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# THEORETICAL IMPLICATION OF LIGANDING REACTIONS IN AXONAL SODIUM CHANNEL GATING

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## ABSTRACT

Mathematical simulation models for measured permeability properties of axonal $Na^+$ channels point to the presence of bimolecular, as well as net-membrane-field-dependent intramolecular, reaction steps in channel gating reactions. An abstract chemical reaction model is presented, which postulates ligand binding reactions in both $Na^+$ channel activation and subsequent desensitization as observed in voltage-clamp experiments. Membrane capacitance currents due to the movement of charged or dipolar gating structures also indicate that the early phase of the gating charge movement is dictated by local, rather than net-membrane-field-driven reactions, and therefore reflect energy input from chemical sources which are an integral part of the membrane.

## KEYWORDS

Electrochemical gating model; asymmetric membrane capacitance.

## INTRODUCTION

Two fundamentally different sets of ideas dominate the thinking of workers concerned with identifying the molecular events which regulate the ionic permeability of axonal membranes. On the one hand is the traditional electrophysiological viewpoint, that gating dynamics of $Na^+$ and $K^+$ channels are driven solely by electrodiffusive processes, involving the spatial redistribution of charged or dipolar membrane components in response to changes in the net membrane electric field. This view received strong support from the work of Hodgkin and Huxley (1952), which demonstrated clearly that the kinetics of $Na^+$ and $K^+$ currents responsible for generation of action potentials are critically dependent on the membrane field.

An alternate and somewhat more involved gating concept was proposed by Nachmansohn (1953) and later extended by Neumann, Nachmansohn and Katchalsky (1973) and Neumann (1974), in which gating of axonal channels was envisioned to involve a "biochemical control cycle", similar to the acetylcholine-regulated permeability system of synaptic membranes. We have recently extended this biochemical model further, in order to better define the respective roles and connection between chemical and membrane-field-driven processes in the case of axonal $Na^+$ channels (Dorogi and Neumann, 1980).

Although axonal ionic channels have not yet been purified to an extent suitable for biochemical identification techniques, electrophysiological measurements of the channels can provide useful information as to the nature, even if not to the identity, of molecular gating components and events. Due to the polyphyletic nature of membrane science, terminological differences may shield the full meaning of electrophysiological findings from biochemists. Certainly, the usual formalism in which electrophysiological data is expressed reflects a different way of thinking and a different view of which parameters are the most important. Here we recall some interesting properties of axonal membranes, determined from voltage-clamp studies, which suggest the presence of chemical energy sources in the gating machinery of axonal $Na^+$ channels.

## LIGANDING REACTION IN SODIUM CHANNEL ACTIVATION

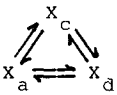
At rest, electric potential inside the giant axon of squid is about -70 mV relative to the potential of the external solution. When this voltage difference, V, is stepped to a more positive value in a voltage-clamp experiment, there follows a dramatic rise in the membrane's $Na^+$ conductance ($g_{Na}$), i.e., permeability, which is followed by a subsequent decline of $g_{Na}$ back to negligible values. This sequence of events is also associated with a concurrent desensitization process, so that $Na^+$ channels are again responsive to further depolarizations only after a period of recovery at a more negative value of V. Therefore, three states of $Na^+$ channels are identifiable: during step depolarizations, channel gating units can be envisioned to transit from an activatable state $X_a$ to a desensitized state $X_d$, via the metastable $Na^+$-conducting state $X_c$;

$$X_a \rightleftharpoons X_c \rightleftharpoons X \tag{1}$$

with

$$g_{Na}(V,t) \alpha \left[X_c(V,t)\right]^n, \tag{2}$$

where t denotes time, square brackets refer to concentration and n models the observed delay in the rise of $g_{Na}(V,t)$ following onset of depolarization. For squid giant axons n=3 is adequate (Rawlings and Neumann, 1976). After V is returned to a more negative value, there follows a redistribution of gating units away from state $X_d$ back to state $X_a$, but $g_{Na}$ remains zero during recovery. Hence scheme (1) has to be modified in order to cover salient features of $Na^+$-channel gating behavior by a cyclic three state model,




(3)

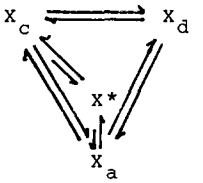
which suggests membrane-field-dependent intramolecular transitions among three gating unit states. However, more subtle aspects of gating are not explainable by such a scheme.

