NOVEL KINETICS IN THE SODIUM CONDUCTANCE SYSTEM PREDICTED BY THE AGGREGATION MODEL OF CHANNEL GATING

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ABSTRACT Special voltage-clamp pulse protocols are given that make differential predictions for the kinetics of models based on a simple sequential, simple cyclic, and an aggregation scheme. Detailed kinetic time-courses for the discriminating pulse protocols are numerically derived from the differential equation system that describes the aggregation model.

INTRODUCTION

The concept of aggregation gating is based on the assumption that the ionic pathways responsible for the permeability changes that underly the nerve impulse are formed by a number of subunit proteins. An aggregation channel opens when these subunits assemble in a configuration that creates a pore in the membrane and closes when the pore-forming aggregate changes into a nonconducting configuration. The kinetic scheme of the elementary reactions underlying this hypothesis features two parts (Fig. 1). First, it consists of a reversible first-order voltage-dependent reaction step between inert and active monomer conformation. This step is then followed by reversible voltage-independent aggregation steps involving active monomers. Aggregation is the process in which a number of subunit molecules (monomers) reversibly combine with each other, one by one, to form aggregates (oligomers). If the first aggregation step (reversible formation of a dimer from two active monomers) is energetically in favor of the dimer, the time-course of higher aggregates is biphasic and resembles the inactivating conductance time-course of the voltage-clamped sodium conductance system.

Although the aggregation process has sequential aspects, its kinetic behavior is different from a sequence of opposing first-order reactions, because every consecutive aggregation step involves a monomer either in the form of a reactant or a product. Because of monomer involvment in all reactions, these are kinetically coupled in such a way that feedback between the various reactions can occur. This leads to autocatalysis-like effects and makes the conductance behave in a state-dependent manner. In other words, an aggregation-based gating system is not only voltage dependent, but its conductance kinetics also depend on the perturbations that the system has experienced in its immediate past.

In this paper I wish to (*a*) point out certain unusual kinetic consequences of the aggregation model assuming high dimer stability, (*b*) devise voltage-clamp protocols based on such special kinetics which result in time-courses that could distinguish between this model and the class of cyclic and sequential schemes (Armstrong, 1969; Goldman, 1976; Bezanilla and Armstrong, 1977), and (*c*) present detailed examples of the kinetic response for these pulse sequences,

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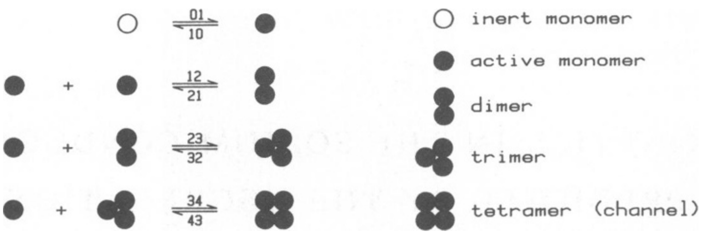

FIGURE 1 Elementary reactions of the aggregation gating model. Reversible voltage-dependent conformational change (reactions 01 and 10) and reversible voltage-independent aggregation steps (reactions 12, 21, 23, 32, 34, and 43) are the eight elementary reactions (left) assumed to occur between the five states (right) in which the subunits of each hypothetical aggregation gating site can exist.

which are numerically derived from the system of differential equations that describes the aggregation model.

CONCEPTUAL BACKGROUND AND DIFFERENTIAL PREDICTIONS

Aggregation Model vs. Other Models

There are a number of different types of reaction schemes that can potentially account for the basic kinetic response of the sodium conductance system. Cyclic and sequential reactions are the two most frequently mentioned schemes.

