BRIEF COMMUNICATION

n-ALKANOLS POTENTIATE SODIUM CHANNEL INACTIVATION IN SQUID GIANT AXONS

G. S. OXFORD AND R. P. SWENSON, *Department of Physiology, University of North Carolina, Chapel Hill, North Carolina 27514, and Department of Physiology and Pharmacology, Duke University Medical Center, Durham, North Carolina 27710 U.S.A.*

ABSTRACT The effects of *n*-octanol and *n*-decanol on nerve membrane sodium channels were examined in internally perfused, voltage-clamped squid giant axons. Both *n*-octanol and *n*-decanol almost completely eliminated the residual sodium conductance at the end of 8-ms voltage steps. In contrast, peak sodium conductance was only partially reduced. This block of peak and residual sodium conductance was very reversible and seen with both internal and external alkanol application. The differential sensitivity of peak and residual conductance to alkanol treatment was eliminated after internal pronase treatment, suggesting that *n*-octanol and *n*-decanol enhance the normal inactivation mechanism rather than directly blocking channels in a time-dependent manner.

The *n*-alkanols and the *n*-alkanes exhibit anesthetic actions on nerve (1, 2) and have been suggested as useful compounds for examining the mechanisms of general anesthesia (1, 3). Recent studies on both natural and artificial membranes using saturated solutions of the *n*-alkanes have revealed correlations between the structural properties of phosphatidylcholine-cholesterol bilayers, nerve blocking activity, and alkyl chain length (2). Although they afford both simple structure and increased aqueous solubility, the series of *n*-alkanols have not been examined in a similar fashion. Octanol, however, has been studied independently in artificial bilayer membranes (3), squid giant axon (4), and frog neuromuscular junction (5). The latter study suggested that changes in end-plate channel gating kinetics occur in the presence of octanol. Prompted by these experiments, we began a detailed examination of the influence of *n*-alkanols upon sodium channel function in internally perfused squid giant axons. Our most prominent observation thus far is that *n*-octanol and *n*-decanol, in micromolar concentrations, enhance sodium channel inactivation in that these alcohols almost completely eliminate steady-state current while only partially reducing peak transient sodium current.

Experiments were performed by using giant axons from *Loligo pealei* obtained at the Marine Biological Laboratory, Woods Hole, Mass. Conventional axial wire voltage clamp methods, including series resistance compensation (6, 7), were employed, and the axons were internally perfused by the roller method. Axons were bathed in an artificial seawater (ASW) containing 445 mM NaCl and 5 mM *N*-2-hydroxyethylpiperazine-*N*-2-ethane sulfonic acid buffer, pH 8.0. In some experiments the external sodium concentration was reduced to

one-third by equimolar substitution with tetramethylammonium (TMA^+). No experiments to examine alkanol effects on potassium channels were attempted and thus current flow through these channels was prevented by the removal of potassium ions. Two potassium-free internal perfusates were used. The first contained 275 mM CsF, 20 mM Na glutamate, 350 mM sucrose, and 30 mM Na phosphate buffer, pH 7.3. The other solution contained 250 mM CsF, 50 mM Na glutamate, 400 mM sucrose, and 10 mM morpholinopropane sulfonic acid (MOPS) buffer, pH 7.3. No differences were observed in results from the two solutions. Temperature was maintained at 10 ± 0.2°C during all experiments. Alcohol solutions were prepared from 1 M stock solutions in dimethylsulfoxide (DMSO) by injection into rapidly spinning external or internal perfusate. DMSO alone had no effect at concentrations of 2% or less as determined in separate control experiments.

n-Octanol (0.1–1 mM) reversibly decreased peak sodium current when applied either externally or internally with 0.5 mM *n*-octanol reversibly decreasing peak sodium conductance by ~ 33% ($n = 4$). In perfused squid axons a residual steady-state sodium current exists for moderate duration, depolarizing voltage steps as described previously (8–10). In contrast to the partial reduction of peak sodium current, *n*-octanol almost completely eliminates the steady-state component in a reversible manner. Fig. 1 demonstrates this dual sensitivity to *n*-octanol. Similar results were observed with *n*-decanol in the same concentration range. Control experiments revealed the steady-state current to be as sensitive to tetrodotoxin block as peak current, indicating that normal voltage-dependent sodium channels are involved in the effect rather than another unidentified channel type.

