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Kinetic models suggest bimolecular reaction steps in axonal Na^+-channel gating

(ligand binding/conformational changes/acetylcholine/K^+ channels)

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ABSTRACT **Abstract kinetic models that can successfully simulate the ion-permeability features of axonal Na^+ channels suggest the presence of bimolecular reaction steps in the activation of the channels. A chemically plausible interpretation of minimum complexity is described. The implied chemical formalism is highly suggestive of an activator-controlled gating system with strong similarities to the acetylcholine-regulated system. Conformational changes that underlie the ion-conductance changes are suggested to possess a greater sensitivity to the membrane field in axonal parts of excitable membranes than at synaptic parts. This would allow axonal permeability changes to be energetically regulated more conservatively than is observed for synaptic ion channels. Axonal K^+ channels with delayed activation kinetics would serve to reverse the increase in membrane permeability to Na^+ with a minimum of chemical dissipation.**

There have been numerous attempts at expressing membrane permeability changes that underlie nerve excitability in terms of self-consistent kinetic models. Inferences as to the precise physical meaning of parameters appearing in these models are to a large degree subjective, because the set of equations characterizing a particular model have an infinite number of equivalent representations, interchangeable by transformations of variables (1). Deciding which representation, if any, of a particular model has its parameters corresponding one-to-one with physical units and events is therefore largely arbitrary. Nonetheless, some definitive characteristics of the Na^+-channel gating system in axonal membranes have become apparent only as a result of attempts at model simulation of Na^+ currents; these include the finding by Jakobsson (2, 3) that unimolecular models with classical voltage-dependent rate constants may not be able to cover all the electrophysiological data obtained with voltage-clamped axon membranes.

There are connections between this finding and general features of a biochemical gating concept proposed several years ago (4–6). The basis of the biochemical model was derived from molecular notions first proposed by Nachmansohn (5)—namely, that changes in membrane permeability are controlled by conformational changes induced by the binding of low molecular weight ligands to gating macromolecules. The present article describes how numerical simulation studies of axonal Na^+ currents also indirectly suggest the presence of bimolecular reaction steps in axonal Na^+-channel gating.

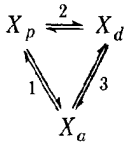
Although biochemical evidence on axonal Na^+ channels is to date insufficient for the proposal of specific chemical reaction models, explanation of some electrical properties of axons appears to require energy contribution from a local chemical source early in the reaction sequence that leads to opening of the channels. A physicochemically plausible explanation is that channel activation is coupled with a bimolecular reaction step involving a ligand whose availability to its receptor is mediated by the membrane field. The chemical interpretation of the minimal mathematical model describing Na^+-channel conductance characteristics resembles that of reaction models proposed for the acetylcholine-regulated permeability system of synaptic membranes, which are based on biochemical as well as electrophysiological data. It is deduced that the reaction model for axonal Na^+ channels must involve concentration-limited and critically membrane-field-dependent reaction rates. Expected similarities and differences between axonal and synaptic gating reactions are discussed here within the framework of a formalistically specific chemical reaction model.

Kinetic models

Basic features of Na^+-permeability changes in voltage-clamped axon membranes are first summarized.

When a sufficiently large depolarizing voltage step is applied to a resting (polarized) membrane—i.e., when the normally negative electric potential of the cell interior (relative to that outside the cell, arbitrarily set at zero) is suddenly changed to a more positive value—there follows a dramatic *transient* increase in the membrane's permeability to Na^+. If the membrane is subsequently repolarized—i.e., reclamped such that the electric potential of the cell interior is again more negative relative to the outside potential—the ability of Na^+ channels to respond to a second depolarization depends, in part, on the strength and duration of the first depolarization step; maintained depolarization "desensitizes" Na^+ channels to further stimuli. Both the extent and rate of desensitization increase as depolarization steps impose progressively more positive voltages.