Cyclic schemes involve one or several closed loops of a chain of reversible first-order reactions. The second law of thermodynamics demands that the total free energy change around a closed reaction loop be zero. This imposes a constraint on the choice of activation energies which restricts the kinetic behavior of small reaction loops, so that they cannot fit the voltage-clamp data (Goldman, 1976). The kinetics of reaction systems featuring several small closed loops or one or two larger ones can be very complex, with multiple over- and undershoots in the time-course of the various concentrations in response to a sudden change in one of the reactant concentrations or one of the rate constants (Higgins, 1967). To make such schemes display the desired kinetic behavior, one or several reaction steps in a loop can be made irreversible. This may eliminate kinetic behavior that has not been experimentally observed, but it precludes a physical interpretation since such a scheme violates the second law of thermodynamics. Moreover, as purely mathematical formalisms designed to structure experimental data, such schemes are too elaborate to meet the generally accepted criterion of abstract modeling that there be a minimum degree of complexity.

Sequential schemes are inherently more flexible than cyclic schemes, but for modeling voltage-clamp data they require specific assumptions that may be difficult to test. For example, when applied to the sodium conductance system, the conducting state must necessarily precede the inactivated state in the chain of first-order reversible reactions. Thus, the transition from inactivated to resting state in response to repolarization can only occur via a conducting state; this results in a current that has not been observed experimentally. This repolarization current can be minimized by assuming appropriate rate constants, but then the other currents cannot be correctly fitted. In spite of this potential problem, sequential schemes seem more promising than cyclic ones and, indeed, have been applied with varying degrees of success to the potassium current (Hill and Chen, 1971*a,b*), the potassium current with induced inactivation (Armstrong, 1969), and the sodium current (Bezanilla and Armstrong, 1977).

Some of the unusual features of an aggregation scheme can be demonstrated by comparing it with a sequential scheme. The aggregation scheme involves several *second-order* reactions while all elementary reactions in a sequential scheme are *first order.* In other words, if an aggregate of subunits is said to be involved in a sequential process, it is understood that the aggregate behaves like a single reactant at all times, and never does what aggregates do best, namely aggregate and disaggregate. To illustrate this, assume that four subunit molecules are permanently associated into an aggregate. This aggregate is proposed to conduct ions when all four subunits bind by way of next-neighbor interaction to form a channel structure (configuration y_4), but does not conduct when one (configuration y_3), two (configuration y_2), three (configuration y_1), or all (configuration y_0) of the next-neighbor bonds are broken. If one assumes that all four bonds between the subunits can form or break independently of each other with a forward rate constant k_1 and a backward rate constant k_2, then the scheme takes the familiar form introduced by Armstrong (1969):

$$y_0 \underset{k_2}{\overset{4k_1}{\rightleftharpoons}} y_1 \underset{2k_2}{\overset{3k_1}{\rightleftharpoons}} y_2 \underset{3k_2}{\overset{2k_1}{\rightleftharpoons}} y_3 \underset{4k_2}{\overset{k_1}{\rightleftharpoons}} y_4.$$

At the risk of repetition, whereas this scheme can be interpreted as describing an aggregate that can exist in a sequence of different configurations, it does not describe an aggregation *process.*[1] Unlike the final product of an aggregation process, the final product of such a sequential process cannot display inactivation kinetics in response to a sudden change in one of the reactant concentrations or rate constants.[2]

In order to introduce inactivation-like features into the sequential scheme, Armstrong (1969) added a reversible first-order step.

$$y_0 \underset{k_2}{\overset{4k_1}{\rightleftharpoons}} y_1 \underset{2k_2}{\overset{3k_1}{\rightleftharpoons}} y_2 \underset{3k_2}{\overset{2k_1}{\rightleftharpoons}} y_3 \underset{4k_2}{\overset{k_1}{\rightleftharpoons}} y_4 \underset{k_4}{\overset{k_3}{\rightleftharpoons}} y_5.$$

Attempts to fit various basic kinetic data with a sequential scheme featuring an additional inactivation step have been made in the case of induced inactivation in the potassium conductance (Armstrong, 1969) as well as in the case of the sodium conductance (Bezanilla

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[1] Under the special conditions that the sum of the concentrations of all five configurations is 1 M and k_1 and k_2 are α_n and β_n, respectively, of the HH formalism for the potassium conductance in the squid axon (Hodgkin and Huxley, 1952), the y_4-kinetics follows exactly Hodgkin and Huxley's n^4-kinetics (FitzHugh, personal communication cited by Armstrong, 1969). Without these constraints, the scheme behaves qualitatively, but not quantitatively, like the HH formalism for the potassium conductance.