The apparent sensitivity of peak (I_p) and steady-state (I_{ss}) sodium currents to *n*-octanol and *n*-decanol can be compared in terms of the fractional current remaining during alcohol treatment. The relative magnitudes of I_p and I_{ss} after alcohol treatment were compared to control values by computing the ratio of I_p to I_{ss} in the alcohol, each expressed as a percent of

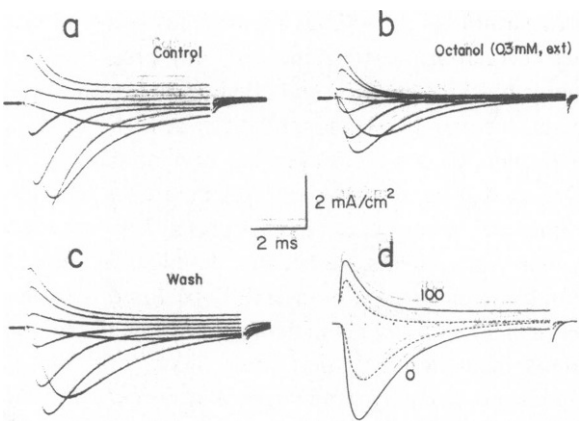

FIGURE 1 Effects of *n*-octanol on sodium current-time records. (*a*) Family of current-time records from an axon bathed in ASW and perfused with 275 mM CsF, 20 mM Na glutamate, 350 mM sucrose, 30 mM Na phosphate buffer, pH 7.3. The voltage steps were applied in 20-mV increments from −40 mV to +100 mV. (*b*) The same family of currents after external exposure to 0.3 mM *n*-octanol for 10 min. (*c*) Recovery of sodium currents after a 15 min wash in octanol-free ASW. (*d*) Traces of current-time records from the same axon at 0 and +100 before (solid lines) and during (dashed lines) the octanol treatment to illustrate the relative sensitivities of peak and steady-state sodium currents to block.

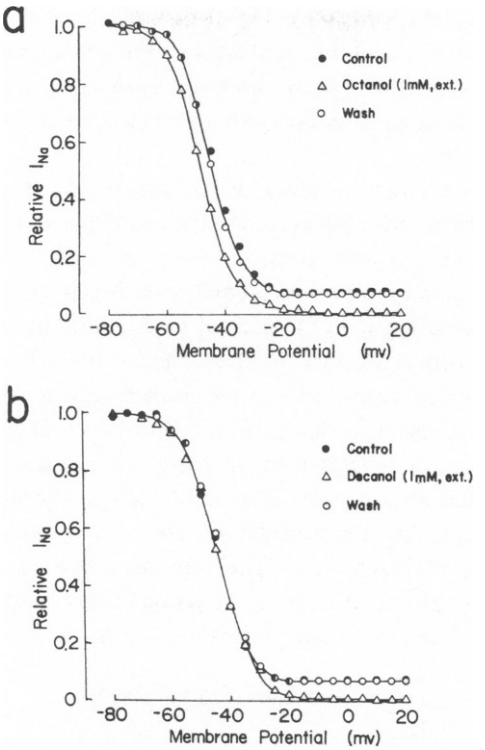

FIGURE 2 Voltage-dependence of sodium channel inactivation in octanol and decanol. Conditioning voltage steps of 50 ms in duration preceded test pulses to 0 mV. The magnitude of sodium current during the test pulse relative to the maximum current in the absence of a conditioning step is plotted as a function of the conditioning step amplitude. (*a*) Relative currents before (●), during (△), and after (○) exposure to 1 mM *n*-octanol applied externally. (*b*) Relative currents before (●), during (△), and after (○) exposure to 1 mM *n*-decanol applied externally.

the control measurements ($\%I_p$:$\%I_{ss}$). A value of 1.00 would clearly be predicted for no differential sensitivity to octanol. The ratio of $\%I_p$:$\%I_{ss}$ in 1 mM *n*-octanol = 5.28 ± 1.3 (n = 6). The ratio for 0.2 mM *n*-decanol = 2.71 ± 0.96 (n = 4). Both measurements were made for potential steps to +100 mV from a holding potential of −80 mV.

The dramatic enhancement of sodium channel inactivation can also be observed in conditioning prepulse experiments where the magnitude of peak sodium current during a step of 0 mV is measured as a function of 50 ms conditioning voltage and reveals a region of incomplete inactivation at positive membrane potentials (7–10) which is reversibly eliminated by either *n*-octanol or *n*-decanol (Fig. 2). The voltage sensitivity of sodium inactivation is relatively unchanged by the alcohols at these concentrations. Fig 2 *a* illustrates one case where a small reversible shift of the curve in the hyperpolarizing direction was found. This shift was the largest observed for this concentration range; however, somewhat larger shifts were observed at higher concentrations in crayfish axons.[1] In addition, no changes in the voltage-dependence of sodium channel activation were observed over the concentration range explored here.

[1]Swenson, R. P. Unpublished observations.

The kinetics of inactivation were only slightly changed despite the dramatic enhanced closure of sodium channels. The time constant for sodium current decay (τ_h) at +100 mV was 1.1 ± 0.19 ms before and 0.85 ± 0.2 ms after exposure to 0.5 mM octanol (n = 6). This change in kinetics is in the same direction although considerably smaller than observed for end-plate current kinetics (5).

