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Effects of N-alcohols on potassium conductance in squid giant axons

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Abstract. (*1*) The effect of bath application of several short chain N-alcohols on voltage-dependent potassium conductance has been studied in intact giant axons of *Loligo forbesi* under voltage-clamp conditions.

(*2*) All tested alcohols (methanol, ethanol, propanol, butanol, heptanol and octanol) were found to depress potassium conductance only at concentrations much larger than those necessary to reduce sodium conductance. The efficacy of the different molecules was correlated with the carbon-chain length. In all cases the effects were found to be at least partly reversible.

(*3*) Low concentrations of propanol (100 m*M*) or heptanol (1 m*M*) were found to increase potassium conductance whereas higher concentrations had the usual depressing effect. The two alcohols were found to induce a slow inactivation of the potassium conductance.

(*4*) A detailed analysis of the time course of the turning-on of the potassium current for various pulse potentials in the presence of TTX revealed that, for membrane potential values more positive than -20 mV, the time constant of activation was reduced in the presence of propanol or heptanol. The delay which separates the change in potential and the turning-on of the potassium current, which was systematically analysed for different pulse and prepulse potential values, was increased by the two alcohols, the curve relating this delay to prepulse potential being shifted towards larger (positive) delays.

(*5*) This high degree of complexity in the effects on potassium conductance suggests that the alcohol molecules modify several more or less independent mechanisms associated with the turning-on of the potassium current.

Key words: Alcohols, K^+ conductance, squid axon

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Introduction

Alcohols are often taken as models in the study of anaesthesia, mainly because of their simple chemical structure and the existence within this family of groups of compounds which differ from each other by small modifications (number of carbons in the molecules, branchings, etc.).

Whereas it is now generally accepted that local anaesthetics such as procaine or benzocaine exert their effects through a direct effect on the ionic channels (Hille 1980), the precise mode of action of the alcohol molecules remains hypothetical.

In a very interesting series of experiments on crayfish and squid axons, Swenson and Oxford (1980) analysed the mode of action of N-alcohols from C6 to C10 on the sodium current and the related "on" gating current. From these experiments it was found that the block induced by the alcohol molecules closely resembles that described for local anaesthetics, therefore suggesting a common mode of action. They also observed that the two components of the sodium current were unequally affected, the delayed component (steady state current) being more sensitive than the peak component, suggesting effects on the kinetics. This observation was the starting point of our experiments since it indicated that a better understanding of the mode of action of alcohols could not be reached without taking into account not only the blocking potency of the molecules but also their effects on the kinetics, as well as the effects of concentration, carbon-chain length, etc. Our approach has been to analyse quantitatively the mode of action of a homologous series of alcohols on the turning-on and turning-off of the the ionic conductances in intact squid axons. In this paper we shall report the effects on the potassium current. The effects on the sodium current will be reported in a subsequent paper.

The effects of alcohols on the potassium conductance have been studied less than their effects on sodium conductance. This comes from the fact that the local anaesthetic properties of alcohols result from the interaction of these molecules with the functioning of the sodium channels. It is not, however, unreasonable to believe that the mode of action of alcohol molecules on the two categories of ionic channels is somewhat similar.

Compared to the sodium system, the potassium system is simpler since, under normal conditions, the turning-on of the potassium current is not complicated by inactivation. It is also significantly slower and therefore enables more precise kinetic studies.

In their study on the effects of several alcohols on the properties of the squid giant axon, Armstrong and Binstock (1964) reported that short chain N-alcohols depolarized the axonal membrane by a few millivolts whereas octanol slightly hyperpolarized the membrane. This effect was found to be correlated with changes in the resting potassium conductance. The voltage-dependent potassium conductance (gK) was found to be decreased for small depolarizing steps for alcohols between C2 and C5 and large concentrations of octanol. In all cases, the depression of gK was much smaller than that of the sodium conductance (gNa). In a parallel set of experiments on this same preparation, Moore et al. (1964), using the sucrose-gap voltage clamp technique, found that the potassium conductance of the squid axon membrane was reduced by ethanol by about the same extent as the sodium conductance. More recently, Gregory et al. (1979) found, for the squid axon membrane, that 1–4 m*M* concentrations of hexanol reduced the peak (sodium) current by 30–60% and the delayed (potassium) current by 0–25%.

The first part of this study has been devoted to a systematic study of the effects of external application of various concentrations of N-alcohols from C1 to C8 on the potassium current and conductance. The second part is a quantitative analysis of the effects of propanol (C3) and heptanol (C7) on the kinetics of the potassium current.

Methods

Experiments were performed on giant axons (axon diameter ranging from 450 to 800 μm) dissected from the mantles of freshly killed *Loligo forbesi.* The isolated axons were cleaned from adhering small fibres and mounted horizontally across a Perspex chamber filled with artificial sea water.

The axons were studied under current-clamp and voltage-clamp conditions as describing by Kimura and Meves (1979) and Pichon (1981). Unter voltage-clamp conditions, the membrane potential was held at − 60 mV, corresponding to the mean resting potential of the axons in artificial sea water. Test pulses (usually 20 ms long) were preceded by 80 ms hyperpolarizing prepulses to − 80 mV.

Artificial sea water contained 470 m*M* NaCl, 11 m*M* $CaCl_2$ and 55 m*M* $MgCl_2$ and was buffered at pH 7.8 using *Tris.* When needed, the sodium conductance was blocked by adding 0.5 μ*M* tetrodotoxin (TTX, Sigma) to the external solution. Alcohols were dissolved directly in artificial sea water, sonication was used for heptanol and octanol. The osmotic effects induced by large alcohol concentrations were found to be negligible compared to those of similar concentrations of sucrose or glucose. This lack of effect is likely to result from the high permeability of the membrane to these highly lipophilic molecules.

