Stochastic Description of Sodium Channel Gating

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Abstract. A stochastic model of the sodium channel is proposed. Transitions from the resting to the open state of the channel is described by the gamma distribution. The open state is temporary with an average open time τ, and the channel proceeds to the inactivated state. The channel can be represented by two identical control molecules which undergo conformation transitions under changes of the electrical field. The gating of the channel is analyzed and its relation to the gating current is proposed. The movements of the control molecules are not identical with the charge movements. Charged parts of control molecules move in the electrical field of the membrane and make their conformation energetically possible. The model is represented by a set of differential equations, and explicit solutions for long depolarizing voltage steps are found. Parameters are determined to fit literary experimental data.

Key words: Stochastic model — Sodium channel — Gating process

Introduction

Excitability is the essential attribute of a living system. One of the most important forms of excitability is the generation of the nerve impulse. Our knowledge about the mechanism of the nerve impulse derives primarily from experimental and theoretical results of Hodgkin and Huxley (1952) on the squid giant axon. The structural basis of excitability are selective, voltage dependent ionic channels. The first excitability type described was the sodium one. This type of excitation process is expressed by the action potential generated by temporary changes of permeabilities for sodium and potassium ions. The sodium channel, which plays the central role in the generation of nerve impulses, is a voltage-regulated integral membrane protein. The voltage dependence implies the existence of a molecule which is able to respond to the cell membrane potential because it, or parts of it, have a charge or a dipole moment. Movements of a such molecule due to changes in membrane potential produce an electric current, a displacement current, also called gating current.

We still have no knowledge on the molecular structure of the sodium channel. Its function on the molecular level has been only derived from voltage clamp

experiments, noise analysis, gating currents and patch clamp experiments and from the phenomenological mathematical description by Hodgkin and Huxley. In Hodgkin and Huxley's (1952) model, sodium conductance, g_{Na}, is described by

$$g_{Na} = \bar{g}_{Na} \cdot m^3 \cdot h \tag{1}$$

where $\bar{g}_{Na}$ is the conductance expected when all channels are open. In the most popular physical interpretation, it is imagined that a sodium channel becomes conducting after three subunits, m, have made independent first order transitions from the resting to the activating positions, and closes when a subunit, h, has moved from the resting to the inactivating position or as soon as the first m particle has reverted to the resting position. Since in this model the sodium channel derives its voltage dependence entirely from those of the m- and h-particles, the entire gating current should be due to them. However, one of the first definite insights to emerge from the study of gating currents is that any proposed physical interpretation of the Hodgkin-Huxley equations for sodium conductance is now ruled out. They failed both kinetically and in the description of the steady state (Almers 1978).

A number of experimental results have accumulated (see Goldman 1976), including patch clamp experiments (Aldrich et al. 1984) which are not concordant with the m^3h kinetics and with the assumption of independent activation and inactivation. To overcome this discrepancy, several multistate models were constructed (e.g. Goldman 1976; Jakobsson 1976; Bell and Cook 1979; Hoyt 1984). The proposed model provides a new possibility to explain observed phenomena. Its advantage is a simple fitting to experimental data and a comprehensive molecular interpretation.

Model of channel gating and results

The excitable biological membrane about which there is most experimental information is undoubtedly the membrane of the squid axon. The voltage clamp technique made possible direct measurements of ionic currents and their dependence on the membrane voltage and ion concentrations. The model is constructed with the voltage clamp data obtained by Hodgkin and Huxley (1952) on the giant axon of squid. These experiments are the best documented and there also are gating currents measurements on the same preparation.