Salient features of the evolution of and recovery from desensitization and kinetics of the Na^+-permeability change can be simulated with the following three-state model (2, 7):



[1]

This model represents intramolecular transitions among three states of a hypothetical Na^+-channel regulating unit. The distribution (concentration) of these states is dependent on the membrane potential, V, defined usually as the difference between the electric potential of the cell's interior and that outside the cell. At very negative V, the activatable nonpermeable state X_a is favored. As V grows more positive (depolarization), the nonpermeable and desensitized state X_d becomes the most stable. When a resting membrane (for example, $-70 < V < -60$ mV in the case of squid giant axon) is subjected to a rapid depolarization, some gating units are thought to redistribute

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from X_a to X_d; the observed permeability increase, usually expressed as a transmembrane Na^+ conductance, g_{Na}, is taken to reflect the passage of units through state X_p via pathways 1 and 2 in scheme **1**. The nonlinear growth of g_{Na} after rapid depolarization suggests a higher-order correlation between g_{Na} and the concentration of state X_p. For squid giant axon, a cubic proportionality is adequate (8), $g_{Na}(t,V) \alpha [X_p(t,V)]^3$, in which square brackets denote concentration. The exponent suggests three independent permeability-regulating units per Na^+ channel. For the most part, pathway 3 models recovery from desensitization ($X_d \rightarrow X_a$) and dominates when V is returned to a sufficiently negative value.

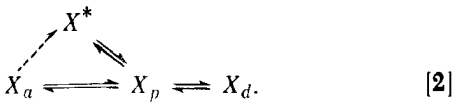
There are at least two observations that cannot be consistently modeled by scheme **1**. Either one, or both, have been observed with most types of axons studied, and hence they warrant incorporation into any generally applicable kinetic model of Na^+-channel gating.

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. During a second depolarization to $V = V_2$, g_{Na} is observed after Na^+ channels have been allowed to "equilibrate" at a less-positive voltage, V_1 (9). The quantity of interest is the peak value of $g_{Na}(t,V)$ during the second pulse, $g_{Na,p}(V_2)$, because $g_{Na,p}(V_2)$ is a reflection of the equilibrium fraction of units in state X_a at the voltage V_1.

When one compares the calculated quantities $g_{Na,p}(V_2)/g^{max}_{Na,p}(V_2)$ at different values of V_2 for a fixed value of V_1, where $g^{max}_{Na,p}(V_2)$ is the maximum value attainable (when V_1 is very negative), it is found that this ratio increases as V_2 becomes more positive. On the other hand, when the six rate constants in scheme **1** are selected so as to model the g_{Na} increase and decrease during a maintained depolarization and recovery kinetics at the same potential, the same rate constants are unable to model the V_2 dependence seen in two-pulse experiments and also to satisfy the requirement of detailed dynamic balance (2, 7). The latter property must hold for "equilibrium" gating models such as scheme **1**.

A second observation that is anomalous with scheme **1** is that some axon preparations during a maintained depolarization show a rate of decline in $g_{Na}(t,V)$ that is faster than the concurrent rate of desensitization. Numerical simulation studies indicate that a separation of the two phenomena cannot be described by scheme **1**, because state X_p can be emptied only into state X_d (3).

As pointed out by Jakobsson (1–3), both of these observations can be formally modeled if the depolarization pathway, $X_a \rightleftharpoons X_p \rightleftharpoons X_d$, actually encompasses an "excited" state, X^*, so that the depolarization model takes the form



[2]

The observed increase in $g_{Na,p}(V_2)/g^{max}_{Na,p}(V_2)$ with increasing V_2 can be explained if the permeable state X_p is fed from *two* sources, X_a and X^* but with different V dependencies. During small to moderate depolarizations, the $X^* \rightarrow X_p$ transition would dominate, whereas at larger depolarizations the $X_a \rightarrow X_p$ transition would be also important (3).

The inclusion of such an excited state also covers the observation that X_p can empty faster than X_d can fill, i.e., that the g_{Na} decline can occur faster than g_{Na} desensitization, because some units would traverse from X_p back to X_a, rather than directly on to X_d (3).

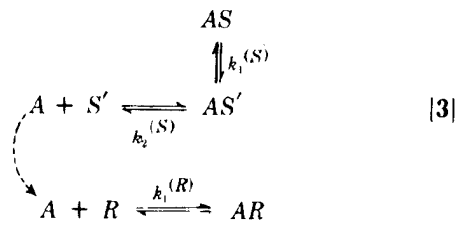
Because the $X^* \rightarrow X_p$ transition must occur more readily than the $X_a \rightarrow X_p$ transition, state X^* must be less stable than state X_a during depolarization. Effectively then, depolarization "splits" the gating units initially in state X_a into two energetically distinct populations. It is very important to note that only a fraction of the units can make the $X_a \rightarrow X^*$ transition; in other words, the $X_a \rightarrow X^*$ transition must be both rapid (microsecond range) and also short-lived. Otherwise, state X_a would be totally depleted, and the desensitization "anomalies" described above could not be described with model **2**. Therefore, modeling of the electrophysiological observations requires that the fraction of gating units attaining state X^* after onset of a rapid depolarization be determined by a finite short-lived energy source.