In order to ensure a reasonably good time resolution for the time course of the ionic currents and reduce potassium accumulation, the temperature in the bath was maintained between 2° and 3 °C. Special care was taken to avoid temperature changes during the experiments.

Curve fitting procedure

The potassium current traces recorded in the presence of TTX were fitted to the following equations:

$$I_K = \bar{g}_K (V_m - V_K)\, n^4 \quad (1)$$

$$n = n\,\infty - (n\,\infty - n\,o)\exp(-t/\tau_n)\,. \quad (2)$$

The leak current was subtracted and, when needed, the noise in the trace was reduced using a smoothing procedure. To reduce errors due to potassium accumulation in the space between the axons and the Schwann cells (see Frankenhaeuser and Hodgkin 1956), the curve fit was limited to the earlier phase of the potassium current. This portion of the curve was subtracted from an adjustable plateau value and its fourth root extracted. The resulting curve was then linearized and fitted to a straight line using a least squares procedure. The parameters of the linearized curve were used to calculate the potassium activation time constant and the potassium activation delay (ϑ) which was observed under most experimental conditions (Pichon et al. 1984). Under those conditions and in contrast with the original observations of Cole and Moore (1960) on intact axons of *Loligo pealei* and the more recent finding of Keynes et al. (unpublished experiments cited in Keynes 1983) on perfused axons of *Loligo forbesi,* we consistently obtained a good fit of the experi-

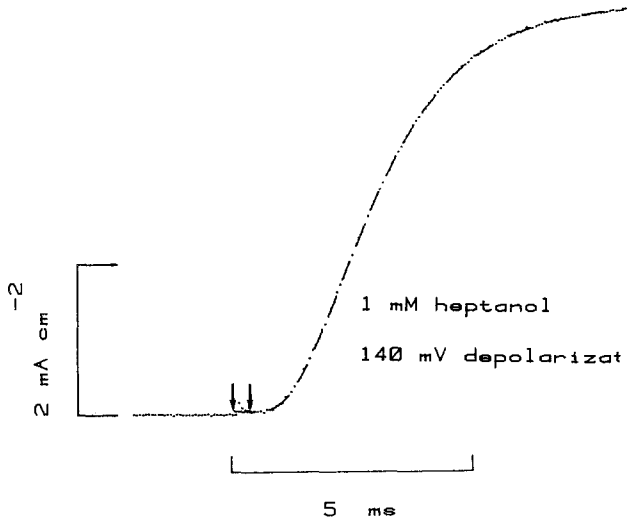

Fig. 1. Example of fit of a potassium current trace with the Hodgkin and Huxley kinetics plus a positive delay. The membrane normally held at −60 mV was hyperpolarized to −80 mV for 80 ms, then depolarized to +80 mV for 20 ms. The *dots* correspond to the experimental (unretouched) recording, the *interrupted line* to the computed curve obtained as described in the method section. This fit (correlation coefficient of 0.9999 for 160 points) was obtained with $I_{max} = 5.35$ mA cm^{-2}, $\tau n = 1.376$ ms and a positive delay (ϑ, enclosed between the two *arrows*) = 0.337 ms. Temperature: 2 °C. For clarity, only every other experimental *point* is shown

mental data (correlation coefficient around 99 for 100 points or more) with n^4. An example of such a fit is shown in Fig. 1.

Results

General effects of alcohols on the ionic currents

In a first series of experiments, we studied the effects of several concentrations of alcohols from methanol (C1) to octanol (C8) on the leak current (I_l) and both sodium and potassium currents. The general effects of these molecules are summarized in Table 1. Apart from methanol, which was found to poison irreversibly the chamber and electrodes and increase the leak conductance, all alcohols were found to modify reversibly the ionic conductances without changing the leak. Large concentrations of all alcohols decrease both the peak (sodium) current and the delayed (potassium) current. This is illustrated in Fig. 2 for butanol and octanol. In all cases, the potassium current was reduced less than the sodium current and, interestingly, the time course of inhibition of the two conductances was found to be clearly different (especially for longer chain alcohols such as octanol): the potassium current is reduced later during exposure and reappears earlier following return to alcohol-free solution. The efficacy of the alcohol molecules was found to

Table 1. Effects of various concentrations of N-alcohols on the peak (sodium) and the delayed (potassium) current corresponding to a (20 ms long) 80 mV depolarization to + 20 mV from a holding potential of − 60 mV in voltage-clamped giant axons of *Loligo forbesi*

Alcohol	Concentration [m*M*]	Percent of inhibition of	
		INa	IK
Methanol (C1)	1,250	23%	20%
Ethanol (C2)	360	31%	22%
	900	69%	27%
Propanol (C3)	75	19%	–
	100	22%	5%, +20% *
	150	30%	–
	200	–	17%
	250	55%	–
	300	58%	–
	400	75%	–
	500	–	48%, 58%
	1,000	–	77%, 79%
Butanol (C4)	1.1	1%	1%
	11	6%	6%
	28	44%	13%
	56	60%	33%
	110	78%	48%
Pentanol (C5)	9.35	38%	–
Heptanol (C7)	0.2	22%	–
	0.5	57%	–
	1	70%	+4%, +13% *
	2	–	10%, 0%
	4	–	77%, 56%
Octanol (C8)	0.06	33%	–
	0.24	76%	–
	0.6	100	17%

* increase

increase with carbon chain length. This effect was, however, not as striking as for the sodium current and long chain alcohols were comparatively less effective than short chain ones in blocking the delayed current (Table 1). Thus, at equilibrium, and for a depolarizing pulse to + 20 mV, 56 m*M* butanol inhibited the peak inward current by 60% and the delayed outward current by 33% whereas 0.6 m*M* octanol which totally inhibited the peak inward current reduced the delayed outward current by only 17%. For reasons which will be become clear later in this paper, this comparison only holds for comparatively large alcohol concentrations.

