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The Interaction of Homologous Series of Alkanols with Sodium Channels in Nerve Membrane Vesicles

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Summary. The potency of members of the homologous series of alkanols to block ^{22}Na uptake through sodium channels stimulated by veratridine was studied in membrane vesicles obtained from lobster walking leg nerves. A cut-off was revealed at the level of 1-undecanol. However, secondary isomers of inactive primary homologues, such as 5-dodecanol and 5-tridecanol, were able to block ion flux. From the concentration required for an equipotent effect, it was calculated that the standard free energy for adsorption of primary alkanols is -725 cal/mol CH_2. Furthermore, since the concentration required for an equipotent effect for primary isomer was found to be lower than that obtained for secondary isomers, it is concluded that the latter are less potent than the former. The similarity between this set of results and those obtained in intact frog sciatic nerve (J. Requena et al., *J. Membrane Biol.*, **84**:229–238, 1985) offers further support to the notion that the procedure employed to isolate the membrane vesicles does preserve the Na channels. However, the mechanism of alcohol inhibition of the Na channel in isolated membrane vesicles would seem to be somewhat different from that preferred in axons. While in vesicles the block needs to be thought in terms of a reduction in the number of conducting Na channel, in axons this is considered to be the less likely mode of action, mainly because under veratridine it is not possible to invoke a shift in the steady-state activation or inactivation.

Key Words excitability · nerve · alcohols · sodium channel · membrane vesicles · general anesthesia · cut-off effect

Introduction

Knowledge about the structure and function of the Na^+ channel at the molecular level has advanced, thanks to the solubilization and purification of Na^+ channels from different sources (Agnew et al., 1978; Barchi, Cohen & Murphy, 1980; Hartshorne & Catterall, 1981; Lombet & Lazdunski, 1984; Villegas et al., 1988), to their functional reconstitution into liposomes or planar lipid bilayers (Villegas et al., 1977; Hartshorne & Caterall, 1981; Weigele & Barchi, 1982; Rosenberg, Tomiko & Agnew, 1984*a*,*b*; Hartshorne et al., 1985) and, more recently, to the sequencing of its DNA precursor (Noda et al., 1984).

The sources of Na channels for solubilization, purification and reconstitution studies have been mainly vertebrate nerve and muscle plasma membranes (Agnew et al., 1978; Barchi et al., 1980; Hartshorne & Catterall, 1981; Weigele & Barchi, 1982; Lombet & Lazdunski, 1984; Rosenberg et al., 1984*a*,*b*; Tamkun, Talvenheimo & Catterall, 1984; Hartshorne et al., 1985). However, the functional reconstitution was first achieved with crude membrane preparations from lobster nerves incorporated into soybean liposomes (Villegas et al., 1977; Villegas, Villegas & Suárez-Mata, 1981).

The functional relationship between the native and the reconstituted Na channel and the receptor sites for neurotoxins, has been well studied in vertebrate and invertebrate preparations. Tetrodotoxin (TTX) (Kao, 1966), veratridine (VER) (Ulbricht, 1969), and batrachotoxin (BTX) (Albuquerque, Daly & Witkop, 1971) are the most common toxins used to characterize the Na channel. TTX and saxitoxin share the same receptor site and block reversibly the ion flux through the Na^+ channel. The lipid-soluble polycyclic compounds VER, batrachotoxin, aconitrine and the grayanotoxins affect the voltage dependence of activation and inactivation of the Na channel. They displace the voltage required for activation toward more negative values and prevent channels from inactivating (Catterall, 1980). However, there has been no systematic investigation of the action of anesthetics either at the isolated Na channel level or a comparative study of its action in vesicles and in intact nerves. In fact, there only appears to be a couple of papers on the interaction of vesicles with experimental general anesthetics (Rosenberg et al., 1984*a*; Willow et al., 1986).