[2] The reason that the rising concentration of the final product of a sequential process cannot overshoot its new steady-state concentration in response to change in one of its parameters can readily be demonstrated with an example. In the sequential scheme above the rate of change of the final product, y_4, is given by $dy_4/dt = k_1y_3 - 4k_2y_4$. It is apparent from this expression that the concentration of the final product increases when the build-up term k_1y_3 is greater than the decay term $4k_2y_4$ and, conversely, decreases when the decay term is greater than the build-up term. In the sequential scheme the switch from the first to the second kinetic mode needed to produce the biphasic time-course of the sodium conductance could occur only if the concentration of y_3 should get depleted to such an extent that the build-up term can no longer keep up with the decay term. This can never happen, though, because a decrease in the concentration of y_4 immediately results in a corresponding increase in the concentration of y_3 which, in turn, increases the build-up rate of the concentration of y_4, thus preventing a net decrease in its concentration.

and Armstrong, 1977). Schemes that combine sequential with cyclic features were proposed more recently (Armstrong and Bezanilla, 1977; Armstrong and Gilly, 1979). It is noteworthy that these schemes resemble somewhat the scheme of transitions between the subunit configurations in a single aggregation gating site.

Experimental Tests for the Aggregation Hypothesis

In the aggregation scheme the conducting aggregate is built up from an active monomer and the aggregate that precedes the conducting aggregate, which in turn, also has its origin in the combination of an active monomer and its own precursor aggregate, and so on. In other words, a change in the availability of active monomers because of a voltage change has an effect on the number of conducting aggregates not only in a direct, but also in an indirect way via intermediate aggregates. The fact that there is a direct and an indirect component in the voltage dependence of the conductance manifests itself in the kinetic response to voltage perturbations immediately before or during the clamped voltage pulse.

However, these kinetically characteristic state-dependent features of the aggregation process are displayed only if the voltage-dependent first-order step from inert to active monomer is faster than the subsequent aggregation of active monomers and, therefore, is not the rate-limiting step. If the first-order step is rate-limiting, the various aggregate pools have time to equilibrate with the changing supply of active monomers in response to a change in voltage. For all practical purposes, they can be considered to be at steady-state with the pool of active monomers. Since at steady-state the *n*-mer concentrations are always proportional to the *n*th power of the concentration of active monomers (*n*th power law of aggregation), the *n*-mer kinetics derived from the aggregation model under the constraint of a rate-limiting voltage-dependent step are essentially first order raised to the *n*th power, or, in other words, are Hodgkin-Huxley (HH) kinetics. The fact that HH kinetics fairly accurately describe the basic voltage-clamp data from biological membranes suggests, within the framework of the subunit hypothesis, that in biological excitable membranes the step from inert to active monomer is slow compared with the aggregation steps.

This sets the stage for a series of experimental tests for the aggregation concept. If it is true that the voltage-dependent step is rate-limiting, then a sufficiently large depolarizing voltage should increase the rate of change of this reaction to such an extent that it ceases to be rate-limiting. In other words, if the model applies to biological membranes, they may display some of the more complex aggregation kinetics at higher voltages. Unfortunately, the application of high voltages to the axon can easily lead to electrode polarization and dielectric breakdown of the membrane. If the voltage pulses are of very short duration, however, membrane and electrode damage should be minimal. Voltage-clamp protocols that have originated from such considerations are shown in Fig. 2.

