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Molecular events and energy changes during the action potential

(nerve thermodynamics/membrane structural changes/dissipation function/initial heat of activity)

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ABSTRACT **A novel interpretation of the existing data concerning the energy changes associated with nerve impulse propagation is proposed. The main conclusion is that the negative phase of the initial heat of activity cannot be accounted for without recourse to conformational changes in membrane proteins. It stems from analyzing and computing the energy changes associated with ionic flows, capacitive currents, and structural changes in membrane gateways. A close quantitative agreement with microcalorimetric measurements was achieved.**

Since the now classical measurements of heat production in nerve by A. V. Hill, it is a well-established fact that the energy changes associated with nerve activity can be divided into two phases: (*i*) an initial heat of activity, and (*ii*) heat production associated with the recovery processes.

The initial heat of activity expresses the external dissipation of free internal energy due to the specific processes occurring in the nerve fiber during the action potential (*AP*) (1, 2). In the apparent order of their appearance, these processes are:

(*i*) structural changes in the membrane as a result of the biochemical events in the membrane proteins induced by the changes in the electrical field;

(*ii*) redistribution of electric charges in the membrane, phenomenologically termed the capacitive current (I_c) which expresses at the microscopic (molecular) level both the redistribution of electrons on the membrane lipids (the "dielectric" of the membrane capacity) and the intramembranous currents associated with the actuation of the gateways; and

(*iii*) ionic flows through specific channels (gateways), obviously implying ionic interchanges between the axoplasm and the interstitial fluid.

Besides these three types of events there is a reversible ionic exchange of Ca^{2+} for K^+ between the axoplasm and the membrane, extensively discussed by Tasaki (3). These Ca^{2+} movements have been discussed by others (4) in terms of early and late Ca^{2+} currents. The contribution of these events to the thermal changes appears to be of the order of a few percent (see below).

Owing to the authoritative domination of the ionic theory, only processes *ii* and *iii* have been considered, under various names such as condenser theory, local circuit heat, and ionic mixing and interchange (5–7). The energetics of structural changes occurring in the membrane itself have been practically neglected. On the basis of a rough approximation, Ritchie (6) rejected acetylcholene (AcCh) hydrolysis as an explanation for the residual (net) heat, but this cannot restrain further investigations of molecular correlates of the initial heat of activity. An attempt by Wei (8) to ascribe the heat production and absorption in the nerve axon to the displacement of dipoles in the electric field would imply the association of all the energy changes solely with the intramembranous currents, which seems to be unrealistic. Indeed, the agreement he showed with calorimetric data relies on an arbitrary choice of dipole characteristics. Coster (9) recently considered the electromechanical stresses in the membrane under the influence of the changing electric field. Without discarding the possibility of approaching the problem on these lines, we can nevertheless observe that none of the "uniformistic" or "continuum" approaches such as Peltier effect, electromechanical stresses, and even the flip-flop of a continuous population of dipoles comply with the image of microscopical diversity of the cell membrane that has emerged from recent studies (10).

The classification of the processes occurring in the active axon as presented above could have a structural relationship if one assumes that the items in *i* (above) are related to specific proteins in the membrane, those in *ii* are mainly (but not exclusively) dependent on the membrane lipids, and those in *iii* express the changes in the ionic compartments and the frictional interaction of the ions with the membrane.

Although our purpose is to focus on the transitions in the membrane proteins, for the sake of completeness we shall also consider the other contributions to the initial heat of activity. The usual symbols used are listed in the Abbreviations footnote. All the computations were made with the same values of the parameters characterizing the squid giant axon as previously used (11). Throughout this paper we constantly refer to an action potential elicited by a 20 mV depolarization.