Since the examination of interactions of a homologous series of compounds with the unit responsible for the excitation has provided some mechanistic insight into the process (Armstrong & Binstock, 1964; Haydon, Elliot & Hendry, 1984), it seemed appropriate to extend the investigation into isolated nerve membrane vesicles containing Na channels. Moreover, such study would also help to understand the nature of the cut-off effect of general anesthesia. In effect, Requena et al. (1985) have advanced a hypothesis based upon the activity of structural isomers of members of the homologous series of functional derivatives of alkanes. It was argued that since they have similar chemical and physical properties (more specifically, an almost identical mean molecular volume), they should have nearly equal potency in blocking nerve impulse, i.e. the cut-off point should only depend upon the number of aliphatic carbons in the chain, but not on the isomeric arrangement. This was found not to be the case in frog sciatic nerves. Thus occurs the interest to further explore this question in an artificial system composed of isolated nerve membrane vesicles containing Na channels and exposed to members of the homologous series of alkanols as experimental general anesthetic agents.

Materials and Methods

Plasma Membrane Preparation

Plasma membranes were isolated from the walking-leg nerves of the lobster *Panulirus argus*. Up to 50 g wet weight of nerves were used at a time. The procedure is similar to that previously reported (Barnola, Villegas & Camejo, 1973; Correa, Villegas & Villegas, 1987). Briefly, the nerves were kept after dissection in an ice-cold 0.33 M sucrose solution containing 2 mM $MgCl_2$ and buffered with 10 mM Tris-HCl (pH 7.5). The amount of sucrose solution was adjusted to 2 ml per g of wet tissue and then the nerves were minced in a blender at 12,000 rpm for 1 min for each 10 g of tissue. The minced tissue was next homogenized with 10 strokes in a 55-ml glass-Teflon® pestle at 9700 rpm. The sucrose solution then adjusted to a final volume of 10 ml per g of wet nerve. The homogenate was centrifuged at 65,000 × *g* for 40 min. The pellets were resuspended in sucrose solution, homogenized again and adjusted to a final volume corresponding to 4.5 ml per g of wet nerve. This homogenate was layered on top of a solution containing 1.12 M sucrose, 2 mM $MgCl_2$, and 10 mM Tris-HCl (pH 7.5), and centrifuged in a Beckman swinging rotor at 65,000 × *g* for 60 min. Plasma membrane banding at the interface on top of the 1.12 M sucrose solution were collected, diluted threefold with 10 mM Tris-HCl (pH 7.5) containing 2 mN $MgCl_2$, and centrifuged at 65,000 × *g* for 40 min. The membrane pellets were resuspended in 0.35 M sucrose, 10 mM Tris-HCl (pH 7.5), homogenized by hand in a glass-Teflon pestle homogenizer and kept at −70°C until use. The temperature during the isolation procedure was 2 to 4°C. The final protein concentration was approximately 10 mg/ml. Protein measurements were done according to the procedure described by Lowry et al. (1951). Lipid content of the membrane vesicles was derived from the protein-to-lipid ratio (32 : 68) determined for this preparation by Barnola et al. (1973).

Preparation of Membrane Vesicles

Froxen plasma membranes were thawed, and then diluted fivefold in a solution containing 300 mM KP_i, 1 mM NaP_i, 2 mM $MgSO_4$ (pH 7.4) to a final protein concentration of about 2 mg/ml. The membrane suspension was then incubated for 10 min at 30°C under continuous agitation and centrifuged at 50,000 × *g* for 10 min at 4°C. The supernatant was discarded and the pellet was resuspended in the same solution, rehomogenized and kept in ice until use.

Experimental Procedure and ^{22}Na Uptake Assays

The membrane vesicles resuspended in the 300 mM KP_i, 2 mM $MgSO_4$ and 1 mM NaP_i (pH 7.4) were diluted to half with the same solution with the test alcohol added, usually, at double the desired final concentration. The vesicles with the alcohol were then incubated for 1 hr under continuous agitation at 4°C. At the end of this period, 20 μl of the vesicles suspension were diluted in 180 μl of a solution made of 300 mM choline chloride, 1 mM NaP_i, 2 mM $MgSO_4$ at pH 7.4 containing the ^{22}Na label, the test alcohol and the neurotoxins.