Because chemical rate constants can depend only on the instantaneous value of the membrane field, which remains constant in voltage-clamp experiments, the rate parameter describing the $X_a \rightarrow X^*$ transition cannot be an elementary chemical rate constant. On the other hand, it is physicochemically plausible that the kinetic parameter describing the $X_a \rightarrow X^*$ transition reflects the short-lived availability of some substance that becomes available to the permeability-regulating units as a result of a depolarizing shift in the membrane field.

Chemical gating model

The formal $X_a \rightarrow X^*$ transition represents a destabilization of some of the gating units, and this must be compensated for by energy input somehow resulting from the act of changing the net transmembrane electric field (1). This quality of scheme **2** is difficult to comprehend in a physicochemical sense, and leads one to question traditional attempts at modeling of Na^+-channel gating reactions in terms only of membrane-field-dependent intramolecular reactions. The only elementary alternative to purely intramolecular processes are bimolecular reactions; the rate parameter of the $X_a \rightarrow X^*$ step would then be only an apparent rate coefficient involving concentration-dependent terms. For instance, the fundamentally bimolecular reaction $B + C \rightarrow D$ may be modeled monomolecularly by $B \rightarrow D$, with an apparent rate coefficient $k' = k[C]$, which is concentration dependent. Scheme **2** suggests that the $X_a \rightarrow X^*$ transition requires a binding reaction involving an "activator" ligand, A.

Because the $X_a \rightarrow X^*$ step must be very rapid, A should be a low molecular weight activator. When the membrane is at rest, A must be somehow sequestered, otherwise it would be lost through self-diffusion or other binding reactions. Upon depolarization, A must be released from storage and allowed to bind with the permeability-regulating unit; this requires that the applied change in membrane field induce a rapid structural change in the storage units (4). Hence, the first steps in activation are suggested to be a rapid structural change followed by a binding reaction. A representative reaction model is

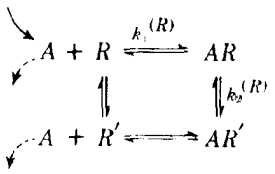


[3]

in which R denotes the activatable, nonpermeable state of the permeability-regulating unit; $k_1^{(S)}$, $k_2^{(S)}$ and the rate parameter $k_1^{(R)}[A]$ would each be on the order of, or greater than, 10^6 sec^{-1}. For example, if $k_1^{(R)} \approx 10^8$ M^{-1} sec^{-1}, a typical value for diffusion-limited bimolecular reactions, then $[A]$ must be $\geq 10^{-3}$ M near the binding site immediately after depolarization.

The sequence of reactions shown in scheme 3 would precede the appearance of g_{Na}, and they correspond in total to the $X_a \rightarrow X^*$ step of scheme **2**, in which X_a is specified by R and X^* corresponds to the sum $A + R$. Activation of the permeability unit can be initiated by ligand binding to receptor sites R, so that the number of units activated depends on the number of units in state R and on the amount of stored activator ($[AS]$) at the time of onset of a depolarizing pulse. Of the three rate constants shown explicitly in scheme 3, $k_1^{(S)}$ must be sensitive to the membrane field, because a depolarizing shift in the field must shift the $AS \rightleftharpoons AS'$ equilibrium toward AS'. In contrast, the reaction $A + R \rightleftharpoons AR$ would be driven dominantly by chemical affinity forces. Binding of A must serve to facilitate transition of the permeability-regulating unit to the permeable configuration by reducing the required thermodynamic activation energy. In supplying A, membrane depolarization makes an "energy source" available to the permeability-regulating unit; the fraction of units "excited" to state AR would depend critically on the finite and short-lived availability of A.