One such observation concerns experiments designed to evaluate the distribution of gating units between the two stable states $X_a$ and $X_d$, as a function of V. This involves experiments in which $g_{Na}$ is observed during a second depolarization step, to a voltage $V_2$, after $Na^+$ channels have been allowed to "equilibrate" at a less positive voltage $V_1$. In such two-step experiments, the quantity of interest is the peak value of $g_{Na}$ seen during the second pulse, $g_{Na,p}(V_2)$ as a function of $V_1$, because $g_{Na,p}(V_2)$ is a reflection of the steady-state fraction of gating units in state $X_a$ at voltage $V_1$.

Interesting conclusions follow when one compares the quantity $(g_{Na,p}(V_2)/g_{Na,p}^{max}(V_2)$, where $g_{Na,p}^{max}(V_2)$ is the maximum value of $g_{Na,p}(V_2)$ possible, i.e. the peak $g_{Na}$ observed when $V_1$ is very negative, for different values of $V_2$. It is found that the ratio $g_{Na,p}(V_2)/g_{Na,p}^{max}(V_2)$ varies with $V_2$, such that the ratio increases with more positive $V_2$ for a fixed value of $V_1$. On the other hand, when the six rate constants of reaction model (3) are fixed at values which can model both $g_{Na}$ rise and fall, as well as recovery kinetics, at a particular value of V, the same rate constants are unable to model the observed dependence of the normalized peak ratio on $V_2$, seen in the two-pulse experiments (Goldman, 1975; Jakobsson, 1976).

It has been pointed out by Jakobsson (1973), that modelling of this observation, among others to be described below, requires modification of the

depolarization reaction pathway from the form $X_a \rightleftharpoons X_c \rightleftharpoons X_d$, so as to include an "excited" state: the successful model has the form




(4)

(Jakobsson, 1978). The fact that the ratio $g_{Na,p}(V_2)/g_{Na,p}^{max}(V_2)$ increases as $V_2$ becomes more positive appears to require, that following onset of depolarization, the conducting state $X_c$ be fed not only from the activatable resting state $X_a$, but also from a different state $X^*$. Both $X_a \longrightarrow X_c$ and $X^* \longrightarrow X_c$ transitions would be driven by depolarization, but the $X^* \longrightarrow X_c$ reaction would dominate at nominal depolarizations, while the $X_a \longrightarrow X_c$ reaction would become comparable only for large depolarizations.

This implies that state $X^*$ is "less stable" than state $X_a$ during depolarization, characterizing it as an "excited" state relative to $X_a$. However, because steady-state values of $g_{Na}$ are generally negligible, state $X^*$ cannot be populated prior to application of the depolarizing step; furthermore, its population following step depolarization must be practically instantaneous (micro seconds), because its kinetics are not apparent in the kinetics of $g_{Na}$ (100μs time range). Importantly, population of state $X^*$ requires energy input from some source other than the membrane field, because the field drives gating units away from state $X^*$ to state $X_a$ during depolarization.

The slower evolution of desensitization over that of $g_{Na}$ decline during depolarizing pulses in some nerve preparations suggests that some gating units "fall back" to state $X_a$ after attaining state $X_c$. Such a phenomenon indicates that in some axon preparations state $X_c$, as well as state $X^*$, is energetically less favorable than state $X_a$ during depolarization (Jakobsson, 1978). In these cases the $X_a \longrightarrow X^* \longrightarrow X_c$ pathway would be totally responsible for $g_{Na}$ and an energy source other than the membrane field would be clearly required.

The nature of the suggested energy input mechanism is evident from the above arguments. It has been noted that $[X^*]$ must rise practically instantaneously following onset of a depolarization step, so that early values of the rate parameter describing the $X_a \longrightarrow X^*$ transition must be very large; however, the amplitude of $g_{Na}$, and hence the attained value of $[X^*]$, increases progressively with increasing size of the depolarization step, so that the state $X_a$ is only fractionally depleted by the $X_a \longrightarrow X^*$ transition for moderate pulse sizes. This requires that the $X_a \longrightarrow X^*$ reaction be short-lived. Since the membrane field remains at a constant value during depolarization pulses, the membrane field cannot be the energy source determining the fraction of gating units attaining state $X^*$. It appears as if the $X_a \longrightarrow X^*$ reaction is limited by the short-lived availability of some substance, which becomes available to the gating unit as a result of a depolarizing shift in the membrane field.

## ELECTRICAL-CHEMICAL GATING MODEL

These suggested properties of the $X_a \longrightarrow X^*$ transition lead one to question traditional attempts at parametrization of $Na^+$-channel gating properties with only membrane-field-dependent intramolecular reaction steps.

