Biochimica et Biophysica Acta, 598 (1980) 200—205

BBA Report

BBA 71457

THE EFFECT OF *n*-ALKANOLS ON THE STATIONARY CURRENT VOLTAGE BEHAVIOR AND ACTION POTENTIAL OF MYELINATED NERVE

A. TIPPE

Abteilung für Physiologie, Gesellschaft für Strahlen- und Umweltforschung, D 8042 Neuherberg (F.R.G.)

(Received November 9th, 1979)

Key words: Myelinated nerve; n-Alkanol; Stationary membrane current; Action potential; Lipid phase transition

Summary

Stationary current voltage characteristics and the action potential of single myelinated nerve fibres were measured to examine the effect of *n*-alkanols (methanol to octanol) on the electrophysiological function of the axon membrane. K^+-depolarized membranes show alkanol-dependent shifts of V_{Tr}, the membrane transition voltage, whereas in veratridine-depolarized membranes such V_{Tr}-shifts are not observed. In the latter case, *n*-alkanols reduce both the stationary Na^+ current and the conductivity step between the high- and low-ohmic conductivity state of the membrane. Action potential amplitude, however, is less affected by the alkanols as is the stationary Na^+ current. The results are compared with the alkanol-dependent changes of the thermotropic phase transition in phospholipid bilayers.

The *n*-alkanols and *n*-alkanes are known to block the conduction of nervous impulses, thus acting as local anaesthetics [1]. Due to the non-ionic nature and simple chemical structure, these compounds have been suggested as being useful in the study of the molecular mechanism of anaesthesia [2—4]. The experimental results on artificial and biological membranes point to a close correlation between the anaesthetic action and the thermotropic structural phase transition of the lipid matrix in the bilayer membrane [5]. Similar phase transitions have been suggested earlier to explain the general ion conductivity behavior of the nerve membrane [6,7]. Because of the voltage-dependency of these ion conductivities, the assumed phase transitions from a high to a low conduc-

tivity state should be triggered by electrical voltages rather than by temperature. In certain smectic liquid crystals, voltage-dependent phase transitions have already been observed [8]. These transitions occur at electric field strengths which are comparable to those acting on a resting axon membrane ($1 \cdot 10^4$—$1 \cdot 10^5$ V/cm). As in nerve membranes, the current-voltage (I vs. V) characteristics of these liquid crystals show a region of negative resistance indicating a 'transition voltage' analog to the more common 'transition temperature'. Similar conformational changes induced by electric fields have also been observed in biopolymers [9].

According to these facts, an investigation of both the stationary current-voltage behavior and the action potential of nerve fibres under the influence of *n*-alkanols might lead to a better understanding of the role which structural phase transitions of the membrane play in the mechanism of anaesthesia. We therefore studied the effect of *n*-alkanols (methanol to octanol) on the stationary I vs. V characteristic of the Ranvier node membrane and compared the results with the corresponding alkanol-induced changes of the thermal phase transition in phospholipid bilayers [4]. Especially, shifts of the bilayer transition temperature (T_m) were compared with the analogous shifts of the transition voltage (V_{Tr}) separating the two conductivity states of the axon membrane [10]. The anaesthetic effect of the alkanols used was observed by measuring the corresponding action potentials.

Electrophysiological experiments were carried out using single fibres of nervus ischiadicus from *Rana esculenta*. Stationary I vs. V characteristics of the axon membrane were measured by applying the method of impressed voltage [10]. This method allows the recording of one characteristic continuously during approx. 2 min: series of more than 10 curves can therefore be recorded within the lifetime of one single nerve fibre preparation. In order to obtain an accurate measure of V_{Tr}, the axon membrane was depolarized by K^+ or veratridine such that the I vs. V characteristics clearly show a region of negative resistance. To depolarize the membrane (K^+-depolarization) 80 mM Na^+ of the usual Ringer's solution was replaced by equimolar K^+; for the veratridine-depolarization, $1 \cdot 10^{-4}$ g veratridine per ml was added to normal Ringer's solution. The *n*-alkanols (Merck) were added to both of these extracellular solutions. Action potentials were excited in normal Ringer's solution as extracellular solution: the different alkanols were added to this solution. The pH of all solutions was held constant at 7.0 ± 2%.

(1) K^+-depolarization of the membrane (80 mM KCl/Ringer's solution). If the alkanol concentration is below a certain value, which depends on the chain length of the alkanol, all *n*-alkanols investigated (methanol to octanol) reversibly shift V_{Tr} of the K^+-depolarized membrane in the direction of hyperpolarization. The amount of the V_{Tr}-shifts increases with increasing chain length and concentration of alkanols. The conductivity step between the high- and low-ohmic branches of the I vs. V characteristics remains unaffected. Fig. 1 shows, as an example, the original registration of stationary I vs. V curves with normal Ringer's solution, 80 mM KCl/Ringer's solution and 80 mM KCl/Ringer's solution with 8 mM 1-hexanol added to the solution: (the V_{Tr}-shift is given by $\Delta V_{Tr} = V^o_{Tr} - V'_{Tr}$: in this case $\Delta V_{Tr} \approx 5$ mV, V_{Tr} is defined as the membrane voltage at the center of the transition region which separates the