Effects of changing alcohol concentration on potassium current inhibition

Lower alcohol concentrations consistently increase the potassium current. This effect reverses for larger concentrations. Thus, for example the maximum

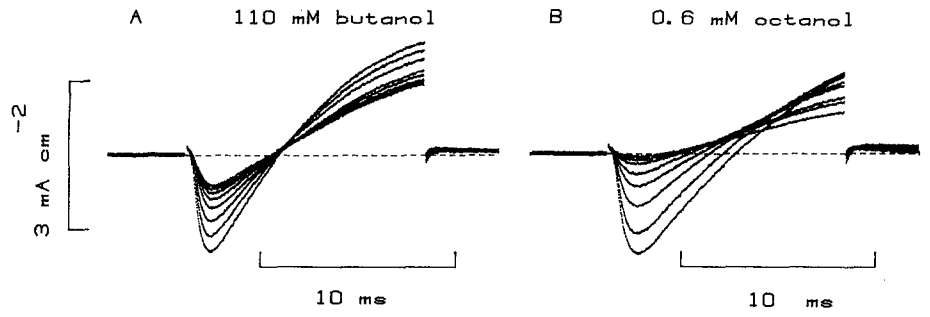

Fig. 2. Effects of 110 m*M* butanol (**A**) and 0.6 m*M* octanol (**B**) on the ionic currents corresponding to a square depolarizing test pulse from − 60 mV to + 20 mV. The current traces were recorded every min following bath application of the alcohol. The currents were not modified during the first 2 min, the first trace thus corresponds to min 0, min 1 and min 2. Both inward and outward currents were reduced but the effect of the alcohol molecules on the inward current were more important than those on the outward current: after 10 min, the reduction of the peak current was of 70% in butanol and 98% in octanol as compared to a reduction of the delayed current of 40% in butanol and 47% in octanol. Temperature: 3.5 °C in **A**, 2.5 °C in **B**

outward current corresponding to an 80 mV depolarization and measured under steady state conditions (i.e. after equilibration) increases by 20% following application of 100 m*M* propanol but drops to 50% of its initial value if the alcohol concentration is subsequently raised to 500 m*M*. Similarly, 1 m*M* heptanol increases the potassium current by 13%, 2 m*M* heptanol has little effect and 4 m*M* decreases the potassium current by 56%. The percentage of increase of inhibition is voltage-dependent (see later). The effects of two concentrations of heptanol on two families of potassium current are illustrated in Fig. 3. Another interesting effect of the alcohol molecules, which is clearly visible on the recordings of Fig. 3, is that it induces a delayed potassium inactivation: after reaching a maximum (which can exceed the maximum potassium current before alcohol application), the potassium current slowly decreases. This effect is especially marked for large depolarizing pulses (between 70 and 150 mV) but does not result exclusively from an increased accumulation of potassium ions in the Frankenhaeuser and Hodgkin (1956) space since it persists when correction is made for changes in V_K.

Voltage and time dependency of the effects of alcohols on the potassium conductance

As mentioned earlier, potassium accumulation is important in our experiments, especially during large depolarizations. Changes in the potassium current can therefore not be directly correlated with changes in potassium conductance, gK. To approximate this parameter, the potassium currents immediately before (I_1) and immediately after (I_2) the end of the pulse were measured. During that interval (16 to 32 μs), the changes in gK and in V_K are negligible and gK approximates $(I_1-I_2)/(V_1-V_2)$ where V_1 is the membrane potential during the pulse and V_2 the membrane potential after the pulse (− 60 mV).

These conductance measurements confirm the previous observations on the currents and clearly show that the effect of the alcohols is time and voltage-dependent.

Thus, in large alcohol concentrations and for a given depolarization, the decrease of the potassium conductance was always larger after 80 ms than after 20 ms (96% against 85% in 1,000 m*M* propanol, 84% against 62% in 4 m*M* heptanol, for example).

The voltage dependency of the effects of alcohols on the potassium conductance is illustrated in Fig. 4 for one propanol and 2 heptanol concentrations. In all cases, the inhibition is larger for small than for large depolarizations. As illustrated in this figure, part of this voltage dependency can be accounted for by shifting the potassium activation curve towards more positive membrane potentials and by changing the maximum potassium conductance, $\bar{g}K$. (The fit is however not good enough to justify any definite conclusion.)

Effects of alcohols on the time course of the potassium current

Besides the already mentioned slow inactivating effect of alcohols on the potassium current, these molecules also delay the turning-on of the potassium current and accelerate the activation phase for large depolarizations. These effects are illustrated in Fig. 5 for three membrane potential values. As mentioned in the method section, the turning-on of the potassium current can be fitted with very good accuracy using the 4th power model for n (correlation coefficients of 0.99 for more than 100 points) and the activation time constants were found

