ETIOLOGY OF THE SUPERNORMAL PERIOD

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ABSTRACT In many excitable cells, there is a time after the action potential when the threshold for eliciting a second action potential is lower than it is in the steady state. The Hodgkin and Huxley (1952) equations predict such a supernormal period. Using their model, it is shown that the supernormal period results from the slow kinetics of the potassium current and does not depend on sodium current activation or inactivation or on the after-depolarization.

INTRODUCTION

After the peak of the action potential in cardiac and nervous tissue, there is a span of time during which no second stimulus, regardless of amplitude, will elicit a second action potential. This is the "absolute refractory period." It is followed by a span of time during which a larger than normal stimulus will elicit a second action potential. This is the "relative refractory period." The refractory periods limit the frequency of action potentials that can be maintained by an excitable cell. Refractoriness is easily understood to arise from inactivation of inward currents and activation of outward currents.

Commonly, there is a time at the end or in the midst of the relative refractory period during which the threshold for producing a second action potential is less than it is in the steady state. This is the "supernormal period" (Adrian and Lucas, 1912; Adrian, 1921). Supernormality has been described in the peripheral nervous systems of invertebrates (Hodgkin, 1948; Zucker, 1974) and of mammals (Adrian and Lucas, 1912; Adrian, 1921; Raymond and Lettvin, 1978). It has been described in many places in the mammalian central nervous system. These include efferents of the caudate nucleus (Kocsis and VanderMaelen, 1979), visual and somatosensory callosal axons (Swadlow and Waxman, 1976; Waxman and Swadlow, 1976), corticotectal axons (Swadlow and Waxman, 1976), parallel fibers of the cerebellum (Gardner-Medwin, 1972; Merrill et al., 1978), lateral Lissauer tract (Merrill et al., 1978), axons of the olfactory peduncle (Ferreyra-Mayano and Cinelli, 1986), and the dorsal hippocampal commissure (Bartesaghi, 1987). It occurs in frog myocardium (Adrian, 1921), mammalian ventricle (Hoff and Nahum, 1938), and in the mammalian myocardial conduction system (Spear and Moore, 1974).

The supernormal period may play a physiological role in neural encoding (Kocsis et al., 1979), perhaps by allowing invasion of otherwise inactive axonal terminals (Chung et al., 1970; Zucker, 1974; Stockbridge and Stockbridge, 1988; Stockbridge, 1988). This hypothesis was considered by Zucker et al. (1986) to account for the profound facilitation observed in crustacean motor synapses.

The supernormal period may also play a pathogenic role in the etiology of cardiac arrhythmias (Spear and Moore, 1974; Goto, 1986).

The etiology of the supernormal period is unclear. Stein (1966) and Swadlow and Waxman (1976) noted its temporal association with the after-depolarization. Zucker (1974) and Raymond and Lettvin (1978) linked periaxonal potassium accumulation to the after-depolarization and the supernormal period, whereas Barrett and Barrett (1982) ascribed the supernormal period to the after-depolarization caused by a passive capacitive current in myelinated axons. Experimental evidence of Bliss and Rosenberg (1979) and Bostock and Grafe (1985) linked activity-dependent changes in the electrogenic sodium pump, the after-depolarization, and the supernormal period. Raymond and Lettvin (1978) considered the hypothesis that the supernormal period results from decreased inactivation of the sodium current. Bliss and Rosenberg (1979) considered the possibility the supernormal period arises from transient increases in the sodium conductance. Examination of the supernormal period produced by a simple membrane model provides another possible mechanism.

The equations of Hodgkin and Huxley (1952) describe the membrane ionic currents underlying the action potential of the squid giant axon. While they are incomplete in some details, such as independence of sodium current activation and inactivation (Goldman and Schauf, 1972), the kinetic description of the potassium current (Cole and Moore, 1960), and in accounting for ion accumulation and depletion effects (Adelman and FitzHugh, 1975), the model does well at predicting the shape of the action potential and the changes in threshold after the action potential in nerve (Stein, 1966). In this study, the Hodgkin-Huxley model was used to identify factors that give rise to supernormality.