In order to model the desensitization properties described in the preceding section, two activation pathways are required, (e.g., the $X_a \rightarrow X_p$ and $X^* \rightarrow X_p$ pathways of scheme **2**); the $X_a \rightarrow X_p$ pathway may be monomolecular and purely membrane-field sensitive. The following overall activation reaction scheme is suggested:



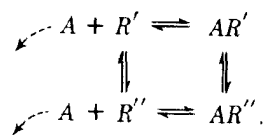
[4]

The conformations R' and AR' are the permeable states, so that for squid axon

$$g_{Na} \propto ([R'] + [AR'])^n$$

is expected. In analogy with scheme **2**, both the $R \rightleftharpoons R'$ and $AR \rightleftharpoons AR'$ reaction pathways are voltage sensitive; however, for small to moderate depolarizations the $AR \rightarrow AR'$ transition would dominate, with the $R \rightarrow R'$ transition becoming comparable only for very positive deviations from the resting potential. On the basis of Jakobsson's computations using scheme **2** (see ref. 1), $k_2^{(R)} \approx \alpha_m$ of the Hodgkin–Huxley model (10), whereas the rate parameter describing the $R \rightarrow R'$ transition would be smaller than α_m. Therefore, it appears that the permeable configuration is induced by ligand binding; alternatively, due to the large field sensitivity of the $AR \rightleftharpoons AR'$ pathway, some permeability units open via the ligand-independent $R \rightarrow R'$ pathway, but the latter type of opening would be an important contribution only when the size of the depolarization is very large.

As described above, a maintained depolarization results in reclosing and desensitization of Na^+ channels. The successive reactions away from states R' and AR' are diagrammed in scheme **5**:



[5]

Both $AR' \rightleftharpoons AR''$ and $R' \rightleftharpoons R''$ reactions must be field sensitive in order to describe electrophysiological observations, and the time constants for both these reactions must be close to $3/\beta_h$ of the Hodgkin–Huxley model (10). In addition, because the entire reaction sequence $AR \rightleftharpoons AR' \rightleftharpoons AR''$ appears to be dominated by the membrane field, these three states cannot differ appreciably in their affinity for A. The practically monophasic field-dependent decline of g_{Na} during maintained depolarization suggests that the $R' \rightarrow R''$ and $AR' \rightarrow AR''$ transitions have very similar kinetics.

The question remains, would enzymatic degradation of A play a role in Na^+-channel conductance regulation? In synaptic membranes, the enzyme acetylcholinesterase is undisputed to play a vital role in reclosing of the ionic channels by irreversibly removing acetylcholine dissociated from the receptor protein (11). In axons, chemical driving forces associated with desensitization can not be resolved from purely electrical properties. Undoubtedly, channel reclosing is strongly influenced by the membrane field, so that rates of reclosing and desensitization during a maintained depolarization would be limited by the strongly field-dependent intramolecular $AR' \rightarrow AR''$ reaction. Once a permeability unit is in state AR'', the activator may be rapidly and irreversibly removed by an enzyme; after a sufficiently long depolarization, all units will be in state R''. In this model, the "desensitization" of a unit is primarily a result of the $AR' \rightarrow AR''$ (or $R' \rightarrow R''$) structural transition, but it is also coupled with the loss of activator to the permeability unit.

The activator A would not be expected to be stored in a reaction space that is accessible to its degrading enzyme, so that the storage unit for A would be separated from the enzyme by a major diffusion barrier (reaction space 1 in Fig. 1). Upon depolarization, A would be released into reaction space 2, which is shared by storage and permeability units. Since desensitization of axonal Na^+ channels occurs only after prolonged depolarization, desensitization associated with removal of A could be possible only after the $AR' \rightarrow AR''$ reaction; this reaction must then translocate A to reaction space 3 that is accessible to the enzyme (Fig. 1).

It has been argued here that even though activation of both axonal Na^+ and synaptic channels appears to involve ligand binding, an important difference appears to be the larger role allocated to the membrane field compared to purely chemical

