BRIEF COMMUNICATION

EXTRACELLULAR pH SELECTIVELY MODULATES RECOVERY FROM SODIUM INACTIVATION IN FROG MYELINATED NERVE

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ABSTRACT The Hodgkin-Huxley kinetic parameters, α_h and β_h, which govern the rate of recovery from and development of sodium channel inactivation, respectively, have been measured as a function of membrane potential and external pH using a three-pulse protocol. α_h but not β_h is substantially accelerated by reducing external pH from 7.4 to 6.4. The α_h vs. voltage curve appears to be selectively shifted in the depolarizing direction by ~ 12 mV for this pH change, giving an apparent h_∞ curve shift of ~ 6 mV in the same direction (less inactivation).

INTRODUCTION

Certain cations such as Ca^{++}, H^+, and Ni^{++} have been thought to modulate sodium channel gating behavior through their effects on membrane surface charge (Frankenhaeuser and Hodgkin, 1957; Hille, 1968; Gilbert, 1971). For instance, after elevating extracellular hydrogen ion concentration one observes an apparent shift of the voltage gating curves that describe activation and inactivation of sodium channels; the membrane appears hyperpolarized by the added hydrogen ions as evidenced by a reduced amount of sodium inactivation at a given membrane potential and an increased threshold for spike initiation. Described herein are results of a new kinetic analysis of the effects of extracellular hydrogen ions on the sodium inactivation process. These experiments show a selective effect of extracellular pH on the rate of recovery from sodium inactivation, α_h, as determined by a three-pulse protocol; low external pH accelerates the α_h process without affecting the β_h process.

MATERIALS AND METHODS

Voltage clamp experiments were carried out on single nerve fibers dissected from the sciatic nerve of the bullfrog *Rana catesbiana* using the vaseline gap procedure of Dodge and Frankenhaeuser as described in Hille (1971*a*). Experiments were carried out at 9-10°C with the node of Ranvier held at a membrane potential where ~ 1/3 of sodium channels were inactivated ($h = 0.66$) under control conditions (pH 7.4). Ringer's solution contained 114 mM NaCl, 2.4 KCl, 2 $CaCl_2$, 7.5 tetraethylammonium chloride (to block g_K), and 10 mM Hepes buffer. Aliquots of this Ringer's solution were titrated to pH 8.4, 7.4, or 6.4 with 200 mM NaOH or HCl for the experiments described below.

The myelinated internodal segments located in the end pools of the vaseline gap chamber were cut in an axoplasmic substitute made up of 100 mM K^+, 13 Na^+, 27 HEPES buffer (pH 7.4), and 100 Cl^-. Fibers were cut in the E and C end pools at a distance of ~ 700 and 1,700 μm from the node of Ranvier. Fibers were cut in this revised axoplasmic substitute rather than the conventional 120 mM KCl because of a recent report by Palti et al. (1979) regarding problems that arise because of diffusion from cut nerve ends. Use of this new intracellular substitute appeared to improve long-term stability and reduce the leak conductance of these preparations. Attempts were made in two experiments to reduce intranodal pH by cutting the internodes in a slightly different axoplasmic substitute containing 50 mM Hepes buffer at pH 6.4 and 18 mM Na^+ instead.

RESULTS AND DISCUSSION

External protons block sodium permeability (Hille, 1968; Woodhull, 1973) in addition to their hypothesized surface charge screening effects with which this study takes issue. Such channel blocking effects were observed in this study; smaller peak sodium currents were routinely recorded at reduced external pH levels. The kinetic changes underlying the effects of extracellular H^+ on the inactivation process are specifically examined in experiments described below.