The ^{22}Na uptake assays were done following the procedure described by Gasko et al. (1976). The ^{22}Na uptake determinations were initiated by the addition of the ^{22}Na-labeled external solution (1 μCi ^{22}Na/ml) at room temperature (20–22°C) to the suspension of membrane vesicles, and ended by the addition of 5 μl of ice-cold 50 μM TTX. 175 μl of this mixture were then immediately passed through a Pasteur pipette-size column of Dowex 50W-8, 50-100 mesh (Sigma Chemical Co., St. Louis, MO). The Dowex columns were preequilibrated with 10 mM Tris-HCl (pH 7.5) and right before use pretreated with 100 μl of 10 mg/ml bovine serum albumin (Albumin Stock Solution, Sigma Chemical Co., St. Louis, MO), followed with 3 ml of ice-cold 0.5 M sucrose solution buffered with 10 mM Tris-HCl (pH 7.5). The sample from the vesicles was eluted with 2 ml of the 0.5 M sucrose solution. The material eluted from the Dowex columns was received in counter vials, 5 ml of scintillating liquid was added and the samples were analyzed in a Beckman scintillation counter, model LS 7800. From the specific activity, the ^{22}Na uptake can be calculated and the results could either be expressed in nmol of Na per mg protein per min, or in a percentage basis with a control experiment as reference.

Reagents

All reagents were obtained from Sigma Chemical Co. ^{22}Na as NaCl in water solution, carrier free, 13.22 mCi/ml, was obtained from New England Nuclear, Boston, MA. The neurotoxins used were the following: citrate-free tetrodotoxin (TTX) (mol wt 319.28) from Calbiochem, San Diego, CA, and veratridine (VER) (mol wt 673.81) purified in our laboratory according to Kupcham et al. (1953), from commercial Veratrine obtained from Sigma. Stock solutions of the toxins were prepared as follows: 50 μM TTX dissolved in 10 mM acetic acid and 37.5 mM VER in 95%

ethanol. The TTX and VER stock solutions were kept at 4°C. The final concentration used in the assay were 1 μM for TTX and 0.75 mM for VER. Since ethanol from the veratridine stock solution was allowed to evaporate, under a stream of N_2, from the test tube where the assay would take place, its final concentration in the reagent mixture was negligible. Alcohols were either purchased commercially or, when necessary, synthesized as described in Requena et al. (1985). In all cases, if purity was less than 98%, they were fractionally distilled under reduced pressure in order to achieve the desired purity of >99%. Purity was checked by gas-liquid chromatography using a Varian 1440 unit equipped with a flame ionization detector and stainless steel columns packed with Chromosorb W (80 to 100 mesh) as support and SE-30 (3% or FFAP 5 to 10%) as stationary phase.

Results

The ^{22}Na uptake was calculated from the influx measured after the first 30 sec of exposure to the ^{22}Na-containing solution. This time was chosen because as shown by Correa et al. (1987) it is the shortest period in which measurements can be made with precision and also, because influx calculated from measurements made after the first 30 sec are more affected by a ^{22}Na efflux component and/or by dissipation of any ionic gradient.

The ^{22}Na uptake was determined in the absence of toxins (control), in the presence of 0.75 mM VER and in the presence of the same concentration of VER plus 1 μM TTX. The difference between the uptake of ^{22}Na in the presence of VER alone and the uptake in VER plus TTX is considered to represent the ^{22}Na entry through the Na channel, and for the purpose of this work is defined as 100% Na channel activity. Values for net uptake of ^{22}Na were of the order of 10 nmol/(mg of protein · min). These are similar to that previously determined by Correa et al. (1987) utilizing similar experimental procedures.

In order to quantify the action of the alcohols on the Na channel activity, the set of measurements just described was carried out in the presence of a given alcohol concentration. The reduction of the ^{22}N uptake due to the alcohol was referenced to the uptake value measured in the absence of the test alcohol and this expressed, throughout, as percent inhibition of the Na channel activity.