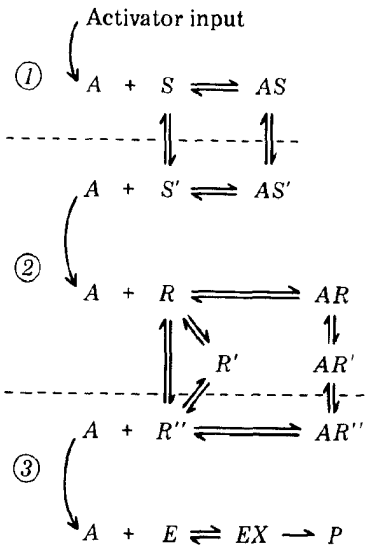


FIG. 1. Overall reaction model for axonal Na^+ channel, showing an essentially sequential translocation of activator A from reaction space 1 (activator storage and release reactions) through reaction space 2 (activator-dependent and -independent channel control reactions) to reaction space 3 (activator removal reactions) during a maintained depolarization. R is the activatable-closed gating unit state, R' and AR' are activated (conducting) states, and R'' corresponds to the desensitized state after removal of A.

driving forces in the axon. In this connection, it may be important that during an action potential, the axon membrane is depolarized only for a short interval of time because of the restoration of the resting membrane field by the delayed outward K^+ current (10). After a short-term depolarization, most units could close via the $AR' \rightarrow AR$ transition (i.e., by a transition which retains A in reaction space 2), and activator may in large part be recovered by the storage units. Because the various intramolecular transitions described in Fig. 1 are suggested by electrophysiological experiments to be very sensitive to the membrane field, the restoration of the membrane field following the opening of K^+ channels may normally effect the reclosing of Na^+-channel permeability units via the $AR' \rightarrow AR$ pathway. Therefore, whereas synaptic channels may close essentially by the enzymatic removal of acetylcholine, the closing of Na^+ channels in axonal membranes during an action potential may possibly be rendered more efficient by other factors in addition to the enzymatic removal of activator.

Hence, the role of the K^+ channel may be to conserve A, by indirectly reversing the $AR \rightarrow AR' \rightarrow AR''$ reaction sequence at AR'—i.e., before A is translocated to the degrading enzyme. For such a conservation method to be feasible, the $AR \rightleftharpoons AR'$ pathway must have evolved a greater sensitivity to the membrane field than to chemical driving forces; in fact, such an increased field sensitivity is suggested by g_{Na} properties. Near-conservation of A during a normal action potential would allow for repetitive firing with little drain on the activator store. In contrast, maintained depolarization would deplete the immediately available supply of A.

The parallel evolution of increased field sensitivity and a K^+ channel with delayed opening kinetics may indicate that axons have modified a synaptic communication system to the needs of a regenerative, much less dissipative, signal-conduction mechanism.

Recovery would require two processes: replenishment of the activator-storage state, AS, and return of the permeability-regulating unit to the R configuration, both of which must be membrane-field sensitive. This suggests inherently biphasic recovery kinetics, but the two recovery rates may differ by orders of magnitude. The return pathway from state R'' to state R must proceed by a pathway that is distinct from the $R \rightleftharpoons R' \rightleftharpoons R''$ pathway, because g_{Na} does not appear during recovery. Recovery is modeled by the direct $R \rightleftharpoons R''$ pathway shown in Fig. 1.

Discussion

A proposed chemical scheme for a membrane process about which only limited chemical information is available is shown in Fig. 1. The model is constructed solely on the basis of the axon membrane's response to rapid voltage shifts. Suggestion of a ligand-binding reaction prior to channel opening follows as a chemically reasonable interpretation of necessary mathematical qualities of the class of models that can successfully simulate channel desensitization properties. It is required that in a monomolecular representation Na^+-channel gating involves a very rapid transition away from equilibrium ($X_a \rightarrow X^*$) and that the rate parameter describing this "nonequilibrium" transition be short-lived (i.e., that the rate parameter be a function of time). The former of these requirements is a violation of the second law of thermodynamics, whereas the latter requirement shows that the rate parameter of this mathematical "reaction" step cannot correspond to an elementary rate constant, which by definition must be time independent.

On the other hand, it is always possible to express bimolecular reactions in monomolecular form, in which case the rate parameters include reactant concentrations and hence can be time dependent. In addition, the otherwise unrealistic energy requirement can be satisfied if the permeability-regulating unit is not an isolated system but is energetically coupled to other membrane units. In chemical reaction systems, this coupling usually reflects that a common reactant is involved in separate reactions; therefore, it may be that one of the reactants in a fundamentally bimolecular channel-opening reaction functions as an intermediary among the directly observed permeability unit and other membrane units.

A gating concept based on storage, receptor, and enzymatic degradation units, with acetylcholine as the intermediary, is widely realized for the regulation of synaptic ionic channels. Therefore, it is very suggestive that the ionic conductance increase responsible for the action potential appears to be regulated by a similar mechanism.