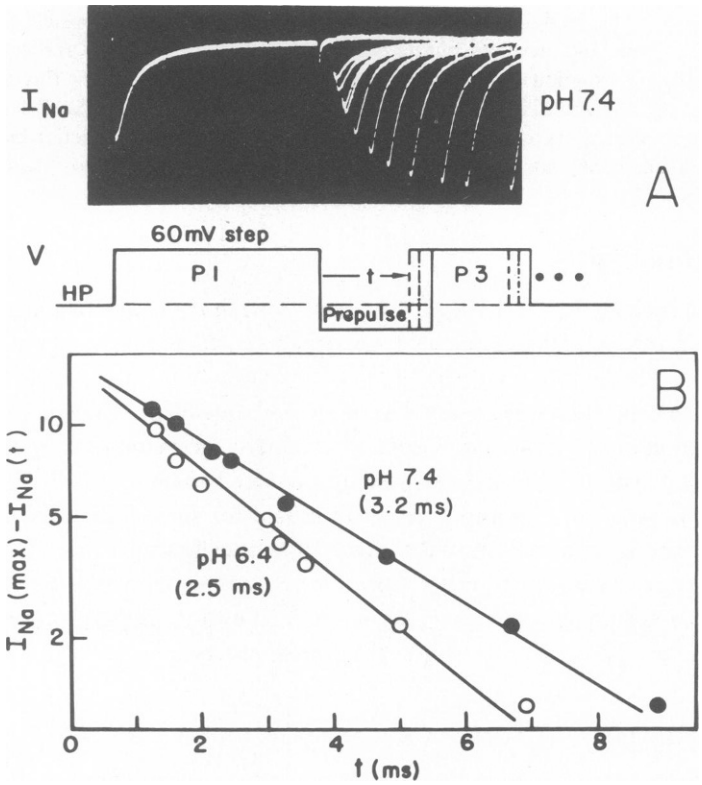

FIGURE 1 Recovery from sodium inactivation during −30-mV prepulse as a function of external pH. (A) Conditioning depolarization, P1, of 60-mV amplitude and 20-ms duration produces sodium inactivation. A similar depolarization, P3, measures the partially recovered sodium current after a variable delay time, t. (B) Semilogarithmic plot of [peak I_{Na}(max) − peak I_{Na} (t)] vs. delay time t between pulses 1 and 3 yields time constant governing recovery from inactivation for the indicated external pH level. HP, holding potential. Arbitrary current scales.

Results of Three-Pulse Experiments

Fig. 1 A shows the basic three-pulse protocol used to evaluate the effects of voltage and time on the recovery from inactivation produced by the conditioning pulse 1. According to Fig. 1 B the essentially monoexponential process associated with recovery from inactivation is faster at lower external pH. A small (few 10ths of a millisecond) delay in recovery from inactivation (Chiu, 1977) was often observed but this delay did not contribute to the pH-induced changes in inactivation rate constants reported here.

Steady-state sodium inactivation curves were shifted by 6-8 mV in the depolarizing direction by increasing H^+ 10-fold (pH 7.4–6.4). Fig. 2 A shows an example of such an inactivation curve shift; these results are very similar to those reported by Hille (1968) for similar preparations. If this inactivation curve (h_∞) shift is produced by a general effect of external hydrogen ions on negative surface charges, then the related τ_h (time constant) curve

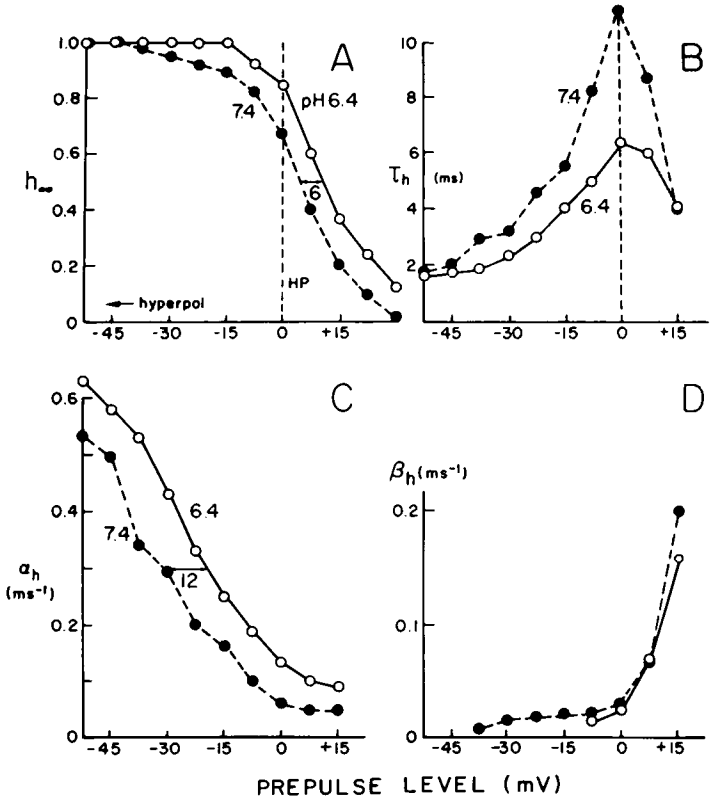

FIGURE 2 Two descriptions of the kinetics governing recovery from sodium inactivation. Prepulse of varying amplitude (abscissa) and durations are used to control recovery from sodium inactivation. (A) 50-ms-long prepulse applied to measure steady-state inactivation (h_∞) curves at two levels of external pH. Apparent 6-mV shift of h_∞ curve. (B) The prepulse duration required for peak I_{Na} on a subsequent depolarization to recovery to half its maximal final level for a given prepulse amplitude is also measured and plotted as τ_h ($t_{1/2}/0.693$). (C and D) h_∞ and τ_h values for a given prepulse level are used to calculate the rate constants β_h,α_h that govern the rate of development of and recovery from inactivation, respectively. According to the surface charge screening hypothesis both α_h and β_h curves should shift by 6 mV. However, only the α_h curve is shifted, by about twice that amount. A single experiment is illustrated here with similar results obtained in other experiments; see Table I for compilation of results.