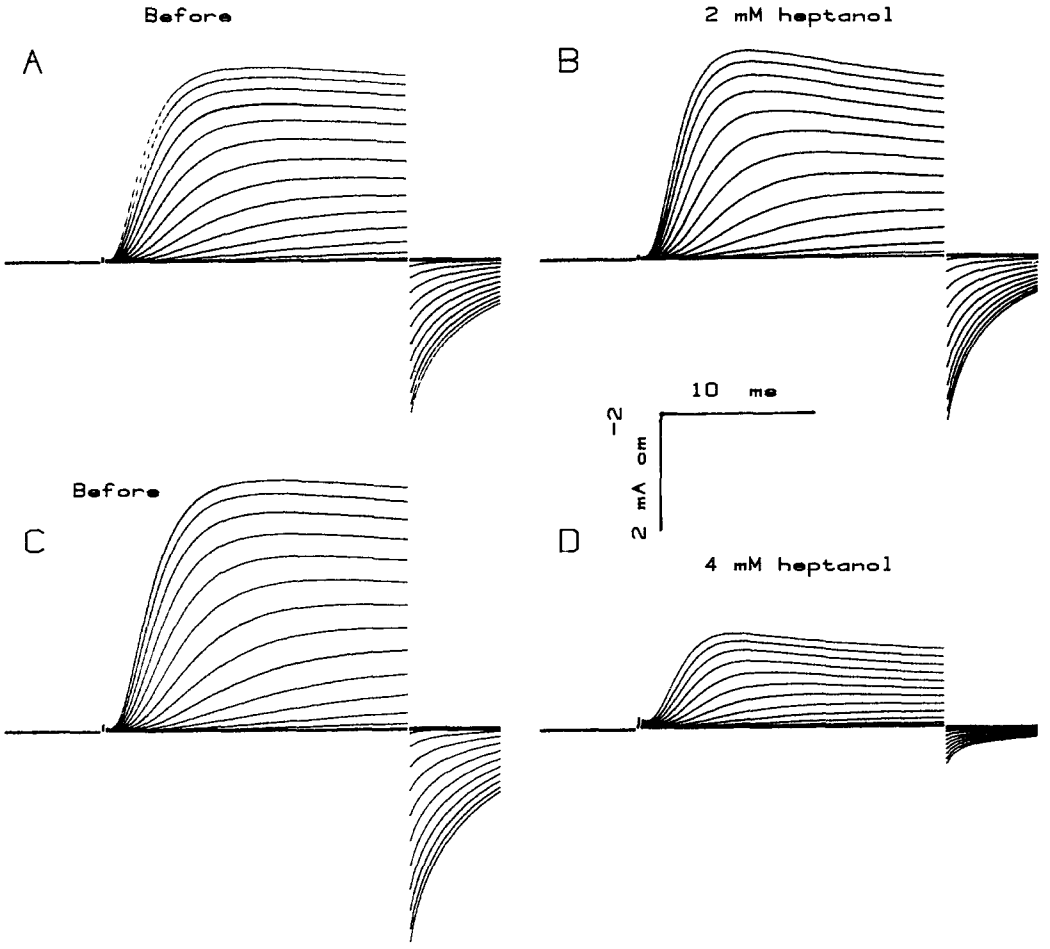

Fig. 3A–D. Effects of two concentrations of heptanol on the time course of the potassium current in two intact squid axons superfused with a solution containing 1 μM TTX. **A and C:** before; **B and D:** following application of respectively 2 mM (**B**) and 4 mM (**D**) heptanol in the external solution. The current traces correspond to square depolarizations of the axonal membrane from its holding value of − 60 to + 90 mV by 10 mV steps. The 20 ms test pulses were preceded by an 80 ms conditioning prepulse to − 80 mV. Temperature: 3.5 °C (**A and B**), 2.5 °C (**C and D**)

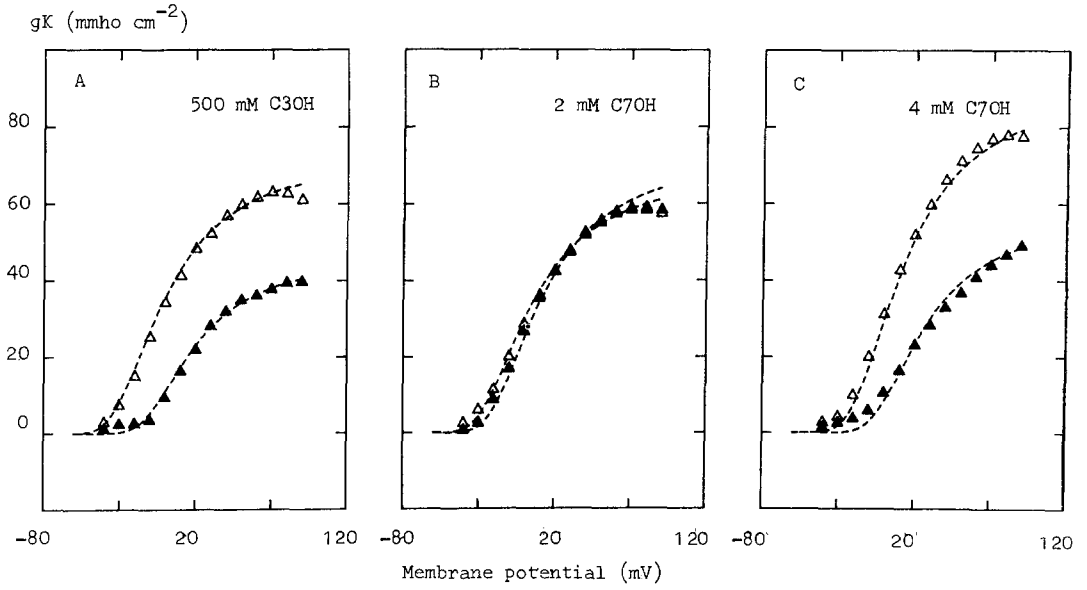

Fig. 4A–C. Effects of 500 mM propanol (**A**), 1 mM heptanol (**B**) and 4 mM heptanol (**C**) on the potassium conductance at the end of 20 ms depolarizing pulses in intact squid axons. *Open triangles:* before alcohol application; *filled triangles:* at least 30 min after application of the alcohol. The experimental data were tentatively fitted using the Hodgkin and Huxley (1952) model *(interrupted lines)*. In all three cases, part of the effects on the conductance could be accounted for by changing $\bar{g}K$ and shifting the activation curve towards more depolarized potentials: 36% decrease in $\bar{g}K$ and 20 mV shift in **A**; 6% increase in $\bar{g}K$ and 7 mV shift in **B**; 37% decrease in $\bar{g}K$ and 14 mV in **C** (more modifications are however clearly needed to reproduce the effects of 4 mM heptanol) Temperature: 2 °C