Kinetic behavior derived from the model for such special pulse protocols is shown in Fig. 3. The detailed assumptions that form the basis of these calculations are given in Baumann and Easton, 1980. Specifically, a temperature of 10°C, a gating charge of 1, a total subunit concentration of 1 M, frequency factors of 10,000 reactions/s (reactions/s-liter in the case of bimolecular reactions), and activation energies of 0 kJ/mol are assumed with the exception of the voltage-dependent step from chemically inert to active monomer where the activation energy is 2 kJ/mol, and the dimer-to-monomer reaction where it is 15 kJ/mol. Currents are

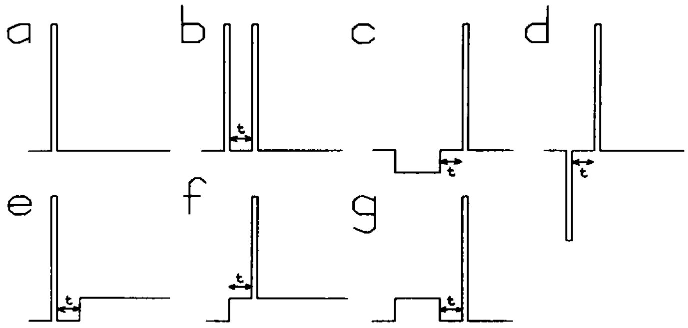

FIGURE 2 Voltage-clamp pulse protocols designed to reveal the kinetics due to aggregation. All pulse protocols begin at the resting potential. There are two kinds of pulses shown. The short (10–200 μs) pulses to a high voltage (100–250 mV) are referred to as brief perturbation. The long pulses are conventional voltage-clamp pulses. (*a*) Depolarizing brief perturbation. (*b*) Two depolarizing brief perturbations in sequence with varying duration between them; t = 10μs–10 ms. (*c*) A hyperpolarizing pulse of varying duration to various voltages followed by a depolarizing brief perturbation; t = 0–10 ms. (*d*) A hyperpolarizing brief perturbation followed by a depolarizing brief perturbation; t = 0–10 ms. (*e*) A depolarizing brief perturbation followed by a depolarizing pulse; t = 10 μs–10 ms. (*f*) A depolarizing brief perturbation during a depolarizing pulse; t = 0–10 ms. (*g*) A depolarizing pulse followed by a depolarizing brief perturbation; t = 0–10 ms.

given in arbitrary units obtained by multiplying the tetramer concentration by the driving force (in Volts) times a factor of 10,000. The assumed reversal potential is 55 mV. For these computations a numerically simple parameter set was chosen. Deviations from this set such as the assumption of values for the gating charge and the concentration different from 1, and for the activation energies different from zero can increase the execution time of such computations by several orders of magnitude. It is thus for computational reasons that the voltage-dependent step is assumed to be relatively fast. Assuming a relatively slow voltage-dependent step would result in qualitatively similar, but smaller kinetic effects.

CONCLUSION

Experimental protocols are presented that result in unusual kinetic features when applied to the aggregation model under the condition of slow dimer-to-monomer rate. These features are not a result of a special combination of the remaining rate constants, but are inherent to the aggregation concept. The specific parameters assumed for the computations were chosen for computational convenience. An activation energy of 0 kJ/mol results in a rate constant of 1. Higher activation energies would result in smaller rate constants. The resulting slower time-courses would then have to be rescaled by assuming higher frequency factors. The only crucial assumption for the model computations is a high energy barrier from the dimer to the monomer. This results in high dimer stability. Under these circumstances monomers that are freed when the conducting tetramer disassembles tend to get locked up in the form of favored but nonconducting dimers and, in the terminology of Hodgkin and Huxley (1952), this leads to an inactivating time-course.