The establishment of an electrochemical potential difference across the membrane of the vesicles is considered to be a convenient experimental condition (Correa et al., 1987). An ionic gradient of a permeable cation which would in turn generate a diffusion potential was thus established. In essence, since K^+ ions are readily permeable through plasma membranes, a diffusion potential for this ion was created diluting the K^+-loaded vesicles in solutions containing a less permeable cation like choline (or Tris). The details for the generation of the potential difference across the membrane vesicles were taken from Correa et al. (1987).

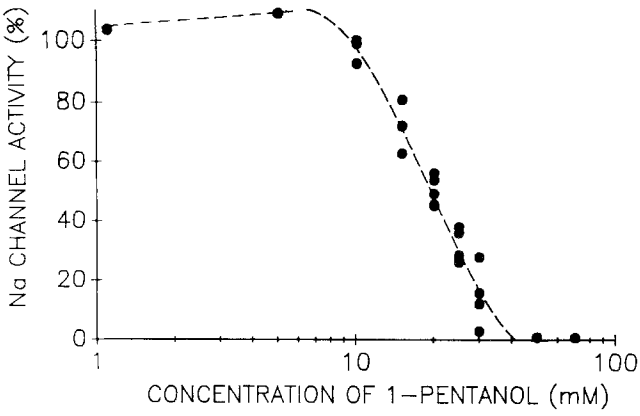

Fig. 1. Dose-response curve for 1-pentanol. The Na channel activity of the nerve membrane vesicles is plotted against the concentration of 1-pentanol in buffer (logarithmic scale). The Na channel activity equal to 100% corresponds to the value of the VER-induced, TTX-sensitive ^{22}Na uptake in the absence of test alcohol. Each point represents one measurement. Line corresponded to best-fitted polynomial. Membrane lipid concentration 19.3 mg/ml

Optimal experimental conditions were established in a series of preliminary experiments for membrane lipid concentration, VER and TTX concentration, time of incubation and experimental protocol. The flux parameters measured in control experiments corresponded with values published in similar preparations (Correa et al., 1987).

Potency of Alcohols

Figure 1 shows the effect upon the uptake of ^{22}Na into membrane vesicles of various concentrations of 1-pentanol in the buffer solution. Vesicles were exposed to the different alcohol concentration for 1 hr at 4°C and then the Na uptake was determined in the presence of 0.75 mM VER and 0.75 mM VER plus 1 μM TTX. The Figure summarizes all the results available for this alcohol, some 36 points carried out in eight experimental runs. The protein content in these experiments was 9.6 mg/ml of buffer or its equivalent of 19.3 mg of lipid/ml. The relationship between the concentration of the alcohol and the Na channel activity, in terms of a reduction in ^{22}Na uptake, is described by a dose-response type of sigmoid curve with an apparent inhibition constant of 19.5 mM 1-pentanol.

The concentration of VER (0.75 mM) used in the experiment of Fig. 1 is 20 times higher than the apparent affinity constant (0.035 mM) determined for the experimental preparation. Nevertheless, in order to discard any competition of alkanol with

Table. Effect of alkanols on Na channel activity

Alcohol	Solubility (mM)	Number Expts.	Half block	
			Vesicles (mM)	Sciatic (mM)[a]
1-pentanol	177.00	36	19.54	19.0
3-pentanol	—	42	52.4	51.0
1-heptanol	13.5	15	1.08	1.8
1-nonanol	1.1	40	0.32	0.14
1-nonanol[b]	—	8	0.13	—
5-nonanol	2.0	45	0.83	0.55

[a] Data from Requena et al., 1985.
[b] Interpolated from data at lipid concentration 4.8–9.5 mg/ml. All other expts. at 19.3 mg lipid/ml.