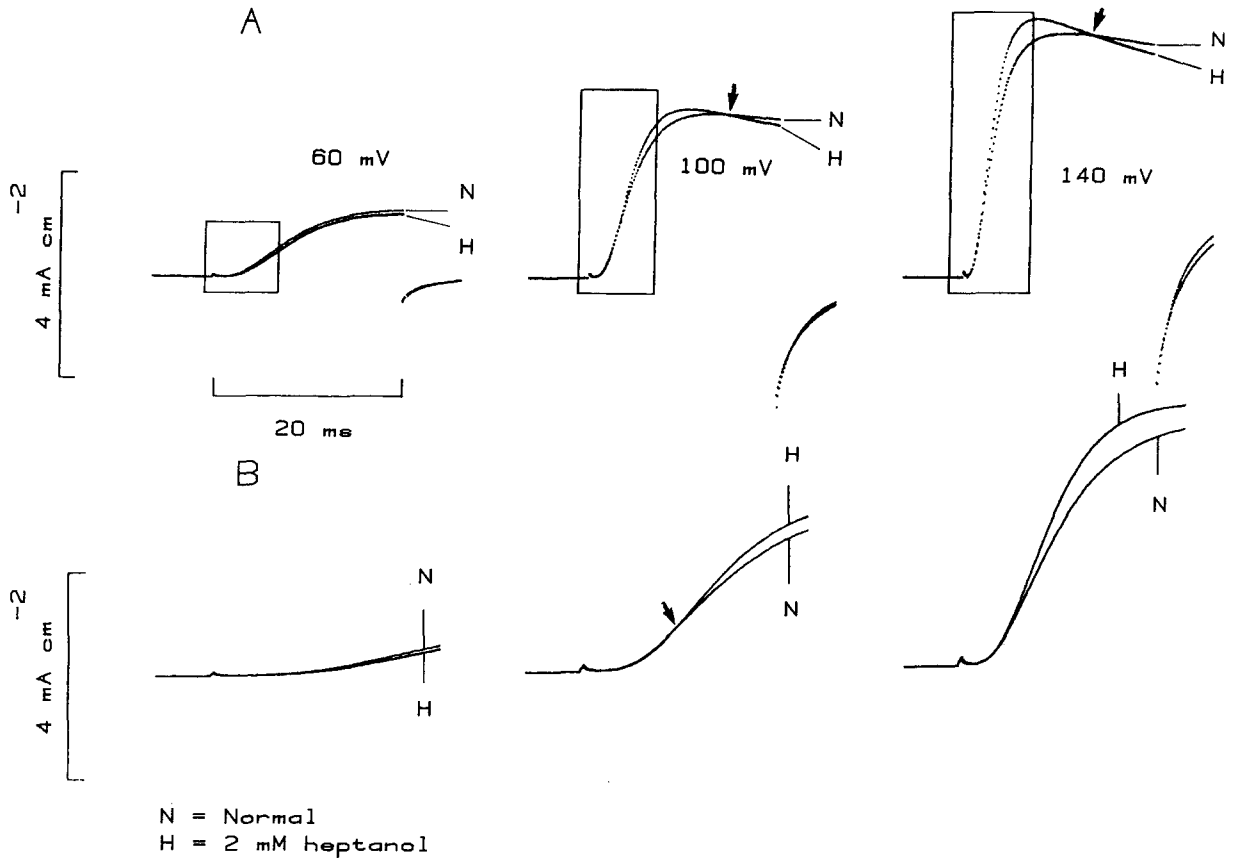

Fig. 5 A and B. Modifications of the time course of the potassium current by 2 m*M* heptanol. The tracings in **B** correspond to the enlarged (boxed) portion of the corresponding tracings in **A**. *N*: before, *H*: 20 min after external application of 2 m*M* heptanol. For 100 and 140 mV depolarizations, the alcohol increases the current, speeds-up its turning-on and induces inactivation. Arrows indicate the intercepts of the two current records. Temperature: 2 °C

to agree reasonably well with those of Hodgkin and Huxley (1952). However, in nearly all cases, the linearized current traces were found to cross the zero current line before or, more often, after the turning-on of the stimulating depolarizing voltage pulse, indicating the existence of a delay. Whereas negative delays corresponding to the non negligible value of n at the holding potential level are predictable from the original Hodgkin and Huxley (1952) formulation, positive delays are not. They were, however, first observed by Cole and Moore (1960) and more recently by Keynes and Kimura (1980), Moore and Young (1981) and Clay and Shlesinger (1982) following membrane hyperpolarization. Our experiments indicate that they also exist under normal conditions when the test pulse is preceded by a hyperpolarizing prepulse to − 80 mV. The overall delay varies with pulse and prepulse potential. For a membrane held at − 60 mV and stepped for 20 to 80 ms to − 80 mV before application of the test pulse, the delay, which is negative for small depolarizations, reverses sign around − 20 mV, reaches a peak value between − 10 and 20 mV, then slowly decreases for larger depolarizations (Fig. 6B). Under similar conditions, when the prepulse is changed but the test pulse maintained constant (Fig. 6D), the activation delay which is largely positive for hyperpolarizing prepulses changes sign between − 60 and − 40 mV and becomes negative for depolarizing prepulses. The curve relating activation delay and prepulse potential is steeper for depolarizing prepulse potentials than for hyperpolarizing prepulse potentials.