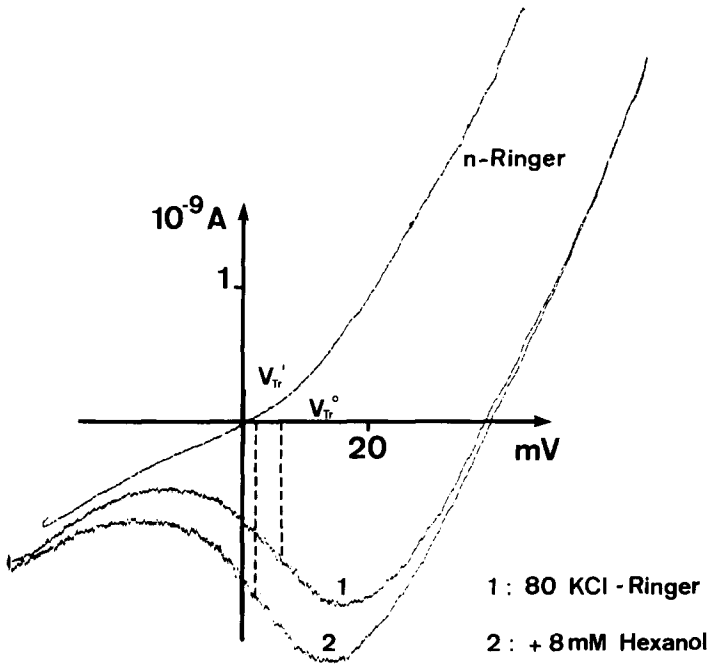

Fig. 1. Original registration of stationary I vs. V curves of the nerve axon membrane demonstrating the effect of n-alkanols. Curve 1 corresponds to 80 mM KCl-Ringer's solution as extracellular solution; V°_{Tr} indicates the respective transition voltage. V'_{Tr} indicates the transition voltage of curve 2 which corresponds to 8 mM 1-hexanol added to the 80 mM KCl-Ringer's solution. The I vs. V curve for normal Ringer's solution (n-Ringer) is shown in addition.

two conductivity states of the membrane [11]). Isomeric alkanols also produce V_{Tr}-shifts, but the effect is smaller (2-hexanol) than in the normal alkanol or even changes the sign (3-hexanol).

(2) Veratridine-depolarization of the membrane. The alkaloid, veratridine, is known to increase the steady-state Na^+ permeability of axon membranes [12]. Dissolved in normal Ringer's solution, veratridine therefore depolarizes the Ranvier node membrane if the nerve fibre is bathed in this solution. Contrary to the results obtained with K^+-depolarized membranes, V_{Tr} of the veratridine-depolarized axon membrane will not be shifted by adding n-alkanols to the external Ringer's solution. On the other hand, the amplitude of the veratridine-induced depolarization and therefore the stationary Na^+ permeability of the membrane will be reduced by all alkanols used. The amount of this veratridine antagonizing effect increases with chain length and concentration of the alkanols. This effect is seen in Fig. 2. The high-ohmic branch of the I vs. V curve remains unchanged, whereas for the low-ohmic branch the electrical resistivity of the membrane increases with increasing concentration and chain length of the alkanols. With isomeric alkanols such as 2-hexanol and 3-hexanol qualitatively the same effects are observed. The effectiveness of the alkanols referred to the same concentration, however, decreases in the following sequence: 1-hexanol > 2-hexanol > 3-hexanol.

(3) The n-alkanols increase the threshold voltage for stimulating action potentials and decrease the action potential amplitude. These effects are again

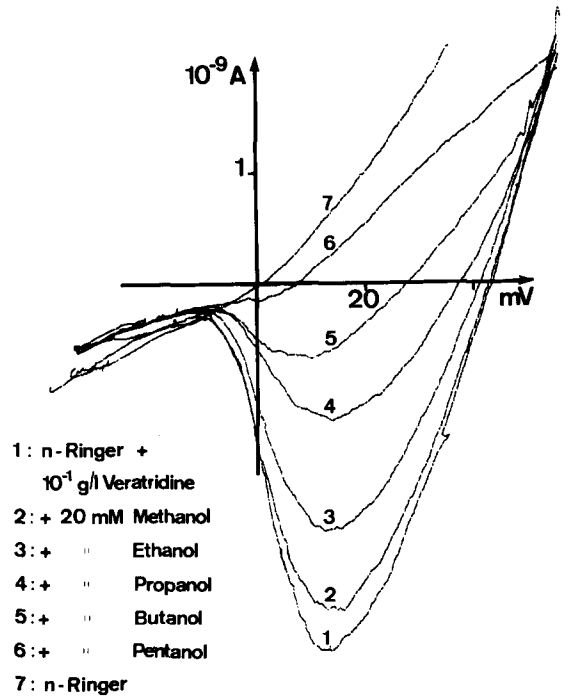

Fig. 2. Effect of different *n*-alkanols (20 mM) on the stationary I vs. V curve of the veratridine-depolarized axon membrane ($1 \cdot 10^{-1}$ g/l veratridine in normal Ringer's solution).

increased with increasing concentration and chain length of the alkanols. But, the alkanol-induced reduction of the action potential amplitude is not directly correlated to those of the stationary Na^+ current. Alkanol concentrations which already block the stationary Na^+ current only partially reduce the action potential amplitude. This can be seen by comparing Fig. 2 with Fig. 3 which shows action potentials measured at the same alkanol concentrations as used in the experiment of Fig. 2.