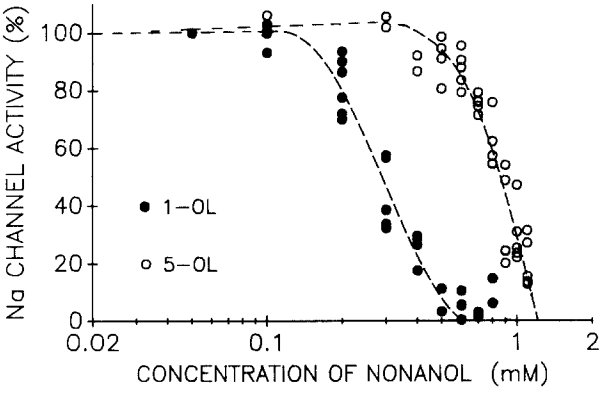

Fig. 2. Dose-response curve for nonanol. The Na channel activity of the nerve membrane vesicles plotted against the concentration of 1-nonanol (filled circles) and 5-nonanol (open circles) in buffer (logarithmic scale). The Na channel activity equal to 100% corresponds to the value of the VER-induced, TTX-sensitive ^{22}Na uptake in the absence of test alcohol. Each point represents one measurement. Lines correspond to best-fit polynomial. Membrane lipid concentration was 19.3 mg/ml

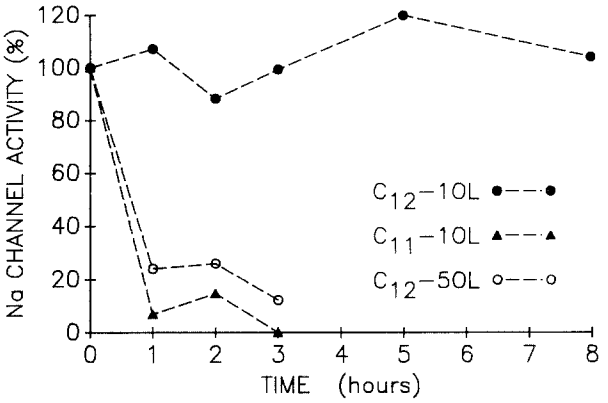

Fig. 3. Time course of Na channel inhibition around the point of cut-off. The Na channel activity of the nerve membrane vesicles plotted against time of exposure of the membrane vesicles to buffer saturated with long-chain alcohols. Filled circles (●) correspond to 1-dodecanol (solubility = 0.010 mM), open circles (○) to 5-dodecanol (solubility = 0.036 mM) and filled triangles (▲) to 1-undecanol (solubility = 0.063 mM, taken from Requena et al., 1985). The Na channel activity equal to 100% corresponds to the value of the VER-induced, TTX-sensitive ^{22}Na uptake in the absence of test alcohol. Each point represents the mean of five determinations. Membrane lipid concentration was 19.3 mg/ml

VER, a set of control experiments looking for an effect of [VER] on the apparent inhibition constant of an alcohol was carried out. An apparent inhibition constant of 19 mM (±2 mM as limits) was determined for 1-pentanol throughout the VER concentration range of 0.075 to 1.5 mM. Thus, it would appear that pentanol and VER do not compete for the same site in isolated nerve membrane vesicles containing Na channels.

A similar effect to that shown for 1-pentanol on Na channel activity was observed for other members of the primary homologous series of alkanols. The results for these are summarized in the Table.

From the concentration of members of the primary series of alkanols required to block by 50% the uptake of Na by the vesicles, a free energy of adsorption of −725 cal/mol CH_2 can be calculated. This value was obtained as the slope of the linear regression on the number of carbons in the hydrophobic chain of the concentration determined for an equipotent effect. This treatment follows the analysis of Haydon and Urban (1983) for the reduction of Na current by alkanols in squid giant axons. The calculated free energy of adsorption of primary alkanols in lobster nerve membrane vesicles is very similar to that observed in frog sciatic nerve (−705 cal/mol CH_2) by Requena et al. (1985) and in squid giant axons (−730 cal/mol CH_2, Haydon & Urban, 1983).

In the Table the concentration of various alkanols required to reduce by 50% the amplitude of the compound action potential in frog sciatic nerves is also listed (from the work of Requena et al., 1985). For very alcohol tested, a comparison of this equipotent parameter with that detected in lobster nerve membrane vesicles reveals that these are very similar. Nevertheless, it should be mentioned that a reduction in 50% of the compound action potential amplitude is not necessarily equal to a reduction in 50% of the ^{22}Na uptake through the Na channel, even though they are quite related. In fact, it has been shown (Haydon & Urban, 1983, 1986) that alkanols block both Na and K currents. The effect on the former current is higher than that on the latter current by a factor of 2, however.