The time constant of activation, τ n, changes with pulse potential as predicted from Hodgkin and Huxley (1952) although the experimental curve relating τ n to Vm was found to be consistently shifted towards more depolarized potentials. As first shown by Cole and Moore (1960) for hyperpolarizing prepulse potentials, the time constant of activation is almost independent of prepulse potential (Fig. 6C). This is true for the entire prepulse potential range between − 100 and − 20 mV.

External application of propanol or heptanol consistently and reversibly modify both τ n and the activation delay.

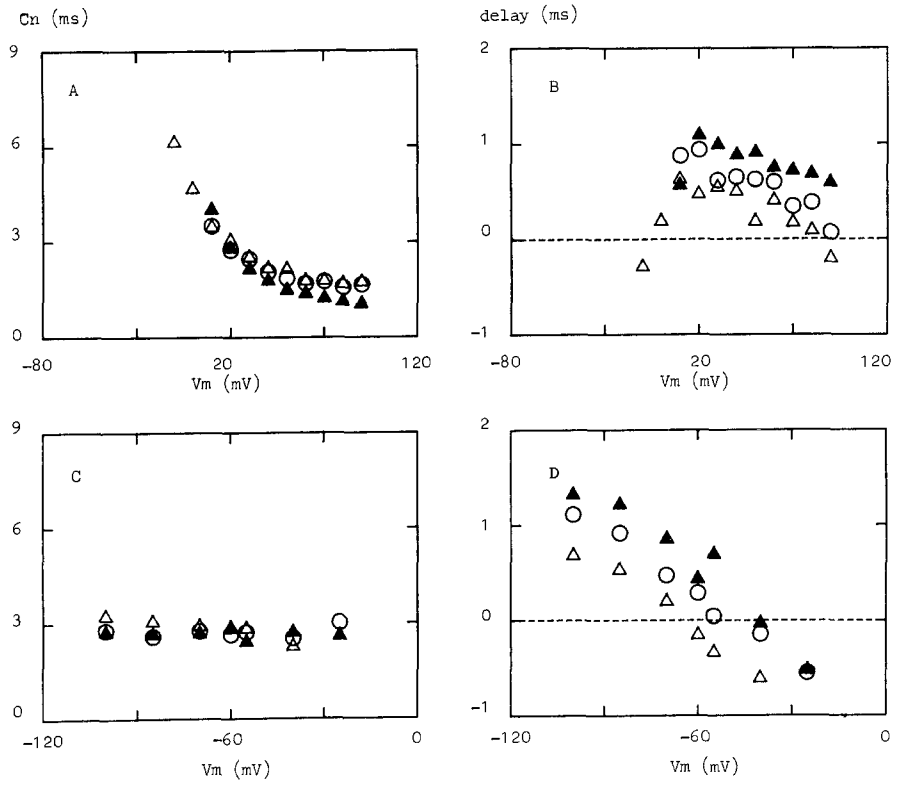

Fig. 6A – D. Effects of 500 m*M* propanol on the time constant of activation of the potassium current (τ n) and the potassium activation delay. *Open triangles:* before; *filled triangles:* more than 40 min after external application of propanol; *circles:* more than 20 min following return to alcohol-free solution. Prepulse duration: 80 ms; pulse duration: 20 ms. Prepulse size: 20 mV hyperpolarizing in **A** and **B**, variable in **C** and **D**; pulse size: variable in **A** and **B**, 80 mV depolarizing in **C** and **D**. Temperature: 2 °C. For test potential values more positive than +20 mV, propanol decreases τ n and increases the delay. This effect is partly reversible

Thus, 500 m*M* propanol (a concentration which reduces the potassium conductance at 20 mV by 55%) shifts the τ n versus pulse potential curve towards more positive potentials and shorter time constants (Fig. 6A): The two curves cross each other at 20 mV and τ n is 40% shorter than τ n before alcohol application (1.05 ms against 1.73 ms) at 90 mV. This shift is reversible following return to alcohol-free solution.

The curve relating the activation delay to membrane potential is shifted upwards towards larger (positive) delays. For test membrane potentials between 20 and 90 mV, this shift approximates 0.40 ms. This effect is only partly reversible following return to alcohol-free solution (about 30% recovery more than 20 min after return to alcohol-free solution). Propanol has no effect on the τ n versus prepulse potential relationship which remains almost linear (Fig. 6C). On the other hand, the alcohol shifts the delay versus prepulse potential relationship towards more positive delays (Fig. 6D). Thus, for a prepulse potential to − 55 mV, the delay which is slightly negative before alcohol application (− 0.34 ms) reverses polarity and reaches 0.69 ms following external application of propanol. The zero intercept of the curve relating the activation delay to the prepulse potential is shifted by about 30 mV from − 70 mV to − 40 mV. This effect on the delay is only partly reversible following return to alcohol-free solution.