Some similarities and important differences between synaptic and axonal gating properties need to be summarized here. The kinetics of end-plate currents are known to be sensitive to both membrane field and reactant concentrations (12, 13). Unlike axonal g_{Na}, the early growth phase of end-plate currents appears to be dominated by the concentration of acetylcholine near receptors (e.g., see ref. 12) rather than directly by the membrane field. Hence, in the synaptic receptor, transition from the activatable, closed state to the open configuration appears to be chemically induced by acetylcholine. On the other hand, the subsequent open-to-closed transition displays kinetics that are appreciably slowed by membrane hyperpolarization, and as with axonal g_{Na}, end-plate currents undergo a simple exponential decay (12, 13). The decay kinetics of end-plate currents are not only voltage sensitive but also are slowed in the presence of high concentrations of neostigmine, which is known as an inhibitor of acetylcholinesterase (13).

Both axonal Na^+ and synaptic ionic channels exhibit desensitization under extreme conditions: in the axon, desensitization evolves during a membrane depolarization, whereas a similar phenomenon is observed with synaptic receptors after prolonged exposure to appropriate ligands (11, 12). As with axonal Na^+ channels, activation of synaptic receptors represents a transition between activatable-closed and desensitized states (14, 15), and the ion-permeable configuration appears to be an intrinsically metastable transient state for both types of channels (11).

Therefore, it appears that axonal Na^+ channels, which underlie the generation of an action potential, and the system that is responsible for the increased ionic permeability caused by the arrival of an action potential at a synapse can be covered with very similar reaction models. Principal differences between axonal and synaptic receptor systems appear to reflect an increased sensitivity of the axonal system to the transmembrane field, which may serve to minimize the amount of activator ligand lost to the Na^+ channel during action potentials. At the synapse, potassium ion conductance, $g_K(t)$, and $g_{Na}(t)$ appear to have practically identical kinetics (16) (however, see ref. 12, p. 230), whereas in the axon $g_K(t)$ increases appreciably only after $g_{Na}(t)$ has "peaked" and is mainly responsible for the return of V to its resting value (10).

As already noted, even though Na^+-permeability characteristics can be analyzed to find the correct class of models that are representative of axonal Na^+-channel gating (i.e., models with bimolecular as well as monomolecular reactions), identification of gating molecules can come only after successful chemical isolation and purification of Na^+-channel components. In this connection, several biochemical properties of axons also suggest an acetylcholine-like gating system. For example, it has been observed that once permeability barriers are removed, axonal conduction is sensitive to both acetylcholine

and curare, that acetylcholine is released from cut nerves, and that the enzyme acetylcholinesterase is present on the axolemma (e.g., see ref. 17). It has also been found that α-bungarotoxin, a neurotoxin believed to bind selectively to nicotinic acetylcholine receptors, also attaches to lobster axon-membrane fragments (17).

Most recent is the finding that tetrodotoxin and veratridine, which affect the electrical activity of intact axons, also influence the phosphorylation/dephosphorylation cycle of some proteins in the axolemma; this suggests a connection between the phosphorylation state of membrane proteins and membrane conduction properties (18).

Metabolic contributions to the axonal gating processes are possible. It is recalled that pharmacological and biochemical studies are notoriously difficult because of diffusion barriers. Electronmicrographs of squid giant axon sections show the excitable membrane protected by thick Schwann cell layers covered by a basement membrane (19), which in many cases is impervious to externally applied metabolites and metabolic inhibitors. Furthermore, the peripheral axoplasm adjacent to the membrane, which is not extruded in perfusion experiments, accounts for about 80% of axoplasmic ATP production (20).

The arguments presented here propose that an acetylcholine-mediated chemical system or one fundamentally similar be actively considered as a plausible candidate for the regulation of axonal permeability changes. Thus, the rapid transient permeability changes in axonal and synaptic parts of excitable biomembranes appear to be specialized cases of a more general chemically dissipative control principle that is based on activator–receptor interactions.

We send best wishes to Prof. David Nachmansohn on the occasion of his 81st birthday. The comments of Dr. E. Jakobsson were very helpful in the design of the chemical model. Financial support of the Deutsche Forschungsgemeinschaft Grant NE 227 is gratefully acknowledged.

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