should also shift along the voltage axis in a similar manner. However, time constant curves were considerably depressed (accelerated) at low pH. To ascertain the reason for this unexpected result a more complete analysis was made for the Hodgkin-Huxley rate parameters, α_h, and β_h, that model voltage and time-dependent inactivation behavior. Results of several such analyses showed that a simple voltage shift was not involved in generating the h_∞ curve shifts depicted in Fig. 2 A. The rate of recovery from inactivation, α_h, appears to be selectively altered by reduced external pH (Fig. 2 C and D) with the apparent 6-mV h_∞ shift explained by a 12-mV shift of the α_h curve. Results from several preparatons are given in Table I, which includes a few results at pH 8.4 as well. Results of experiments at pH 7.4 and 6.4 provide a statistically significant demonstration of a selective effect of reduced pH on the α_h process at the holding potential.

Fig. 3 shows a theoretical reconstruction of the effects of reduced extracellular pH on the sodium inactivation process. A selective α_h shift of 12 mV for this external pH change (7.4–6.4) satisfactorily accounts for the general shape of the results presented here (Fig. 2 A and B).

Other Results

The rates of development of inactivation observed during depolarizing steps of from 40 to 80 mV amplitude were compared at extracellular pH levels of 7.4 and 6.4. Results were inconsistent but the inactivation rate constant usually decreased. In five experiments β_h during

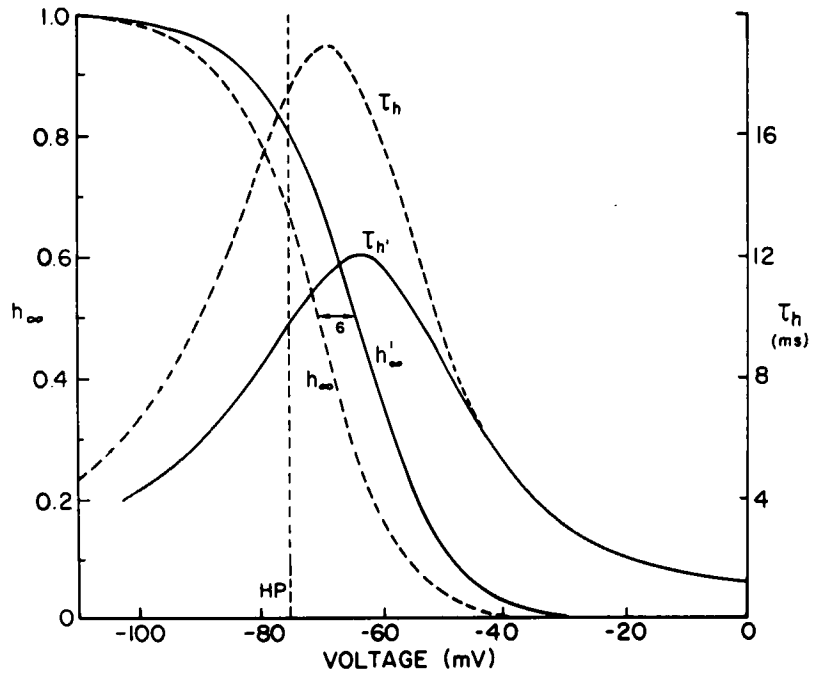

FIGURE 3 Modeled effective of selective α_h shift on the steady-state sodium inactivation curve and related time constants. Node of Ranvier model for 10°C (Hille, 1971*b*) is used to simulate normal set of parameters h_∞ and τ_h (dotted lines for pH 7.4), then α_h is shifted 12 mV to generate primed parameters (solid lines, pH 6.4). It is clear that measurement of h_∞ curve shift by itself cannot be used to distinguish between the possibility that both α_h and β_h curves are shifted by 6 mV (surface charge screening) or that α_h is selectively shifted by 12 mV. Kinetic measurements are required to distinguish these possibilities.

a 60-mV depolarization decreased from 0.21 ± 0.05 ms^{-1} at pH 7.4 to 0.16 ± 0.05 at pH 6.4. Small but variable potassium currents could have contributed to the lack of consistency in these measurements (Woodhull, 1973), although leak subtractor settings did not change with the external pH manipulations used here. Carbone et al. (1978) have studied the effects of external pH changes on sodium channel inactivation (in squid nerve) and have reported a 25% reduction in β_h observed during similar depolarizing steps when pH was reduced from 8 to 5.2. A 15-mV shift of their β_h curve occurs for this very large pH change. They did not, however, report results of three-pulse experiments designed to extract α_h values, so further comparisons cannot be made.