When the voltage across a resting excitable membrane is changed stepwise to a value sufficiently more positive than the resting level, the permeability of the membrane to sodium rapidly increases and then more slowly declines despite the fact that the membrane voltage is maintained at the positive value which induced channel openings (Hodgkin and Huxley 1952). On the other hand, the gating current at the same conditions exponentially decreases from a finite value to zero

(Armstrong and Bezanilla 1973). This increase and subsequent decrease in sodium permeability implies that the sodium channels can progress through at least three distinct functional states, i.e. resting, activated and inactivated (Hodgkin and Huxley 1952). Thus, a sufficiently positive voltage causes sodium channels to make the transitions from a closed resting state to an open conducting state and closed inactivated state, which is distinct from the resting state.

Because nothing is known about the molecular mechanism of the sodium channel, to describe such a time course, several assumptions should be adopted which have simple implications.

a) First, the sodium channel has only one conducting state with a definite conductance γ.

b) Molecules controlling sodium channels respond to the depolarizing voltage step by a jump from the resting state to the active state, and these jumps are independent. Therefore, they should have an exponential time distribution with the probability density function

$$R(t, \alpha) = \alpha \exp(-\alpha t) \tag{2}$$

where α is the intensity of jumps, i.e. the mean number of jumps per time unit.

c) Let us suppose that p independent transitions of control molecules (of one channel) to the active state allow the channel to open.

d) All the control molecules have the same kinetic parameters. Hence, the opening of channels have the gamma distribution with the density of probability (see, e.g. Rao 1965)

$$G(t, \alpha, p) = \alpha^p \cdot t^{p-1} \cdot \exp(-\alpha t)/\Gamma(p) \tag{3}$$

where $\Gamma(p)$ is the gamma function, α and p are parameters. For $p \geq 2$, the function G has qualitatively the same time course as the sodium conductance.

e) To describe the sodium conductance with the gamma distribution, we shall suppose a temporary conducting state of the sodium channel with an average open time τ. We can use an average channel open time, because τ is by about one order smaller than the time scale of the sodium conductance as can be estimated from the time course of the action potential. The total charge crossing the surface unit of the membrane is then

$$Q_{Na} = N(V)\tau(V)\gamma(V - V_{Na})$$

Its distribution in time, i.e. the membrane current, is

$$I_{Na} = N(V)\tau(V)\gamma(V - V_{Na})G(t, \alpha(V), p)$$

and conductance

$$g_{Na}(t, V) = N(V)\tau(V)\gamma G(t, \alpha(V), p) \tag{4}$$

where $N(V)$ is the number of channels which pass through the open state during a long depolarizing voltage step.

Fitting Hodgkin's and Huxley's experimental data summarized in their Table 2 (Hodgkin and Huxley 1952); we can get parameters of the model for the squid axon membrane. The value $p = 2$ is the most suitable to describe principal features of the sodium conductance, mainly with respect to the time to peak and the descending phase.

To find parameters of the model we can use special properties of the gamma distribution. $G(t, \alpha, 2)$ has a maximum at $t = 1/\alpha$, where

$$\partial G(t, \alpha, 2)/\partial t = \alpha^2(1 - \alpha t) \exp(-\alpha t) = 0$$

and the maximal value is $G_{max} = \alpha/e$.

Because $G(t, \alpha, 2)$ is the only time dependent term in the description of the sodium conductance, then it has to reach the maximum at $t = 1/\alpha$ with the value

$$g_{Na\,max} = N(V)\tau(V)\gamma\alpha(V)/e \tag{5}$$

Now the finding of the parameters of the model is straightforward. Plotting the reciprocal time to peak of the sodium conductance we get the parameter $\alpha(V)$. Data can be fitted with the curve*

$$\alpha(V) = 2.3/(1 + \exp(-0.05(V + 20))) \tag{6}$$

as is shown in Fig. 1. Similarly, plotting $g_{Na\,max} \cdot e/\alpha$ we get the voltage dependence of $N\tau\gamma$. The best approximation gives the curve (Fig. 2)

$$22.56/(1 + \exp(-0.15(V + 34))) \tag{7}$$

A comparison of the sodium conductances computed from this model and the Hodgkin-Huxley model is shown in Fig. 3. The proposed model describes the basic phenomena of the sodium conductance. The difference mainly concerns the raising phase, where the experimental record shows a delay, which is not mimiced by the model. Let us analyse this phenomenon.