The effects of heptanol are very similar to those of propanol as illustrated in Fig. 7 for 4 m*M*, a concentration which inhibits $\bar{g}$K by 37%. The time constant of activation of the potassium conductance for a test depolarization to − 90 mV is decreased by 28% from 1.56 ms to 1.12 ms (Fig. 7A). The potassium activation delay is increased for test depolarizations between 0 and + 90 mV (Fig. 7B). For a depolarization to + 20 mV, τ n in heptanol, which is 18% shorter than before alcohol application, remains almost constant when the prepulse potential is varied between − 100 and − 20 mV (Fig. 7C). The activation delay which is almost nil for a depolarizing prepulse to − 55 mV reaches a positive value of 0.69 ms in the presence of heptanol. Under those conditions, the curve relating the activation delay to the prepulse potential intercepts the zero delay line at − 35 mV as opposed to − 60 mV before alcohol application (Fig. 7D).

One example of the effects of successive applications of 1, then 2 m*M* heptanol followed by a return to alcohol-free solution is shown Table 2. It can be seen that the changes in τ n are proportional to the alcohol concentrations and are partly reversible at potentials more positive than + 20 mV. Thus, for a depolarization to 60 mV, τ n is decreased from 1.68 ms to 1.58 in 1 m*M* heptanol and to 1.41 in 2 m*M* heptanol. Following return to the alcohol-free TTX solution, the time constant goes back to 1.60 ms. The effects on the delay are not as consistent

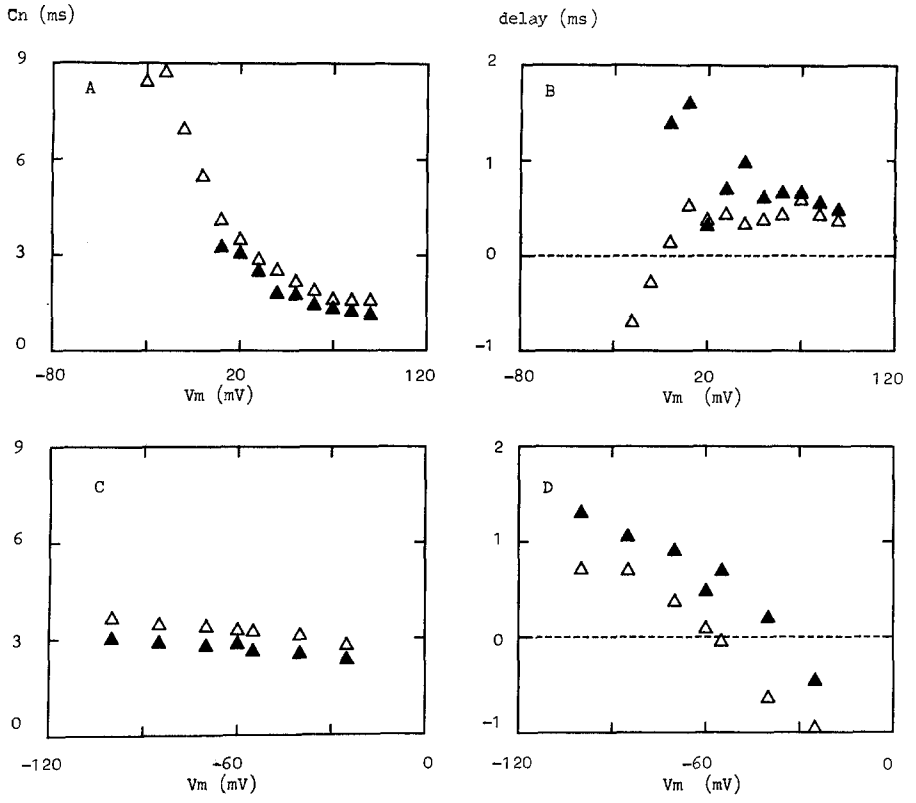

Fig. 7A–D. Effects of 4 m*M* heptanol on the time constant of activation of the potassium current (τ n) and the potassium activation delay. *Open triangles:* before; *filled triangles:* more than 40 min after external application of heptanol. Prepulse duration: 80 ms; pulse duration: 20 ms. Prepulse size: 20 mV hyperpolarizing in **A** and **B**, variable in **C** and **D**; pulse size: variable in **A** and **B**, 80 mV depolarizing in **C** and **D**. Temperature: 2 °C. For test potential values more positive than 0 mV, heptanol decreases τ n and increases the delay

Table 2. Effects of external application of two concentrations of heptanol on the time constant of activation of the potassium current (τ n) and the potassium activation delay (delay) in one giant axon of *Loligo forbesi.* CC indicates the correlation coefficient. The membrane was held at − 60 mV between the pulses. The test pulse to the indicated potential followed a 80 ms conditioning prepulse to − 80 mV. Temperature: 2 °C

V_m	Before			1 m*M* heptanol			2 m*M* heptanol			Following return		
[mV]	CC	τ n	Del	CC	τ n	Del	CC	τ n	Del	CC	τ n	Del
− 20	0.99	6.67	0.09	0.99	7.28	− 0.20	0.99	7.42	− 0.17	0.99	7.80	0.71
− 10	0.99	5.13	0.37	0.99	5.54	0.31	0.99	5.45	0.42	0.99	6.05	0.74
0	0.99	3.95	0.60	0.99	4.30	0.59	0.99	4.13	0.64	0.99	4.50	0.95
10	0.98	3.23	0.53	0.99	3.48	0.52	0.99	3.27	0.67	0.99	3.58	0.84
20	0.99	2.72	0.50	0.99	2.79	0.61	0.99	2.64	0.68	0.99	3.04	0.60
30	0.99	2.34	0.50	0.99	2.30	0.65	0.99	2.22	0.57	0.99	2.27	0.91
40	0.99	2.93	0.50	0.99	1.98	0.61	0.99	1.91	0.59	0.99	2.96	0.68
50	0.99	1.82	0.39	0.99	1.80	0.49	0.99	1.61	0.54	0.99	1.80	0.61
60	0.99	1.68	0.29	0.99	1.58	0.49	0.99	1.41	0.48	0.99	1.60	0.52
70	0.98	1.50	0.33	0.99	1.43	0.46	0.99	1.36	0.40	0.99	1.51	0.37
80	0.99	1.45	0.21	0.99	1.35	0.35	0.99	1.24	0.37	0.99	1.44	0.25
90	0.99	1.44	0.09	0.99	1.31	0.25	0.99	1.20	0.29	0.99	1.40	0.15

and there is almost no recovery. A similar (but relative) lack of reversibility of the increase in delay (as opposed to a good reversibility of the effects on the time constant of activation) is also seen in Fig. 6 following propanol treatment. This difference in reversibility of the two effects suggests that they originate from two different mechanisms.