It is possible that these selective α_h changes (Fig. 2 C and Table I) observed with pH changes could be attributable to the Hepes buffer. A Tris buffer system was therefore used to manipulate external pH in two preparations and gave similar results; that is, α_h increased substantially at pH 6.4 with no change in β_h. Curiously, α_h was 10–20% smaller in Tris buffer at a given external pH in one of these experiments where both buffers were used extracellularly; h_∞ measures were somewhat smaller in Tris buffer at the holding potential, and β_h did not change at all with several buffer and pH changes in this experiment.

DISCUSSION

One possible explanation for the effect of extracellular pH on recovery from sodium inactivation is that the electrochemical gradient for hydrogen ions modulates, possibly through inward H^+ ion flow through the sodium channel, the rate of recovery from the inactivation. Such an explanation is suggested by a report that very alkaline internal solutions substantially reduce sodium inactivation in giant squid axons (Brodwick and Eaton, 1978); this result could be interpreted as a greatly speeded recovery from inactivation (removal of Armstrong and Bezanilla's [1977] "inactivation plug?") because of the large inward hydrogen ion gradient. To test this hydrogen ion gradient hypothesis the internodal segments in the end pools were cut in an axoplasmic substitute buffered to pH 6.4. The rate of recovery from inactivation should be reduced if the inward H^+ gradient is thereby reduced by lower internal pH. α_h at the holding potential was reduced by 29% at external pH 7.4 and by 23% at external pH 6.4 in two such experiments with acidic internal buffers. These results, although interesting, are probably not conclusive. It would be profitable to pursue this issue further with the internally perfused squid axon preparation where internal pH can be changed with greater reliability and a given preparation can easily be exposed to several different internal pH levels.

TABLE I
SUMMARY OF RESULTS FROM SEVEN PREPARATIONS

	pH 8.4	pH 7.4	pH 6.4
h_∞ at holding potential	0.64 ± 0.08*(3)	0.66 ± 0.01 (7)	0.80 ± 0.02 (5)
α_h during −45 mV prepulse	—	0.42‡ ± 0.03 (4)	0.62 ± 0.08 (3)
α_h at holding potential	0.050 ± 0.020 (3)	0.056 ± 0.010 (7)	0.115 ± 0.020§ (5)
β_h at holding potential	0.024 ± 0.004 (3)	0.029 ± 0.005 (7)	0.029 ± 0.007 (5)

*Results expressed as mean ± SEM, number of experiments in parenthesis.
‡Units of milliseconds^{-1} on all rate constants α_h and β_h.
§Significantly different from pH 7.4 parameter at 1% level.

Hille's (1968) results suggest an alternative hypothesis: some titratable group with an acidic pK_a resides on or near the inactivation gating particle and can affect its operation according to its charge. A more extensive set of experiments at several reduced external pH levels might allow evaluation of this alternative hypothesis. Hydrogen ion effects on P_{Na} as well as the rate constants could, however, complicate such experiments.

α_h is selectively increased by reduced extracellular pH. This could imply that a different microscopic process is involved in the establishment of channel inactivation and in recovery from channel inactivation near the resting potential with external protons contributing only to the latter process. Benzocaine, which shifts the h_∞ curve in the direction opposite to low pH as reported here, selectively slows rather than speeds the rate of recovery from inactivation (Kendig et al., 1979). These benzocaine effects are also described by selective changes in α_h with little or no change in β_h. Others have recently reported inactivation curve shifts with temperature (Chiu et al., 1979), which implies different Q_{10}'s for the α_h and ∞_h processes. Further characterization of the α_h and B_h processes with alternations in Ca^{++} levels, temperature, and other pharmacological manipulations should then provide a more complete picture of the microscopic events associated with sodium channel gating. It will therefore be very important to add kinetic measurements of the type indicated in Fig. 2 to the more commonly reported h_∞ curve measurements in future studies of altered sodium channel inactivation.

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