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* In eqs. (6) and (7), experimental data are fitted with the curve

$$f(V) = a/(1 + \exp(-b(V - c)))$$

and parameters can be obtained directly from plotted data. The curve, $f(V)$, saturates and asymptotically reaches the value a. The half value of saturation is at c, and

$$b = 4/(a \cdot f'(c))$$

where $f'(c)$ is the slope at c.

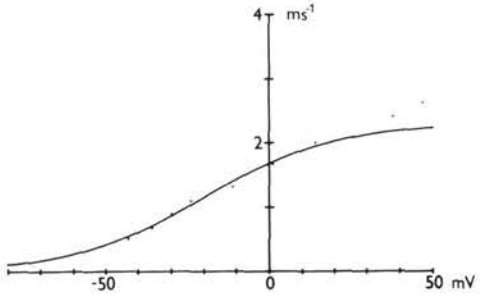

Fig. 1. Experimental data represent reciprocal values of the time to peak of the sodium conductance determined from Table 2 in Hodgkin and Huxley (1952). Data are fitted with the curve $\alpha(V) = 2.3/(1 + \exp(-0.05(V + 20)))$.

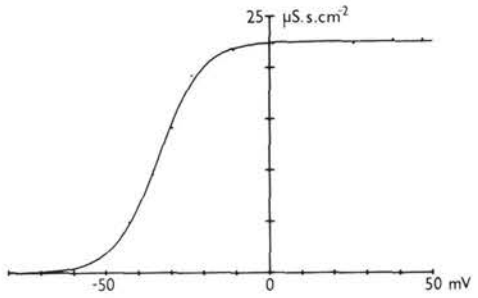

Fig. 2. Experimental points represent $g_{\mathrm{Na\,max}}(V)e/\alpha(V)$, where $g_{\mathrm{Na\,max}}(V)$ are obtained from Hodgkin's and Huxley's (1952) Table 2. They represent the product $N(V)\tau(V)\gamma$. Data are fitted with the curve $22.56/(1 + \exp(-0.15(V + 34)))$.

Including gating currents into the model

The voltage dependence of permeability of the sodium channel implies that this process begins with the movement of a voltage sensor. This movement of the voltage sensor produces a gating current. The time constant of the gating current is almost by one order faster than the time constant of the movement of supposed control molecules, which is $1/\alpha$ (compare, e.g. Fig. 5, Bezanilla and Armstrong 1975). Therefore, the movement of the control molecules is not identical with the charge movement. It could be supposed that charged parts of control molecules move in the electric field of the membrane and make their active state energetically more favourable. Hence, the gating current precedes the movement of the control molecules of the channel.

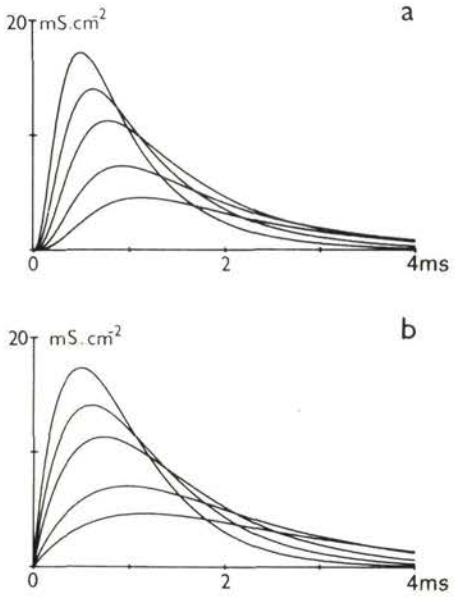

Fig. 3. A comparison of the sodium conductances computed from the Hodgkin and Huxley model (*a*) and from the stochastic model (*b*). The resting potential was −62 mV and depolarizing steps were to 26; 1; −11; −24; −30 mV.

