tance, and bisG10 blocking seem not to have been modified by the reconstitution process. The results also conform to the previous proposal of heterogeneity in channel distribution (5), with 30% of the vesicles having no channels and the rest having one or more. Furthermore, the fact that blocking by bisG10 was observed only after it had been loaded into the vesicles supports the idea of an asymmetric channel that can be blocked only from the inside of the membrane.

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Zn, MULTIPLE ACTIVATION PROCESSES, AND INACTIVATION DELAYS

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It is now well established that the activation and inactivation processes of the sodium conductance, g_{Na}, are coupled together in some way (Goldman, 1976; Armstrong and Bezanilla, 1977). Less clear is the nature of the coupling.

For *Myxicola* axons the coupling seems to be sequential, i.e., at least some fraction of the channels must open before they can inactivate (Goldman and Kenyon, 1982). Inactivation development, determined in this preparation with a two-pulse procedure, proceeds with an initial delay, as expected if inactivation develops subsequent to a precursor process; this precursor (delaying) process has the properties of Na activation.

In an extensive series of experiments in *Myxicola*, the time to peak g_{Na} during the conditioning pulse was found to be proportional to the inactivation delay, as required if inactivation follows sequentially on activation. This demonstrates that the potential dependence of the time constant of the delay process is the same as that of the activation process (Goldman and Kenyon, 1982). The potential dependence of the time constants of inactivation, τ_c and τ_h, differ significantly. Neither the normalized $\tau_h(V)$ nor $\tau_c(V)$ fit the inactivation delay vs. potential data, while the normalized $\tau_m(V)$ describes them well. In a less extensive series of determinations, the extracted time constant of the delay process, $\tau_{delay}(V)$, was found to be in quantitative agreement with $\tau_m(V)$.

We present here new results from *Myxicola* which firmly establish the close correspondence between inactivation delay and Na activation by showing that external Zn selectively slows both processes in parallel.

RESULTS

Fig. 1 presents a typical inactivation determination in the presence of Zn (5 mM). Peak Na currents during a series of fixed test steps in clamped potential were obtained by subtraction of current records obtained with identical protocols in Na-free artificial sea water (ASW). Each test step was preceded by a conditioning potential step of fixed amplitude but varying duration and a 6-ms step back to the holding potential. The peak currents during each of the test steps normalized to that during an unconditioned test step are shown as a function of conditioning step duration.

Inactivation delay, determined operationally as the time at which the unconditioned I_{Na} value intersects the τ_c exponential (solid curve), was 1.081 ms, while for another determination on this same axon under identical conditions, but without Zn, it was 0.594 ms: an increase in delay of 1.82-fold in Zn. Correspondingly, the time-to-half maximum of g_{Na} ($t_{1/2}$) during the conditioning pulse in this same axon increased nearly identically (1.81-fold) in 5 mM Zn, from 0.775 to 1.40 ms. 1 mM Zn had no effect.

These Zn effects on the Na kinetics cannot be attributed solely to changes in surface potential. If the changes in $t_{1/2}$ are assumed to arise entirely from surface potential effects, then τ_c should have increased from this change by

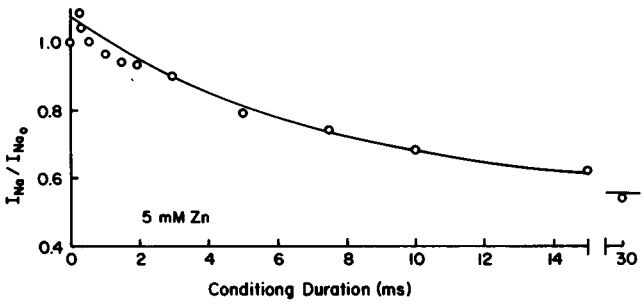

FIGURE 1 Time-course of inactivation development in 5-mM Zn. Normalized peak I_{Na} during a 10-mV test pulse is shown as a function of the duration of a −22 mV conditioning pulse. The solid curve is a simple exponential of time constant (τ_c) of 6.50 ms. Delay is 1.081 ms. 6-ms gap width. Holding potential −110 mV. 2 mM 3,4-diaminopyridine (Aldrich Chemical Co., Milwaukee, WI). Series resistance compensation. Bathing solution (1/2 Na ASW) had the following composition: 220 mM Na, 10 mM Ca, 50 mM Mg, 560 mM Cl, 225 mM Tris [Tris (hydroxymethyl) aminomethane] pH 8.0 ± 0.1. Temperature was 5 ± 0.5°C. Potentials are reported as absolute membrane potential (inside minus outside) and have been corrected for liquid junction potentials. Axon 82M40.