The "time-dependent" reduction or block of sodium current suggested by these results is reminiscent of recent observations with certain local anesthetics (11, 12), pancuronium (13), and strychnine (14) applied internally to squid axons. An "ionic block" mechanism has been proposed for several of these compounds whereby the positively charged drug molecule enters open sodium channels competing with the normal inactivation mechanism for closure of the channel (11, 13). Experimental support for this concept arises from studies using axons in which normal inactivation has been removed by biochemical treatment (7, 15) where these compounds induce a time-dependent decrease in sodium current or "inactivation" (11, 13). Fig. 3 illustrates the effect of n-octanol in an axon after removal of most of the normal inactivation gating by internal pronase treatment. Although the magnitude of peak current was substantially reduced, in this case considerable steady-state sodium current remains after n-octanol treatment. The ratio of $\%I_p$:$\%I_{ss}$ for pronase-treated axons exposed to either n-octanol or n-decanol was 1.09 ± 0.39 (n = 3), considerably less than for axons with intact inactivation mechanisms. The virtual disappearance of enhanced inactivation after pronase-

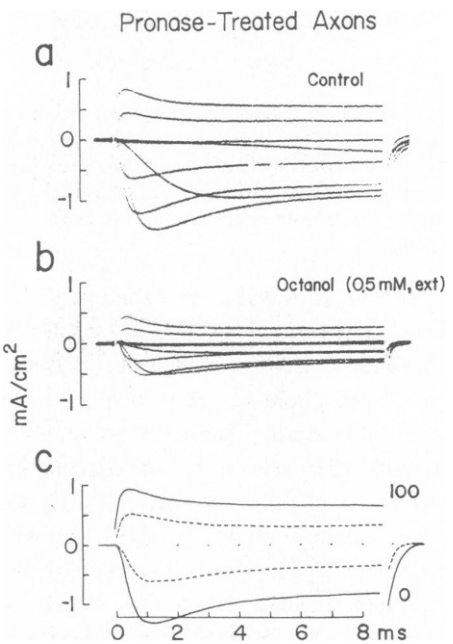

FIGURE 3 Octanol block of sodium currents in pronase-treated axons. (*a*) Family of sodium current-time records from an axon internally perfused with pronase (0.2 mg/milliliter) to destroy a substantial fraction of the normal inactivation behavior of sodium channels. Voltage steps were applied in 20-mV increments from −50 to +90 mV. (*b*) Family of current-time records from the same axon after external exposure to 0.5 mM n-octanol for 10 min. (*c*) Traces of current-time records for the same axon at 0 and +100 mV before (solid lines) and during (dashed lines) the octanol treatment to illustrate the similar sensitivities of peak and steady-state sodium currents to block ($\%I_p$:$\%I_{ss}$ = 1.34).

treatment suggests that the alcohols act by potentiating the normal inactivation mechanism rather than blocking open channels directly. A similar observation has recently been made with the anesthetic QX-314 in squid axons (12).

An analogous choice of mechanisms arises from the work on *n*-octanol at the neuromuscular junction. A definitive decision between the "ionic blocking" mechanism and an alteration of inherent end-plate channel gating could not be made. If the underlying molecular mechanisms for alcohol effects on axon and end-plate membranes are related, our experiments with noninactivating channels support the tentative conclusion of Gage et al. (5) that *n*-octanol speeds end-plate channel gating.

The relation of our observations to the Hodgkin-Huxley (H-H) model of sodium conductance (16) is unclear, as this formulation does not describe the observed steady-state sodium current. Modification to the H-H equations to account for this component have been attempted (10, 18) that suggest that the rate constant α_h may have a significant value at positive membrane potentials. If such a modification accurately describes normal inactivation gating in squid axons, a reduction of α_h at these membrane potentials would promote more complete closure of sodium channels and at least qualitatively account for our observations with *n*-octanol and *n*-decanol.

An alternative explanation for the noninactivating component of sodium current involves a second open state of the sodium channel as originally proposed by Chandler and Meves (17) and more recently suggested by Bezanilla and Armstrong (18). The two open states and their inactivation properties are described kinetically as follows:

$$h_1 \underset{\alpha_1}{\overset{\beta_1}{\rightleftharpoons}} x \underset{\beta_2}{\overset{\alpha_2}{\rightleftharpoons}} h_2,$$

where h_1 represents the normal open state originally described by Hodgkin and Huxley, x is the inactivated state, and h_2 is a normally noninactivating open state. A fraction of the channels that inactivate during a depolarizing voltage step rapidly populate the h_2 state, producing a steady-state current. In such a scheme *n*-octanol might selectively prevent the population of this second open state forcing most of the channels into the x or inactivated state. In this regard *n*-octanol may provide a useful tool by which to determine the existence and properties of such a second open state of the sodium channel.

The observations presented here provide no information concerning the molecular mechanism by which *n*-octanol and *n*-decanol exert effects on the sodium channel. Among the possible mechanisms proposed previously are membrane lipid fluidity changes (19), dimensional changes in the bilayer (2), and perturbation of the membrane electric field by dipole interactions (3). In addition, specific interaction of these molecules with the sodium channel protein as proposed for the neutral local anesthetic benzocaine (20, 21) may perhaps play a role in enhancing sodium inactivation. Further work is required to determine the involvement of these mechanisms in the phenomena reported here, as well as in the reduction of peak sodium conductance.

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