The gating current has an exponential time course as a response to a voltage step change. The amplitude of the gating current is scaled with the steady state distribution of the charge according to the Boltzmann principle. Therefore, the gating current, y_1, fulfills the first order differential equation

$$\frac{dy_1}{dt} = -\beta y_1 \tag{8}$$

with the solution

$$y_1 = C \exp(-\beta t) \tag{9}$$

where $1/\beta$ is the time constant of the gating current.

To include the gating current into the model of the sodium channel we should write the model in the form of a set of differential equations. The function $Ct \exp(-\alpha t)$ is the solution of a system of the first order differential equations with constant coefficients, which has the cannonical form

$$\frac{dy_2}{dt} = -\alpha y_2 \tag{10}$$

$$\frac{dy_3}{dt} = y_2 - \alpha y_3$$

For initial conditions

$$y_2(0) = y_3(0) = 0$$

is

$$y_2 = C \exp(-\alpha t)$$

and

$$y_3 = Ct \exp(-\alpha t)$$

The variable y_2 in the proposed model describes the kinetics of the first moving particles of channels, and y_3 describes the kinetics of channels consisting of two subunits.

When we suppose that a movement of control molecules is preceded by a movement of the charged parts, which is measured as the gating current, one possibility of the complex model is the set of equations

$$\frac{dy_1}{dt} = -\beta y_1 \tag{11}$$

$$\frac{dy_2}{dt} = y_1 - \alpha y_2$$

$$\frac{dy_3}{dt} = y_2 - \alpha y_3$$

where the variables have the same meaning as above. To avoid nonlinearities, the assumption adopted that the rate limiting step of the gating process is movement of control molecules.

The solution of the set of differential equations can be expressed in the explicit form. The solutions for y_1 and y_3 can be checked experimentally. They are

$$y_1 = C \exp(-\beta t) \tag{12}$$

$$y_3 = \frac{C}{\beta - \alpha}(t \exp(-\alpha t) - \frac{1}{\beta - \alpha}(\exp(-\alpha t) - \exp(-\beta t))) \tag{13}$$

The distribution of charged parts of control molecules in the electric field of the membrane fulfills the Boltzmann principle. The number of channels which will pass through the open state is equal to the number of channels where both charged parts have moved. Therefore the steady state distribution of the channels passing to the open state is the square of the steady state charge distribution. This can be estimated from the gating current data presented, e.g. by Bezanilla and Armstrong (1975), and expressed as

$$\frac{1}{1+\exp(-0.04\ V)} \tag{14}$$

When N_0 is the total number of channels and $N(V)$ is the number of channels which are open during a long voltage step V, then, based on our assumptions

$$N(V)=\frac{N_0}{(1+\exp(-0.04\ V))^2} \tag{15}$$

and

$$g_{Na}=N(V)\gamma\tau(V)\frac{\alpha^2\beta}{\beta-\alpha}\left(t\exp(-\alpha t)-\frac{1}{\beta-\alpha}(\exp(-\alpha t)-\exp(-\beta t))\right) \tag{16}$$

The term $\alpha^2\beta$ is derived from the fact that the total number of open channels is $N(V)$. We should suppose that not only N but also τ is voltage dependent. To find parameters of this model is also very easy when we know the voltage dependence of $\beta(V)$ from gating current measurements. From the time to peak of the sodium conductivity $t_{max}(V)$ by Newton's iterative method we have

$$\alpha_{i+1}=\alpha_i+(\exp(-\alpha_i t_{max})\left(1-\alpha_i t_{max}+\frac{\alpha_i}{\beta-\alpha_i}\right)-$$

$$-\frac{\beta\exp(-\beta t_{max})}{\beta-\alpha_i}\Big)\Big/(\exp(-\alpha_i t_{max})\left(-2t_{max}+\alpha_i t_{max}^2-\right. \tag{17}$$