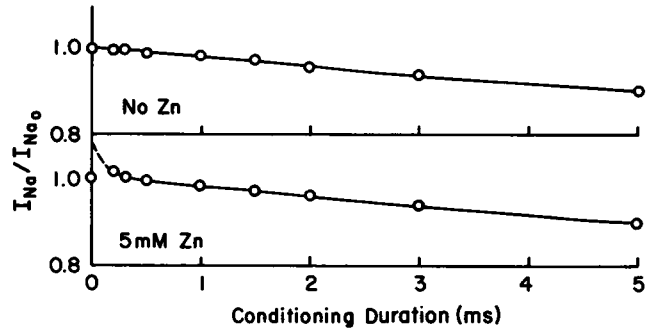

FIGURE 2 First 5 ms of the time course of inactivation development in the presence (bottom) and absence (top) of 5 mM Zn. All conditions otherwise the same as for Fig. 1. Solid curve (top) is the sum of two exponentials with a τ_{delay} of 0.57 ms and a τ_c of 5.65 ms. Bottom curve is the sum of three exponentials with τ_{delay2} of 0.1667 ms, τ_{delay3} of 0.662 ms and the same τ_c as for the top. Axon 82M42.

more than two-fold; however, only a 10% increase was observed, indicating a selective effect of Zn on activation. Such effects are consistent with sequential coupling. Computations with a simple coupled model showed that decreasing the rate constant governing the transition between the resting and conducting states by twofold increased the activation time constant 1.8-fold but inactivation τ by only 7.5%. These results are in agreement with those of Gilly and Armstrong (1982) on squid, where a selective slowing of activation was also observed.

In addition to lengthening the delay, Zn produces two other effects on the inactivation time-course. First, peak I_{Na}, following brief conditioning steps, was always larger than the unconditioned value in 5 mM Zn (Fig. 1). Without Zn, brief conditioning pulses produced either no change or a slight decrease from the unconditioned value (Fig. 2, top; see also Goldman and Kenyon, 1982). Second, in the absence of Zn, the delay process, when resolvable, could be described as a simple exponential of time constant equal to τ_m (Goldman and Kenyon, 1982). With Zn the delay could not be described as a single process. In Fig. 1, moving from longer to shorter conditioning durations, we see that peak I_{Na} values first fall below the τ_c exponential as expected for a single delay process, then curve towards the v_c exponential indicating a second process. As noted, there is a third, faster process producing the increase in peak I_{Na} following brief conditioning pulses which is complete by the first 200 μs of conditioning at this potential. This same pattern was seen in every experiment with Zn.

In Fig. 2 the first 5 ms of two inactivation determinations on another axon with and without 5 mM Zn are shown on an expanded time scale. In the upper part (no Zn) the inactivation time course is well described as just two processes (solid curve) with τ_{delay} of 0.57 ms, which is the *Myxicola* τ_m value at this potential, and a τ_c of 5.65 ms. For the bottom part of the figure the inactivation time course is described as three exponentials (solid curve, extended as a dashed curve) with τ_{delay2} of 0.1667 and τ_{delay3} of 0.662 ms and the same τ_c as in the upper part. There is yet a third delay process too fast to be resolved in these data. Activation in *Myxicola* also develops as three processes (Goldman and Hahin, 1978) one of which was too rapid for clear resolution, providing another correspondence between inactivation delay and Na activation.

These additional effects of Zn are consistent with the proposal of Gilly and Armstrong (1982) that Zn stabilizes the occupancy of the resting state(s). In this view the unconditioned peak I_{Na} would be reduced in Zn owing to a shift in the resting occupancies of the states preceding the conducting state towards rest and away from the conducting state itself. Following brief conditioning pulses occupancy of the preconducting states is now restored to a more favorable distribution for rapid loading of the conducting state, producing the initial rise in the inactivation curve. Similarly, the faster two activation processes become more visible in the inactivation delay as the states more distant from the conducting state are more heavily populated in the presence of Zn.