METHODS

The equations for the membrane ionic currents were the same as those derived by Hodgkin and Huxley (1952). The effects of temperature were confined to the rate constants (α_m, β_m, α_h, β_h, α_n, and β_n), all of which

were said by Hodgkin and Huxley to scale with a Q_{10} of 3. All simulations were repeated at each of 6, 16, 26, and 32°C. The stimulus was a rectangular current pulse of 0.1 ms duration. All variables were stored and all calculations were performed using IEEE double precision floating point arithmetic. The solution evolved in time steps of 2.5 μs. The m (sodium activation), h (sodium inactivation), and n (potassium activation) variables were integrated by a second-order Runge-Kutta method.

The criteria for defining a "successful" membrane action potential are somewhat arbitrary. For this study, the criteria used were depolarization by at least 15 mV and positive first and second derivatives of the membrane potential. A threshold was found by a binary search technique which established two to three significant digits.

RESULTS

Qualitatively similar results were obtained at 6, 16, 26, and 32°C. The reduction in threshold, or supernormality, was most profound at 16°C, and the results are presented in detail for that temperature only. The results were also shown to be insensitive to the duration of the stimulus used to test for the threshold.

Fig. 1 illustrates the phenomenon under study. Part A shows a normal membrane action potential and part B shows the time courses of m, h, and n during that action potential. The after-hyperpolarization was followed by an after-depolarization of <1 mV. m and h each transiently exceeded their steady-state values, and n transiently fell below its steady-state level during the late stages of the action potential. The minimum current necessary to produce a second action potential was determined for various times after this action potential. Part C shows these measurements normalized to the steady-state threshold. The supernormal period was at least partly overlapped by the after-depolarization, by the overshoots of m and h, and by the undershoot in n.

The sodium activation variable, m, is very well correlated in amplitude ($r = -0.98$) and timing ($r > 0.99$) with the relative threshold. This correlation holds at all temperatures and when only the parameters α_m and β_m are each multiplied by a factor of 2.0 or 0.5.

The overshoot in m was found to be a direct result of the after-depolarization. Therefore, elimination of the after-depolarization allows one to determine if either the after-depolarization itself or the overshoot in m is responsible for the supernormal period. A series of simulations were run in which a conditional voltage clamp was employed to prevent the after-depolarization. The first action potential was allowed to proceed normally until the end of the after-hyperpolarization, from which time the potential was held at the resting potential until the time came to apply the current to test for the threshold. As shown in Fig. 2 *A*, supernormality was not abolished by this procedure. Therefore, although both were well correlated with supernormality, neither the overshoot in the membrane potential nor the consequent overshoot in m was primarily responsible for supernormality.

The contribution of the overshoot in the sodium inactivation variable, h, can be assessed by a similar technique.

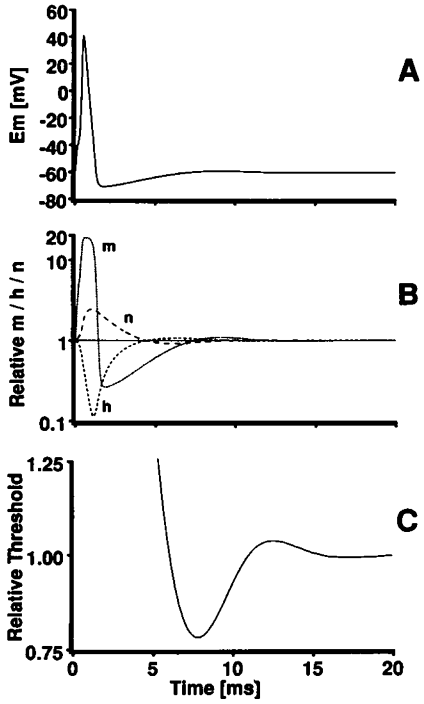

FIGURE 1 (*A*) A normal Hodgkin-Huxley membrane action potential computed for 16°C. Following the after-hyperpolarization, the membrane potential transiently overshot the resting potential (from ~7.3 ms to ~12 ms measuring from the initial stimulus) by as much as 0.8 mV. (*B*) Hodgkin-Huxley state variables m, h, and n (which control sodium conductance activation, sodium conductance inactivation, and potassium conductance activation, respectively) for the same membrane action potential as in *A*. Values for m, h, and n were plotted on a log scale and were normalized to their steady-state values at the resting potential. Variables m and h transiently exceeded their steady-state values after the spike and n transiently fell below its steady-state value at about the same time. (*C*) Time course of changes in the threshold (for a 0.1-ms current stimulus) after the membrane action potential. The threshold current was normalized to the threshold in the steady state. From ~6 ms after the initial stimulus until ~11 ms, the threshold for eliciting a second action potential is less than it is in the steady state, by as much as 22%. After a second period of relative refractoriness, there is a second period from ~16 to 20 ms, during which the threshold is again reduced, but only by a fraction of a percent.