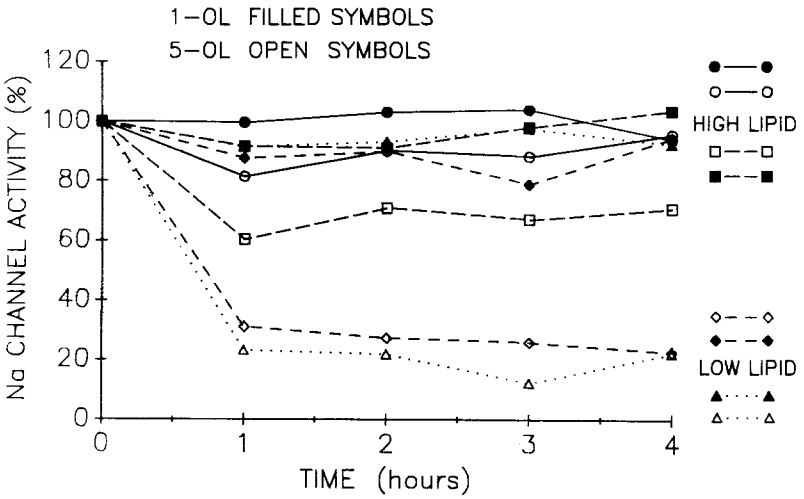

Fig. 4. Time course of channel inhibition by tridecanol at various lipid concentrations. The Na channel activity of the nerve membrane vesicles plotted against time of exposure of nerve membrane vesicles at varying concentrations in buffer saturated with tridecanol. Solubility of 1-tridecanol = 0.0025 mM; for 5-tridecanol = 0.0089 mM (from Requena et al., 1985). Filled symbols correspond to 1-tridecanol while open symbols to 5-tridecanol. Triangles (△) correspond to 2.4 mg/ml of lipid; diamonds (◇) to 4.8 mg/ml; squares (□) to 9.6 mg/ml and circles (○) to 19.3 mg/ml. The Na channel activity equal to 100% corresponds to the value of the VER-induced, TTX-sensitive ^{22}Na uptake in the absence of test alcohol. Each point represents the mean of five determinations

Potency of Structural Isomers

If one looks at the alkanol concentrations required to reduce 50% the uptake of ^{22}Na through the Na channel into the vesicles (*see* Table), it is observed that the primary isomers are more potent than the secondary ones, i.e. a smaller concentration of the primary isomers is needed to induce an equipotent effect. This point is also illustrated in Fig. 2 where dose-response curves for the structural isomers of nonanol are shown. Six times more of the secondary isomer (with the hydroxyl in carbon 5) than of the corresponding primary isomer is required to induce a similar inhibitory effect on ^{22}Na uptake in the vesicles. Each set of experimental points is joined by the best-fit curve, characterized by an apparent inhibition constant of 0.3 and 0.83 mM for the primary and the secondary isomer of nonanol, respectively. A comparison of the results obtained in lobster nerve membrane vesicles about the relative potency of the primary and secondary isomers reveals that they are, once more, very similar to those obtained in intact frog sciatic nerves.

Cut-off Effect

In order to study the behavior of anesthetic agents around the point of "cut-off," a kinetic type of experiment in which a time dependency for the equipotent inhibition is sought has to be inevitably done. In these experiments the alkanol saturating concentration in buffer is chosen as the reference state. Alkanol solubilities were taken from Requena et al. (1985) and, when necessary, listed in the Figure legends. For the experiments, lobster nerve membrane vesicles were exposed to the alcohol for a variable period of time. Figure 3 shows the time course of the Na channel activity for vesicles exposed to undecanol or dodecanol. As can be seen, ^{22}Na uptake in membrane vesicles treated with the primary isomer of dodecanol was not affected in a period of 8 hr of exposure. In fact in a couple of very long experiments, up to 48 hr, no effect on Na flux was detected. However, for undecanol, within less than 1 hr, the saturating concentration of the primary isomer blocked almost completely the uptake of ^{22}Na. Interestingly, the secondary isomer of dodecanol (5-dodecanol) was also capable of reducing Na channel activity.