A further correspondence between inactivation delay

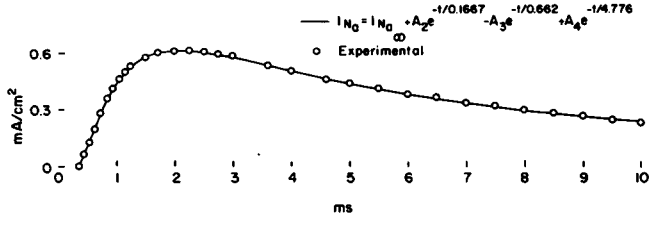

FIGURE 3 Time-course of I_{Na} during the conditioning pulse for the same experiment as in Fig. 2 (bottom). Solid curve drawn according to: $I_{Na} = 0.135 + 1.2326\, e^{-t/0.1667} - 1.8416\, e^{-t/0.662} + 0.867\, e^{-t/4.776}$.

and Na activation is illustrated by the data of Fig. 3. The open circles are the experimental I_{Na} (t) values during the conditioning pulse from the same experiment shown in Fig. 2 (bottom). The solid curve has been computed according to the sequential coupled scheme:

$$A \rightleftharpoons B \rightleftharpoons C \rightleftharpoons D \rightleftharpoons E$$

with A the resting, B and C activated but not conducting, D the conducting and E the inactivated state. I_{Na} (t) is then given by

$$I_{Na} = I_{Na_\infty} - A_1 e^{-t/\tau_1} + A_2 e^{-t/\tau_2} - A_3 e^{-t/\tau_3} + A_4 e^{-t/\tau_4}.$$

For the solid curve of Fig. 3, τ_4 is taken as the measured τ_h from the current record, and τ_3 and τ_2 are just the same τ_{delay3} and τ_{delay2} values that were fitted to the inactivation time-course in the lower part of Fig. 2. τ_1 was not resolved and so was not included in the reconstruction, leaving the first few 100 μs of $I_{Na}(t)$ undescribed. However, for the rest of the I_{Na} time-course the fit is quite reasonable, clearly establishing the detailed correspondence between Na activation and the inactivation delay.

Several interpretations of these data are possible, but the simplest seems to be that at least some fraction of Na channels in *Myxicola* must conduct before they can inactivate.

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SINGLE CHANNEL ANALYSIS OF VOLTAGE-SENSITIVE K^+ CHANNELS IN CULTURED PURKINJE NEURONS

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Recent data suggest that ionic mechanisms intrinsic to the cell membrane, in addition to neuronal circuitry, play an important role in generating and regulating the electrical activity of vertebrate central nervous system (CNS) neurons. For example, the Purkinje neurons (PN) of the cerebellum are characterized by a unique firing pattern comprised of simple and complex spikes in addition to a series of pauses (1). Intracellular voltage recordings from the PN revealed that voltage-sensitive, pacemaker-like activity plays a major role in generating the firing pattern characteristic of this CNS neuronal type (2–5). The intrinsic ionic mechanisms responsible for this activity appear to be localized to both the somal and dendritic membranes, and both regions exhibit spike electrogenesis.

To identify and characterize the ion channel population mediating the intrinsic activity of PN and to facilitate studies using intracellular voltage recording and the extracellular patch clamp technique, a cultured cerebellar model system was developed (4, 5). The cultured PN can be clearly identified on a morphological basis and both the somal and dendritic regions are easily accessible for electrophysiological analysis. Electrical activity characteristic of PN in vivo is demonstrated in the cultured PN (Fig. 1). This activity is significantly altered by K^+ channel blockers, suggesting that K^+ channels play a major role in generating or regulating the intrinsic activity of the PN's.[1] Experiments in progress using the "gigaohm seal" patch clamp technique are directed towards characterizing the somal and dendritic K^+ channel population of PN and identifying the factors (voltage, ions, chemicals) which regulate their activity.

[1]D. L. Gruol. Unpublished results.