Discussion

External application of N-alcohols alters the properties of the (voltage-dependent) potassium conductance in squid giant axons studied under voltage-clamp conditions. These modifications are complex

and seem to be rather independent of those on the sodium system.

The present finding that small concentrations of propanol or heptanol increase the potassium current are consistent with the already mentioned observation of Armstrong and Binstock (1964) that, in this same preparation, 0.52 m*M* octanol increases the resting potassium conductance and reversibly hyperpolarizes the membrane from −56 mV to −60 mV. This increase in the potassium conductance is also consistent with the more recent observations of Tippe (1980) that the stationary current recorded in nodes of Ranvier of the frog in high potassium solution is increased by hexanol.

One interpretation would be that alcohols have the same effects as a hyperpolarization. Under these conditions, an increase in the potassium current could correspond to a relief from inactivation (see later) or to the unveiling of a voltage-dependent potassium conductance somewhat analogous to the early outward current (A current) seen for example in Molluscan neurones (Connor and Stevens 1971). Besides an increase in the number of potassium channels, the observed increase in $\bar{g}K$ could reflect an increase in the mean opening time of each individual channel or an increase in the single channel conductance.

Larger alcohol concentrations induce a steeply concentration-dependent inhibition. The effects on the potassium conductance are in that respect similar to those on the sodium conductance where the Hill coefficient of the dose-response curve was found to be significantly larger that one (Paternostre et al. 1983; Paternostre and Pichon, in preparation). The inhibitory effect of large concentrations of N-alcohols on the potassium conductance are also in agreement with the findings of Armstrong and Binstock (1964) as well as with those of Moore et al. (1964) on ethanol.

As Armstrong and Binstock (1964), we find that the effects of a given concentration of all alcohols with carbon chain lengthes ranging from 2 to 8 are smaller on the potassium system than on the sodium system. This inhibitory effect of large concentrations of alcohol is also consistent with the observation that all alcohols depolarize the axonal membrane to a certain extent and can therefore contribute indirectly through sodium inactivation to the local anaesthetic like blocking effects of these molecules.

The induction of a slow inactivation, which has also been observed following application of N-decanol onto internally perfused squid axons by Haydon and Urban (cited by Haydon et al. 1984), could reflect the fact that alcohol molecules bind preferably to open channels, resembling in that respect TEA derivatives (Armstrong 1969; Armstrong and Hille 1972). This slow decrease in the potassium conductance could also correspond to an enhancement of the normal slow potassium inactivation which follows long term membrane depolarization (Ehrenstein and Gilbert 1966; Meves and Pichon 1977; Pichon et al. 1983). The effects of N-alcohols on the potassium system would in that respect resemble those on the sodium system (Swenson and Oxford 1979; Haydon and Urban 1983 b).

The fact that alcohols have opposite effects on the time constant of activation and the activation delay suggests that these two parameters do not reflect a single and unique mechanism. The decrease in τn which is observed for large depolarizations would correspond to a decrease in the mean opening time of the potassium channels whereas the increase in delay could reflect an increase in the time needed for one channel to open following membrane depolarization. Patch-clamp experiments, now in progress in this laboratory, should provide an answer to that question.

The general effects of N-alcohols on the potassium conductance can fall into three categories: changes in the conductance versus membrane potential relationship, changes in $\bar{g}K$ and changes in the time course. Qualitatively similar effects are obtained by shifting the membrane potential towards more negative values suggesting that alcohols might change the transmembrane electrical field or alter the 'voltage sensor' which is responsible for the opening of the potassium channels.

Alcohols are far less potent than N-alkanes in blocking both sodium and potassium conductances (see Haydon and Kimura 1981; Haydon and Urban 1983 a, b). For example, 0.306 m*M* n-pentane has been found to block the potassium conductance in perfused squid axons by 42% (Haydon and Kimura 1981) and to decrease the time constant of activation of potassium by about 40% (see their Fig. 9). A similar decrease in gK is seen for 4 m*M* heptanol (i.e. a 10 times larger concentration of C7 compound). It is therefore very likely, as pointed out by Haydon and Urban (1983 b) that the mode of action of the two families of compounds on the different components of the nerve membrane is not the same. It has been suggested that N-alcohols act through a rather unspecific effect on membrane lipids (Pichon 1981; Haydon and Urban 1983 b). The effects of alcohols on the potassium conductance or at least some of them, could also originate from direct effects on the ionic channels. There are indications that a part of the ionic channels is hydrophobic and could serve as a non-specific binding site for many pharmacologically active substances such as local anaesthetics or potassium channel blockers (Pichon 1981). The observed cor-

relation between blocking efficacy and carbon-chain-length (see also Seeman 1972) would then reflect the binding properties of this hydrophobic binding site.

In conclusion, N-alcohols modify the potassium conductance in intact squid axons in a complex way which cannot be correlated directly with simple modifications of either the lipid matrix of the membrane or the ionic channels. Experiments on single channels are expected to provide more information in the near future.

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