When it was allowed to follow its natural course for most of the action potential but was prevented from exceeding its steady-state value, a substantial supernormal period (Fig. 2 *B*) remained.

Prevention of the undershoot in the potassium activation variable, n, by this same technique only slightly attenuated the after-depolarization and the overshoots in m and h, but it virtually eliminated the supernormal period (Fig. 2 *C*). As shown in Fig. 1 *B*, n changes more slowly than do m and h, so n peaks only well after the peak depolarization of the action potential. Its recovery is even slower, so that n has only just undershot its resting level near the end of the after-hyperpolarization. This undershoot in n persists during the after-depolarization and is the principal cause of the supernormal period.

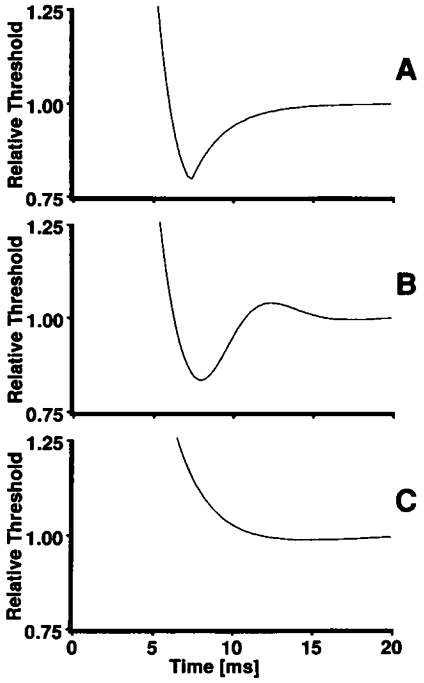

FIGURE 2 (*A*) Changes in excitability after a membrane action potential with identical properties as in Fig. 1, but the small depolarization which follows the after-hyperpolarization was prevented. The maximum reduction in threshold was ~20%. (*B*) Excitability changes after a membrane action potential in which the transient overshoot in *h* was prevented. The maximum threshold reduction was ~17%. (*C*) Excitability changes after a membrane action potential in which the transient undershoot in *n* was prevented. Only about a 1% decrease in threshold was observed.

DISCUSSION

In the present study, a series of hypotheses about the origin of supernormality have been explored using a quantitative model of the ionic currents in squid axons. This model contains no ion-accumulating space and no electrogenic pump, but it does produce a supernormal period. The results of this study show that neither the after-depolarization nor the overshoots in *m* or *h* causes the supernormal period. Rather, in this model, supernormality is caused by the slow kinetics of *n*. Supernormality arises because the outward current remains lower than its resting level for a period of time after the membrane potential and inward current variables have approached their steady-state values. With less outward current to oppose, the stimulus required to bring the membrane to threshold is reduced.

These properties have been demonstrated in a model in which the only outward current is the delayed rectifier, but the only property of that current which is essential for supernormality is that the recovery of the outward current be slow compared with the recovery of the inward current and the after-hyperpolarization of the membrane potential. One would expect other voltage-sensitive potassium currents to produce a similar effect if they had slow kinetics. Less ion-selective channels with reversal potentials somewhere above the normal resting potential (Coronado et al., 1980; Labarca and Miller, 1981; Coronado and Latorre, 1982) could produce supernormality by contributing to the outward current in the late phases of the action potential. Excellent candidates for such a role are cation-selective ion channels whose opening depends upon the slowly changing intracellular calcium concentration (Colquhoun et al., 1981; Yellen, 1982). Supernormality could likewise result from a decrease in a chloride conductance with suitable kinetic properties (Owen et al., 1986).

Other models of neuronal and cardiac action potentials are currently being assessed by similar techniques. For some of these models, the relationship between repetitive activity and excitability changes and between excitability changes and conduction velocity are being explored.

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