$$\left.-\frac{\alpha_i t_{max}}{(\beta-\alpha_i)}+\frac{\beta}{(\beta-\alpha_i)^2}\right)-\frac{\beta\exp(-\beta t_{max})}{(\beta-\alpha_i)^2}\Big)$$

with $\alpha_0=1/t_{max}$

The value α with a good accuracy we will get in less than 5 steps. These values are plotted in Fig. 4 and values

$$N(V)\gamma\tau(V)=g_{Na\,max}/y_3(t_{max}) \tag{18}$$

are plotted in Fig. 5. Because we have already set the voltage dependence of $N(V)$, we can find $\tau(V)$ supposing γ constant. These values are shown in Fig. 6 and they can be fitted by the curve

$$\tau(V)=0.045+\frac{0.9}{\cosh(0.065(V+31))} \tag{19}$$

which is the Eyring rate equation for passage across a single central energy barrier (Keynes and Rojas 1976).

The sodium conductance computed from the model with the included charge movement is presented in Fig. 7. There is a qualitative change on the rising phase, the delay, which better mimics the experimental data.

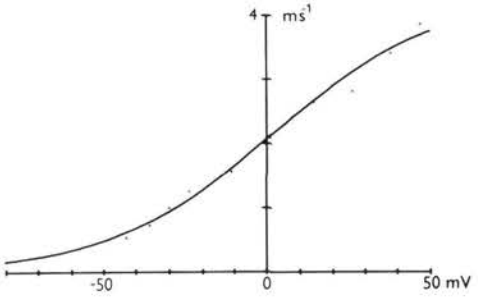

Fig. 4. A voltage dependence of α for the model with gating currents. The time constant $1/\beta$ of the gating current is supposed to be constant with the value $\beta = 10$. Data are fitted with the curve $\alpha(V) = 4.3/(1 + \exp(-0.04(V - 2)))$.

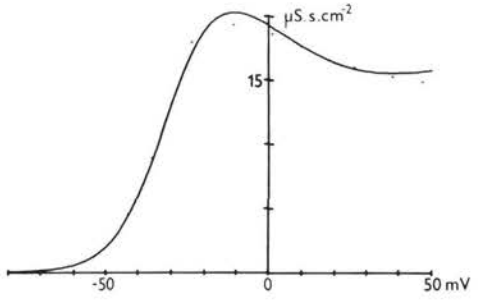

Fig. 5. The values $N(V)\tau(V)\gamma$ for the model with gating currents plotted versus voltage. The solid curve was drawn under assumptions that N is distributed as the square of the charge distribution; $\gamma = 10$ pS, and $\tau(V)$ is fitted as in Fig. 6 (see text).

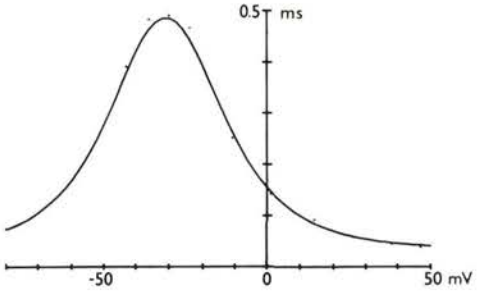

Fig. 6. Voltage dependence of τ for the model with gating currents. The solid curve is $\tau(V) = 0.045 + 0.9/\cosh(0.065(V + 31))$.

Discussion

There are generally two aspects of a mathematical model. The first is, how successfully can a model describe all observable phenomena with minimum of parameters; the other one is, whether the model reflects in some approximation the real mechanism of the process modelled. Let us consider both questions.

