. The final point is that the concentration dependence of the effect appears to saturate. This is best shown by the data for *n*-pentane and *n*-hexane.

Voltage clamp studies [18] indicate that the conductance measured under these conditions is not attributable to a single type of channel. There is a component of the current which exhibits voltage dependence similar in some respects to that of the delayed rectifier. This produces up to two-thirds of the conductance measured under voltage clamp. However, the data presented in Fig. 3 do not indicate a significant contamination of the conductance by 'normal' delayed rectifier channels. These a.c. conductance measurements are made in the steady state at a potential of approx. 10 mV. Under such conditions, current through the delayed rectifier channels can only be inhibited by a reduction in the maximum conductance, $\bar{g}_K$. It has been shown [22] that in voltage clamped axons in high Na, low K asw, 0.9 saturated *n*-pentane reduces $\bar{g}_K$ by approx. 33% but 0.12 saturated *n*-pentanol causes only a 9% reduction. This is the opposite differential sensitivity to alkanes and alkanols to that displayed by the conductance measured during the a.c. bridge experiments reported in Fig. 3. Here the maximum effect of *n*-pentane (for up to 1.0 saturated) is a less than 15% reduction whereas 0.12 saturated *n*-pentanol produces approximately 60% reduction. Moreover, if there was a significant contribution by the relatively insensitive 'normal' delayed rectifier channels to the a.c. conductance then the concentration dependences given in Fig. 3 would be expected to turn up, not saturate. Recent single-channel studies do indicate the presence of more than one type of voltage-gated potassium channel in the squid giant axon [23].

The decline in activity of the *n*-alkanol series in blocking this 'resting' potassium conductance may explain the observation that lower chain length *n*-alkanols produce a substantial depolarisation of the resting squid axon but *n*-octanol has almost no effect [17,24].

In conclusion, there is a potassium conductance in the squid giant axon which can be distinguished from the delayed rectifier channel by its sensitivity to general anaesthetics [18], its differential sensitivity to *n*-alkanols and *n*-alkanes and by the decline in effectiveness of medium chainlength (C_8–C_{11}) members of the *n*-alkanol and *n*-alkyl methyl carboxylic ester homologous series. This axonal potassium conductance is inhibited by general anaesthetics, which contrasts with the anaesthetic-gated potassium conductance recently described in neuronal somata of the snail, *Limnaea stagnalis* [25].

One of us, A.A.E., is a Wellcome Research Training Scholar.

References

1 Führer, H. (1921) Biochem. Z. 115, 235–261.
2 Crisp, D.J., Christie, A.O. and Ghobashy, A.F.A. (1967) Comp. Biochem. Physiol. 22, 629–649.
3 Mullins, L.J. (1975) in Molecular Mechanisms of Anesthesia (Progress in Anesthesiology, Vol. 1) (Fink, B.R., ed.), pp. 237–242, Raven Press, New York.
4 Haydon, D.A., Hendry, B.M., Levinson, S.R. and Requena, J. (1977) Biochim. Biophys. Acta 470, 17–34.
5 Haydon, D.A. and Hendry, B.M. (1982) J. Physiol. 333, 393–403.
6 Meyer, K.H. and Hemmi, H. (1935) Biochem. Z. 277, 39–71.
7 Richards, C.D., Martin, K., Gregory, S., Keightley, C.A., Hesketh, T.R., Smith, G.A., Warren, G.B. and Metcalfe, J.C. (1978) Nature 276, 775–779.
8 Pringle, M.J., Brown, K.B. and Miller, K.W. (1981) Mol. Pharmacol. 19, 49–55.
9 Haydon, D.A. and Urban, B.W. (1983) J. Physiol. 341, 411–427.
10 Requena, J., Velaz, M.E., Guerrero, J.R. and Medina, J.D. (1985) J. Membr. Biol. 84, 229–238.

11 Franks, N.P. and Lieb, W.R. (1985) Nature 316, 349–351.
12 Haydon, D.A. and Elliott, J.R. (1986) Biochim. Biophys. Acta 863, 337–340.
13 Elliott, J.R., Murrell, R.D. and Haydon, D.A. (1987) J. Membr. Biol. 95, 143–149.
14 Elliott, J.R., Haydon, D.A. and McElwee, A.A. (1987) J. Physiol. 384, 21P.
15 Elliott, J.R. and McElwee, A.A. (1988) Br. J. Anaesth. 60, 817–824.
16 Hodgkin, A.L. and Huxley, A.F. (1952) J. Physiol. 117, 500–544.
17 Armstrong, C.M. and Binstock, L. (1964) J. Gen. Physiol. 48, 265–277.
18 Haydon, D.A., Requena, J. and Simon, A.J.B. (1988) J. Physiol. 402, 363–374.
19 Haydon, D.A. and Simon, A.J.B. (1988) J. Physiol. 402, 375–389.
20 Eger, E.I. (1974) Anesthetic Uptake and Action, Williams and Wilkins, Baltimore.
21 Haydon, D.A., Requena, J. and Urban, B.W. (1980) J. Physiol. 309, 229–245.
22 Haydon, D.A. and Urban, B.W. (1986) J. Physiol. 373, 311–327.
23 Llano, I., Webb, C.K. and Bezanilla, F. (1988) J. Gen. Physiol. 92, 179–196.
24 Haydon, D.A., Elliott, J.R. and Hendry, B.M. (1984) Curr. Topics Membr. Transp. 22, 445–482.
25 Franks, N.P. and Lieb, W.R. (1988) Nature 333, 662–664.