If the aggregation concept applies to the gating process in excitable membranes, the predicted unusual kinetic behavior may be detectable given adequate experimental design and

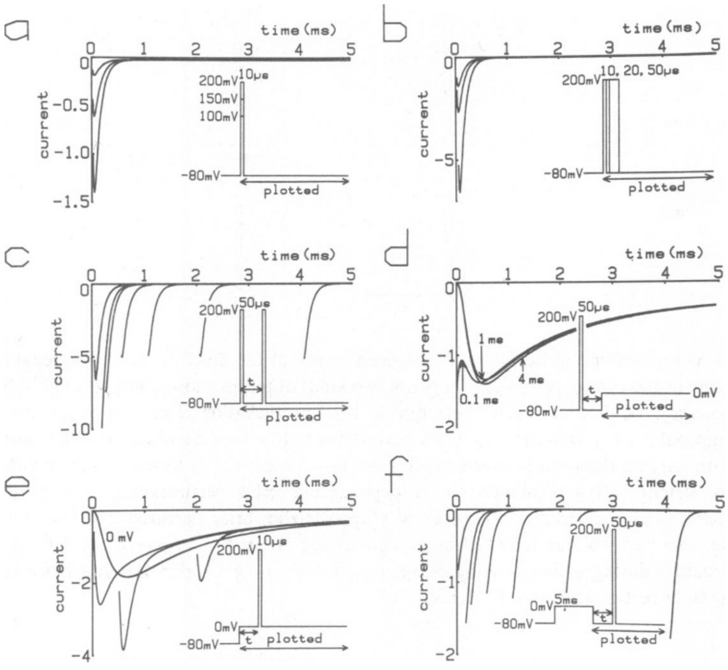

FIGURE 3 Some unusual features in the voltage-clamp kinetics predicted by the aggregation model. The kinetic responses are calculated from the pulse sequences shown in each figure. For details about model parameters see text. The current traces reflect ionic events derived from the aggregation model which does not incorporate capacitative currents or effects due to series resistance. Current traces occurring during the brief pulses are omitted. The pulse sequences in the inserts are not drawn to scale. In order to minimize computation time, parameters were assumed that result in exaggerated kinetic effects. These predictions should be understood as qualitative, but not as quantitative in nature. (*a* and *b*) Recovery from a brief depolarizing voltage pulse is biphasic. (*c*) The peak current due to a second brief depolarizing pulse is higher than the peak current from the first pulse if the delay between the two pulses is short, and lower if the delay is long; t = 0.1, 0.2, 0.5, 1, 2, 4 ms. (*d*) A brief depolarizing pulse followed by a delayed conventional depolarizing pulse results in triphasic kinetics if the delay is short, and biphasic inactivation-kinetics with recovering amplitude for longer delays. In all three cases the current is smaller than the current from the same pulse sequence without the brief prepulse (compare with control [0 mV] in Fig. 2*e*); t = 0.1, 1, 4 ms. (*e*) Brief depolarizing pulse on top of a conventional voltage pulse gives a biphasic response that crosses over the time-course in response to the conventional pulse; t = 0, 0.5, 2 ms. (*f*) Effect of a brief depolarizing pulse after a conventional depolarizing pulse; t = 0.1, 0.2, 0.5, 1, 2, 4 ms. Current is expressed in arbitrary units.

temporal resolution. The data presented in the subsequent paper (Schauf and Baumann, 1981), from experiments performed on voltage-clamped *Myxicola* axons, suggest that the behavior in at least one of the protocols described in Figs. 2 and 3 can in fact be experimentally observed.

Of course, such agreement does not prove that aggregation is involved, but only shows that it could be involved in the gating process. A particular kinetic scheme is not necessarily unique, even if it fits all of the data. With enough patience and time, a large number of kinetic schemes that fit any data can always be constructed. The question, then, should be not only how well a certain scheme can fit certain data, but also how plausible that scheme is. The plausibility of a kinetic model depends on the ease with which it can be interpreted in physical and chemical terms, as well as on its self-consistency, universality, and simplicity.

State dependence (i.e., the property of data to "remember" the voltage-pulse history) can be explained by the aggregation model in terms of only a few gating molecules that have no memory of their own and also have no memory of their interactions with each other (Baumann and Easton, in press). Thus, the value of a model also lies in its ability to provide novel insights into experimental observations and give complex data a simple explanation in terms of a hypothetical underlying mechanism.

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