The proposed model describes the basic phenomena of the sodium conductance. It is easy to find parameters from experimental data due to the statistical nature of the model. For the simpler version, the intensity of conformational jumps of control molecules, $\alpha(V)$, is evaluated as the reciprocal time to peak of the sodium conductance and approximated from eq. (6). This is the only voltage dependent parameter of the gamma distribution, which describes the time course of the sodium conductance. This is scaled with the product of the conductance of one channel, the average channel open time and the number of channels passing open state. The number of channels, N, passing to open state is voltage dependent, and can be derived from Boltzmann's law. Also, both the average open time, τ, as revealed by patch clamp experiments (e.g. Aldrich et al. 1984) and the channel conductance (Fishman et al. 1984), γ, are voltage dependent. Therefore, we have determined the voltage dependence of the whole product, $N\tau\gamma$, from the attained maxima of the sodium conductance, simply from eq. (5), and approximated by eq. (7). Approximations of α and $N\tau\gamma$ by eqs. (6) and (7) are not essential for the model. They serve only for a concise expression of data and are not further interpreted.

Similarly, for the model with gating currents, $\beta(V) = 1/\tau_g$ is determined from the experiment by the exponential fit, and $\alpha(V)$ is computed from eq. (17). The product $N\tau\gamma$, if there is no further information, is determined as in the simpler model.

The model for the giant axon supposses two transitions of control molecules to open one sodium channel. There is no explicit inactivation process because the decay of the sodium conductance is here a consequence of the finite number of the sodium channels, and because their conducting state is temporary.

The essential feature is that the model describes the distribution of the states of the channel as well, and not only the distribution of control molecules as in the case of the Hodgkin-Huxley model.

In the present paper we have dealt only with the response of the channel to the long depolarizing voltage step. The channel passes from the resting state through the open to the inactivated state. To complete the model it is necessary to add the return to the resting state. The most simple possibility is schematically drawn in Fig. 8. The upper row of the diagram shows processes at the resting membrane potential, and the lower one those in the depolarized membrane. With the exception of the transition to the open state of the channel, which has the gamma

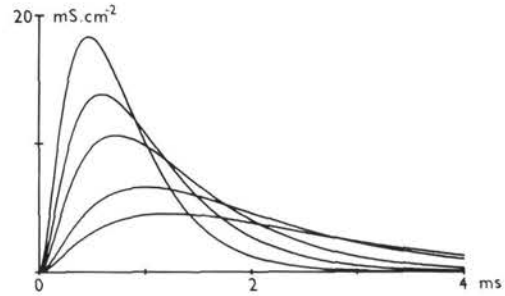

Fig. 7. The sodium conductances were computed from the model with gating currents for long depolarizing voltage steps. The resting potential was −62 mV and steps were to 26; 1; −11; −24 and −30 mV.

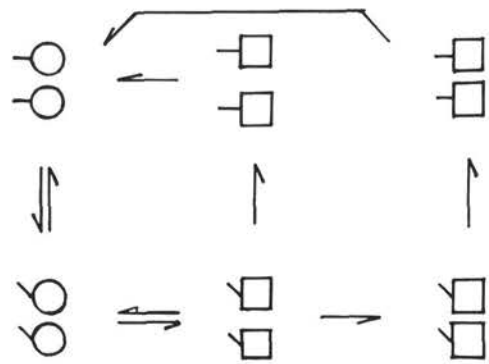

Fig. 8. The proposed model. The upper row represents processes at the resting membrane potential and the lower one those at more positive voltages (depolarized). Circles represent control molecules at the rest and squares indicate activated control molecules. The tail represents the voltage sensing part, which is bent under depolarization and allows the control molecule to reach the active state. Two control molecules in the active state form the temporary open channel. The open channel proceeds to the inactivated state. This pathway is not reversible and when the membrane is repolarized, the system can attain the resting state along a different way.

distribution, all the others are simple exponential processes. This is because the rate limiting step is the conformation of the control molecule and because the channel is in the open state only if both control molecules are activated. An experiment can be designed to determine voltage dependence of all rate constants separately.