Before discussing these different experimental results the main observations should be summarized. In the case of K^+ as the dominant charge-carrier for the membrane conductivity, the *n*-alkanols shift V_{Tr} of the axon membrane whereas the values of the limiting resistances (high- and low-ohmic branches of the I vs. V curves) remain unchanged. Such V_{Tr}-shifts are not observed if, due to veratridine-poisoning of the membrane, the stationary current-voltage behavior will be affected also by Na^+ as charge carrier. Here the *n*-alkanols reduce both the stationary Na^+ current and the conductivity step between the high- and low-ohmic branch by increasing the low-ohmic membrane resistivity. The action potential amplitude and therefore the peak transient Na^+ current, however, is less affected by the alkanols as is the stationary Na^+ current. This is consistent with the fact that veratridine has only a small effect on the nerve action potential [12].

Assuming the high-ohmic branch of the stationary I vs. V curve as correlated to a more ordered (gel) state of the lipid matrix in the membrane and the low-

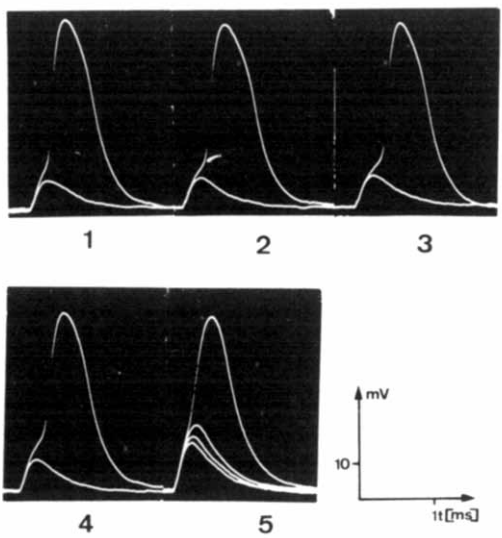

Fig. 3. Action potentials of different alkanols (20 mM) in the extracellular Ringer's solution. 1, no alkanol; 2, ethanol; 3, propanol; 4, butanol; 5, pentanol.

ohmic branch correlated to a more disordered (liquid crystalline) state of the lipids, the observed V_{Tr}-shifts (K^+-depolarization) are comparable to shifts of the phase transition temperature (T_m) produced by *n*-alkanols in artificial phospholipid membranes [4]. Alkanols shifting T_m to lower temperatures widening of the temperature region in which lipids are in liquid crystalline state) analogously shift V_{Tr} to more negative voltages widening of the voltage region in which lipids are in liquid crystalline state). This analogous behavior of V_{Tr} and T_m with respect to alkanol influence includes the effects of different concentrations, chain lengths and isomeric configuration of the alkanols. These observations support our assumption stated above, of a close correlation between stationary *I* vs. *V* characteristics and the morphological state of the membrane.

Interactions of the alkanols with the membrane surface charges (a possible explanation of the V_{Tr}-shifts [11]) are not likely because in recent electrophoretic experiments using membrane vesicles from nerve myelin, alkanol-dependent changes of the electrokinetic potential of these vesicles are not observed (Tippe, A. and Bamberger, S., unpublished results). This supports the proposal that the effective sites of the alkanols are within the aliphatic part of the lipid bilayer [4].

As seen by the alkanol-induced changes in the stationary *I* vs. *V* characteristics the stationary K^+ and Na^+ currents are differently influenced by the *n*-alkanols. But there is a close correlation between V_{Tr}-shift (K^+-depolarization) and reduction of Na^+ current (veratridine-depolarization) for all variations in alkanol concentration and chain length. This fact supports such theoretical models of the molecular mechanism of the alkanol effect on Na^+ pathways in the membrane which assume a triggering of the pathway function (open or closed) by phase transitions of the surrounding lipids [2,3]. The anaesthetic effect of the *n*-alkanols, however, cannot be fully explained by these models because the action potential amplitude only partially correlates to V_{Tr}-shifts and reduction

of stationary Na^+ current. This fact was recently also observed in squid giant axons [13]. The authors suggest that alkanols enhance the normal inactivation mechanism rather than blocking the fast Na^+ channels. Another explanation will be offered by the 'synapse hypothesis' of nerve excitation [14] which assumes membrane pathways for the transient Na^+ current to be located in the paranodal region thus separated from the pathways of the stationary ion currents in the nodal gap.

The author wishes to thank Professor Dr. H. Müller-Mohnssen for his support and encouragement.

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