The only elementary alternative to intramolecular representations is a reaction model which allows for bimolecular reaction steps. One such model, which is in qualitative accord with the above listed properties, is illustrated in Fig. 1. $Na^+$-channel gating is shown to involve three functionally distinct

units: a storage unit for a small activator molecule (A), a receptor unit which can complex with A and is the structural regulating unit of the $Na^+$ channel, and a unit E which can remove A irreversibly from R.

The overall reaction sequence following onset of a depolarizing step pulse is taken to be as follows. Storage units are postulated to exist in either high (S) or low (S') affinity states for A, so that a depolarizing pulse drives both $S \rightleftharpoons S'$ and $AS \rightleftharpoons AS'$ equilibria to the right. Activator molecules are rapidly released from storage via the reactions

$$AS \rightleftharpoons AS' \rightleftharpoons S' + A \quad , \tag{5}$$

after which A can bind to receptor units in state R. Attainment of the $Na^+$-conducting state, AR', proceeds via the reaction pathway

$$A + R \rightleftharpoons AR \rightleftharpoons AR' \quad , \tag{6}$$

e.g., for squid axons

$$g_{Na} \propto [AR']^3 \quad . \tag{7}$$

Formation of AR corresponds to X* of reaction model (4). The reaction $A + R \rightarrow AR$ would be driven by chemical affinity forces, rather than the membrane field. The entire sequence of reactions, involving activator release from storage and "excitation" of receptor units to state AR, would be complete in times on the order of microseconds, which must characterize the $AS \rightleftharpoons AS'$ reequilibration and the lifetime of free A.

Decay of $[AR]$ is prompted by interactions with the membrane field, which drive the reactions

$$AR \rightleftharpoons AR' \rightleftharpoons AR'' \tag{8}$$

to the right, resulting in only a transient appearance of increased conductivity. In order to adequately simulate the amplitude of $g_{Na}$ at very large depolarizations, an alternate pathway is required. This pathway would contribute only for very large depolarizations, and would represent transitions involving the activator-free, but conducting, state R' (c.f. Fig. 1) (Jakobsson, personal communication). Hence, the opening of $Na^+$ channels may proceed via two fundamentally different and competitive pathways: the "role" of the activator molecule would be to induce structural changes in the receptor unit which lead to a conformation from which the $Na^+$-conducting state can be more easily attained. In this way, binding of A is tantamount to the "energy input" suggested above. However, in the absence of activator, gating units can still be forced into the $Na^+$-conducting state by a very large depolarization, i.e., the $X_a \rightarrow X_c$ and $R \rightarrow R'$ pathways of scheme (4) and Fig. 1, respectively. Subsequent discussion will here be limited to the activator-mediated pathways.

During a sufficiently prolonged depolarization (several ms), most activator-receptor complexes enter the AR" state; AR" is the energetically most favorable state under depolarization conditions, and it would be for the most part the sequence of transitions $AR \rightleftharpoons AR' \rightleftharpoons AR''$ which is reflected in the measured $g_{Na}$ transient.

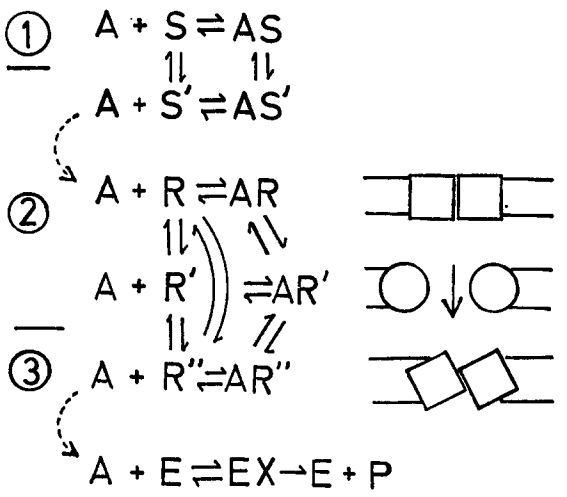



Fig. 1. Chemical reaction model showing sequential transloca- of activator A from reaction space 1 to reaction space 3.

As shown in Fig. 1, receptor units in state AR" would tend to lose activator molecules through an irreversible removal mechanism. The transition to state AR" may physically translocate A from a reaction space shared by S' and R units to a distinct reaction space accessible to the removal unit E, or the AR⟶AR'⟶AR" reaction sequence may convert the receptor from a higher to a lower affinity form for A. In the latter case, it is expected that the required increase in free energy at the binding site is compensated for by more favorable interaction with the membrane field in the AR" form. This is in agreement with the observed membrane-field dependence of $g_{Na}$-kinetics, which tend to be more rapid the greater the depolarization step.