In Figure 4, the results of a similar study carried out with 1-tridecanol and its secondary isomer 5-tridecanol are presented. In this Figure, besides testing the effect of the isomers on Na channel activity, the effect of having various membrane lipid concentrations was investigated. The different lipid concentrations were obtained by adjusting the amount of nerve membrane vesicles present in the buffer solution. The lipid concentration was varied from 2.4 mg/ml up to 19.3 mg/ml. It can be observed that in the case of the primary isomer of tridecanol, no matter what the lipid concentration is, in 4 hr of

exposure (in some cases 8 hr) there is no evidence of Na channel block. However, for the secondary isomer there is a clear inhibition, amounting to 80% of the ^{22}Na uptake but only when the concentration of lipids is 2.4 and 4.8 mg/ml. For higher lipid concentrations of 9.6 and 19.3 mg/ml there are partial inhibitions of 40 and 10%, respectively.

Discussion

Cut-off Effect

Theories to explain the mechanism of general anesthesia revolve around the notion that the phenomenon occurs when a region of the cell membrane is caused to enlarge beyond a critical volume by the adsorption of an inert substance (Mullins, 1954; Miller et al., 1973; Janoff & Miller, 1983). This region has been thought to be hydrophobic and its location and nature is the subject of much research (Franks & Lieb, 1978, 1981, 1982; Janoff, Pringle & Miller, 1981). The effect caused by the adsorption of the inert chemical agent has been argued to occur either locally, at the level of the lipoprotein complex responsible for the gated flow of ions, i.e. the Na channel or, alternatively and less specifically, along the lipid bilayer region of the cell membrane (Seeman, 1972; Haydon et al., 1977*a,b*).

One of the intriguing puzzles in the context of experimental general anesthesia is the cut-off effect (Pringle, Brown & Miller, 1981; Requena & Haydon, 1985). This effect, better found in homologous series of hydrocarbon derivatives, manifests itself as the inability of higher members of a series to act as anesthetics agents. Thus, in alkanols, a classical example, a systematic increase in potency is observed from methanol up to dodecanol while saturated solutions of tridecanol are inactive for exposure periods comparable to the survival time of the experimental preparation (Meyer & Hemmi, 1935; Seeman, Roth & Schneider, 1971; Richards et al., 1978).

The experiments reported herein show that for all alcohols tested, the value for the concentration associated with an equipotent effect are very similar in the intact nerve and in the nerve membrane vesicles experiments. Furthermore, the experimental results in lobster nerve membrane vesicles reveal that the cut-off point rather than occurring at the level of 1-dodecanol as in the intact frog sciatic nerve experiments, occurs at the level of 1-undecanol. It should be stressed that, as a consequence of structural isomerism, in both systems the point of cut-off shifts in secondary isomers to a higher member in the homologous series. Nevertheless, the detected difference in the occurrence of the point of cut-off in nerve membrane vesicles with respect to intact sciatic nerve should not be too worrisome. Indeed, there is in the in vivo system some latitude in the determination of the exact cut-off point (Rang, 1960).

Depletion of Lipids

In order to study the effect of anesthetics around the point of cut-off, in a preparation such as an excitable cell, one needs to determine the time of exposure to an agent required to induce a given degree of block. It is conceivable, thus, that the observation relating loss of blocking activity of alcohols with hydrocarbon chain-length increment might be a reflection of depletion of the molecule from the aqueous phase, this process being caused by the excess amount of lipophylic pockets present in the experimental system and which would act as a sink. For example, in a preparation such as an isolated sciatic nerve, the myelin, far in excess of the exposed regions of axolemma, would deplete the highly nonpolar long-chain hydrocarbon agent from the Ringer's, limiting the access of the experimental anesthetic to the excitable regions.