The model allows for future modification to provide a more detailed description of the channel mechanisms. For instance, due to mutual influence of control molecules within one channel, the time constants of their movement can differ from each other. This would change the relation of the ascending and the descending phases of the conductance, and different time constants could better describe the real situation.

The question, whether the model reflects in some approximation the real mechanism of the process modelled is more complicated. The model may not be unique and agreement between the model and data does not prove that the model actually reflects underlying molecular mechanisms. However, there are several experimental results which are in favour of the proposed hypothesis, expressed by the model. Angelides and Nutter (1984) have found from resonance energy-transfer measurements that at least two TTX receptors are closely arranged. This can be interpreted so that each control molecule has a TTX receptor; this is in accordance with the number of control molecules in one channel of the proposed model. The concurent possibility is aggregation of the channels. This would implicate some form of cooperativity for which there are no convincing experimental data (Neumcke and Stämpfli 1983). The assumption of the model that the channel opens only once per depolarization epoch, then it closes to an inactivated state and does not reopen, was experimentally observed in patch clamp experiments by Aldrich et al. (1984).

The Hodgkin and Huxley model describes very well the basic properties of the sodium channel. However, there are some experimental results which cannot be explained with the simple Hodgkin-Huxley kinetics. These findings concern mainly the channel inactivation. They are, e.g. listed by Goldman (1976), Meves (1978). Goldman (1976) also showed that only a multistate model can explain the observed phenomena. The proposed model represents such a description of the channel kinetics.

Simple experimental situations were described only. To further validate the model, it is necessary to check all other available data and also to draw conclusions for subsequent testing. This is left for further investigations.

References

Aldrich R. W., Corey D. P., Stevens C. F. (1984): A reinterpretation of mammalian sodium channel gating based on single channel recording. Nature **306**, 436—441

Almers W. (1978): Gating currents and charge movements in excitable membranes. Rev. Physiol. Biochem. Pharmacol. **82**, 96—190

Angelides K. J., Nutter T. J. (1984): Molecular and cellular mapping of the voltage-dependent Na^+ channel. Biophys. J. **45**, 31—34

Armstrong C. M., Bezanilla F. (1973): Currents related to movement of the gating particles of the sodium channels. Nature **242**, 459—461

Bell J., Cook L. P. (1979): A model of the nerve action potential. Math. Biosci. **46**, 11—36

Bezanilla F., Armstrong C. M. (1975): Kinetic properties and inactivation of the gating currents of sodium channels in squid axon. Phil. Trans. Roy. Soc. London B. **270**, 449—458

Fishman H. M., Leuchtag H. R., Poussart D. (1984): Nonlinear single-channel sodium conductance in squid axon. Biophys. J. **45**, 46—49

Goldman L. (1976): Kinetics of channel gating in excitable membranes. Quart. Rev. Biophys. **9**, 491—526

Hodgkin A. L., Huxley A. F. (1952): A quantitative description of membrane current and its application to conduction and excitation in nerve. J. Physiol. (London) **117**, 500—544

Hoyt R. C. (1984): A model of the sodium channel. Biophys. J. **45**, 55—57

Jakobsson E. (1976): An assessment of a coupled three-state kinetic model for sodium conductance changes. Biophys. J. **16**, 291—301

Keynes R. D., Rojas E. (1976): The temporal and steady state relationships between activation of the sodium conductance and movement of the gating particles in the squid giant axon. J. Physiol. (London) **225**, 157—189

Meves H. (1978): Inactivation of the sodium permeability in squid giant nerve fibres. Progr. Biophys. Mol. Biol. **33**, 207—230

Neumcke B., Stämpfli R. (1983): Alteration of the conductance of Na^+ channels in the nodal membrane of frog nerve by holding potential and tetrodotoxin. Biochim. Biophys. Acta **727**, 177—184

Rao C. R. (1965): Linear Statistical Inference and its Applications. John Wiley, New York—London—Sydney

Received April 30, 1985/Accepted May 23, 1985