The experimentally observed desensitization of $Na^+$ channels after prolonged depolarization would reflect the absence of A from R due to its removal by translocation or chemical consumption. In the absence of A, recovery of the activatable state, R, requires a different reaction pathway, R"⟶R (c.f. Fig. 1), which electrophysiological experiments would suggest to be field-sensitive and slow (ms time range).

The proposed electrochemical model implies a second recovery mechanism as well, namely, replenishment of the activator storage state AS. Since the identity of the various gating components outlined here cannot be deduced from measurements of electrical properties, notions as to the identiy of A or of its recovery (or resynthesis) shall not be dwelt upon here. It is however noteworthy, that the reaction scheme bears a close formal similarity to that proposed for the acetylcholine permeability system; considerable biochemical evidence has accumulated which also points to the presence of cholinergic constituents in axon membranes (Chester and co-workers, 1979; see also Dorogi and Neumann, 1980).

## EVIDENCE FOR CHEMICAL ENERGY INPUT FROM MEMBRANE CAPACITANCE MEASUREMENTS

Because permeability properties of $Na^+$ channels are dramatically influenced by the voltage difference between the two solutions bathing the membrane, some of the underlying structural changes must involve net charge movement across the membrane, and, furthermore, this charge movement must be asymmetric with respect to de- versus hyperpolarizing voltage steps relative to the resting state.

Indeed, when $Na^+$ and $K^+$ currents are pharmacologically blocked, a small but characteristic asymmetric capacitative charge movement can be resolved. It has been determined with reasonable certainty that a large part of this charge movement is due to $Na^+$ channel activation processes and this capacitative transient has been labelled "gating current" ($I_g$) (Armstrong and Bezanilla, 1977).

The total asymmetric capacitative membrane current measured for squid giant axon is response to a large depolarizing pulse is shown for two cases by solid curves in Fig. 2, showing the cases when $Na^+$ channels are activatable (larger total charge movement) and after $Na^+$ channels are completely inactivated by a preceding depolarization (smaller total charge movement), respectively. The dashed line describes the subtract of the two experimental curves and would presumably represent the actual contribution of gating events, i.e., $I_g$.

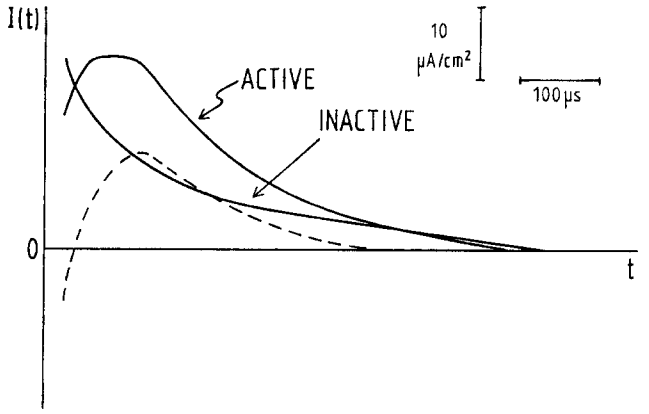



Fig. 2. Solid curves are asymmetric capacitative currents, I(t), for active and inactive $Na^+$ channel cases; taken from Fig. 10 of Armstrong and Gilly (1979). Dashed curve shows difference of the two experimental curves: the presumed activation gating current.

Kinetics of the declining phase of $I_g$ have already been noted to suggest that much of $I_g$ reflects charge movement involved in the release of electrostatic energy built up immediately following depolarization (in the first 100 μs) (Dorogi and Neumann, 1978): this energy buildup would have to be part of a rapid overall reaction which effects a net reduction of energy. It is therefore also extremely interesting that the early part of $I_g$, taken as the dashed curve in Fig. 2, reflects charge movement in a direction opposite to the direction of the later charge movement.

Such behavior is describable with the above-described chemical model: if the later values of $I_g$, above the horizontal axis in Fig. 2, reflect principally the $AR \rightleftharpoons AR' \rightleftharpoons AR''$ reaction steps driven by the membrane field following step depolarization, then early values of $I_g$, below the axis, must reflect charge movement in the opposite direction and hence, this movement must be unfavorable with respect to the membrane field. The early charge movement may be a reflection of energy input from a chemical source soon after onset of a depolarization step, modelled here by the $A + R \rightarrow AR$ reaction.

## CONCLUSION

Abstract kinetic models which are designed for simulation of axonal $Na^+$-channel permeability characteristics appear to require bimolecular reaction steps, i.e., reactions limited by the concentrations of two reactants, not just membrane-field-driven intramolecular reactions. In turn, this suggests that gating of $Na^+$ channels may involve ligand binding to membrane macromolecules as originally proposed by Nachmansohn (1955).

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