The experiments performed in isolated nerve membrane vesicles would seem to be able to offer some insight into this situation. Indeed, on the one hand, they tender a somewhat more controllable experimental environment while, on the other, they permit to assume if not an equilibrium condition, certainly a steady state. Specifically, the experiment with very long-chain alkanols at various concentrations of lobster nerve membrane vesicles show that the blocking effect appears to depend somewhat on the membrane lipid concentrations parameter of the preparation. Thus, for an active secondary isomer of tridecanol, raising the concentration of lipid from 2.4 to 19.3 mg/ml made the inhibitory effect on Na uptake diminish. However, it should be pointed out that in experiments with the primary isomer of dodecanol or tridecanol, a reduction of the lipid content of the preparation and/or very long exposure time did not elicit a blocking effect on ^{22}Na uptake.

It is worth mentioning that the effect of depletion by lipid starts to be visible around octanol. Thus, if an experiment similar to that shown in Fig. 2 is carried out for nonanol but at a different lipid concentration, a shift in the apparent inhibition constant is observed. For the primary isomer, at low lipid (4.8–9.6 mg/ml) a limiting value of 0.13 mM of 1-nonanol can be extrapolated for the apparent inhibition constant. This result is listed in the Table.

Mechanism of Action

It is very unlikely that the described effects of alkanols on isolated lobster nerve membrane vesicles could be the outcome of interactions of the alcohols with the neurotoxins employed in this study and/or its site of action. It is more likely that the reduction in Na channel activity is a direct result of a modification of the channel environment leading to its closure. Nevertheless, it is worth considering the evidence available to support the notion that, if anything, members of the homologous series of alcohols behave in a noncompetitive fashion, with reference to the neurotoxin used either to activate (VER) or to block (TTX) the Na channel present in isolated lobster nerve membranes. There are several lines of evidence sustaining this conclusion: 19 mM of 1-pentanol in the buffer solution, a concentration which is, roughly, 25 times higher than the VER concentration used throughout the experiments, produces the same equipotent effect as does 0.13 mM of 1-nonanol or even 0.063 mM 1-undecanol; control experiments show that the experimentally determined apparent inhibition constant for 1-pentanol were not affected by changes in VER concentration over a wide range; saturating concentrations of alkanols were as effective, if not better, than 1 μM TTX in blocking Na channel activity in vesicles. It should be recalled that the apparent dissociation constant for TTX in the system is of the order of 0.027 μM, while a value of 35 μM was determined for VER. This last dissociation constant is almost twice the value reported in the literature for a very similar system (Correa et al., 1987). Notwithstanding all these, in the experiments reported, the concentrations of neurotoxins employed were, for all practical purposes, saturating and well above the apparent dissociation constant. This ensured the best resolution for the inhibitory effect of alkanols on Na channel activity.

The close relationship between the results presented herein for nerve membrane vesicles with comparable experiments carried out in whole nerve (Requena et al., 1985), could be thought to imply that the VER modified Na channel in isolated membrane vesicles behaves as the native one in an isolated nerve fiber. A point of caution is apropos of this conclusion. It has been shown by Haydon and collaborators (for a review *see* Haydon et al., 1984) that the effect of alcohols occurs in squid giant axon mainly through either shifts in the steady-state activation and inactivation parameters of the Hodgkin and Huxley formulation or through an increment in the rate of inactivation. Yet, these mechanisms of action of alcohols in intact nerve fibers are not possible in isolated membrane vesicles mainly because in the vesicles the Na channels are held open by veratridine. That is, Na channels are fully activated while inactivation is blocked. Thus, the inhibitory effect of alcohol in vesicles has to be thought in terms of a direct block of the Na channel, that is, in a reduction in the Na conductance. In intact nerve fibers, however, this is considered to be the less likely mechanism of action, mainly because it was the less sensitive mode of action to alkanol concentration. In fact, shifts in the steady-state activation or inactivation were the preferred mechanism in squid giant axon.

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