Energy dissipation due to ionic flows during the action potential

Among several possible terminologies, we choose that of *energy dissipation*, bearing in mind that, during all the real processes, part of the free energy of the system is degraded into heat. The intensity of energy dissipation is expressed by Rayleigh's dissipation function Φ—i.e., the sum of the products of the flows times their driving force (12). The contribution of the ionic flows to the initial heat of activity of the nerve is given by the dissipation of energy with respect to the resting state:

$$\Phi_{\text{ionic}} = \sum_i \{J_i \cdot \Delta\mu_i - (J_i \cdot \Delta\mu_i)_r\}$$

$$(i = \text{Na; K; } L).$$

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Abbreviations: *AP*, action potential; AcCh, acetylcholine; imp, impulse; t, time; E, real value of the membrane potential; V, membrane potential normalized so that its resting value, V_r, is zero (in fact, $V_r = 0.01$ mV); V_i (that is, V_{Na}, V_K, and V_L) are Nernst equilibrium potentials at which the ionic currents of Na, K, and leakage are zero; I_i (that is, I_{Na}, I_K, and I_L) are the components of ionic currents per membrane unit area; g_i (that is, g_{Na}, g_K, and g_L) are membrane partial conductances for each type of ion; C, membrane capacity per unit area; I_c, the capacitive current per unit area; Z_i, the valency of the ions; F, Faraday's number (96,500 C/mol).

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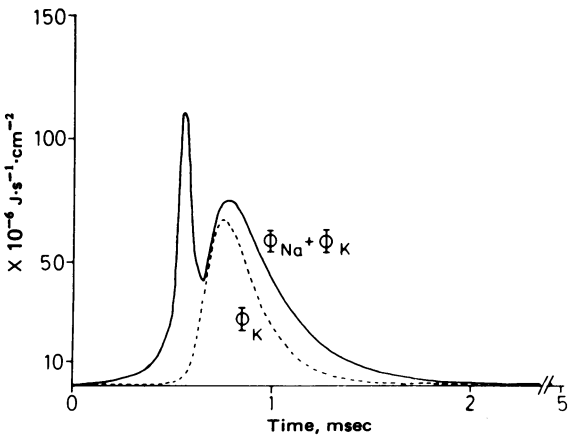

FIG. 1. Energy dissipation due to the potassium flow (Φ_K) and to both sodium and potassium flows ($\Phi_{Na} + \Phi_K$) during an *AP* in the giant axon of the squid

J_i stands for the ionic flows; $\Delta\mu$ are the corresponding driving forces (i.e., the difference between the electrochemical potentials of the ionic species in the axoplasm and in the external solution); and the subscript r denotes the resting state.

After a few simple calculations, the gradients of electrochemical potential can be formed as follows:

$$\Delta\mu_i = z_i F(V - V_i).$$

Taking into account that:

$$z_i F J_i = I_i = g_i(V - V_i)$$

we obtain:

$$\Phi_{\text{ionic}} = \sum_i \{g_i(V - V_i)^2 - g_{ir}(V_r - V_i)^2\}.$$

With this last equation, the values of Φ_{ionic} during the *AP* can be readily computed because V_i and g_{ir} are known constants and the time dependences of g_i and V were already derived according to the phenomonology of Hodgkin and Huxley (11). The results of this computation are represented in Fig. 1 where, in addition to the Φ_{ionic} values, the contribution of the potassium current is also represented. As it appears from Fig. 1, Φ_{ionic} is always positive; this expresses the fact that, during the *AP*, both Na^+ and K^+ flow down the electrochemical gradients.

The overall energy dissipation by ionic flows during the nerve impulse is $\Delta Q_{\text{ionic}} = \int_0^\infty \Phi_{\text{ionic}}\, dt$, which in our case is 49.64×10^{-9} J·cm^{-2}·imp^{-1}. Taking the known specific membrane area of the giant axon as 80 cm^2·g^{-1}, this gives a heat production of 0.95 μcal·g^{-1}·imp^{-1}.

The liberation of Ca^{2+} from its binding protein during the depolarization and the subsequent rebinding during repolarization has found strong experimental support in recent observations with the AcCh receptor (13, 14). It could most probably lead to an evolution and then a reabsorption of heat as proposed by Tasaki (3). But the contribution of this phenomenon to the overall heat changes appears to be negligibly small. If one takes the value quoted by Tasaki, 2.7 kcal·mol^{-1}, for the heat evolved when K^+ replaces Ca^{2+} on the fixed negative charges of the membrane and the inflow of Ca^{2+} as 0.006 pmol·cm^{-2}·imp^{-1} (15) in the squid giant axon whose mean diameter is 500 μm, one obtains 13×10^{-3} μcal·g^{-1}, a value 2 orders of magnitude smaller than not only the value calculated above but also the capacitive and membrane heats (see below).

Energy changes due to capacitive currents

The variations in charge separation on the two sides of the

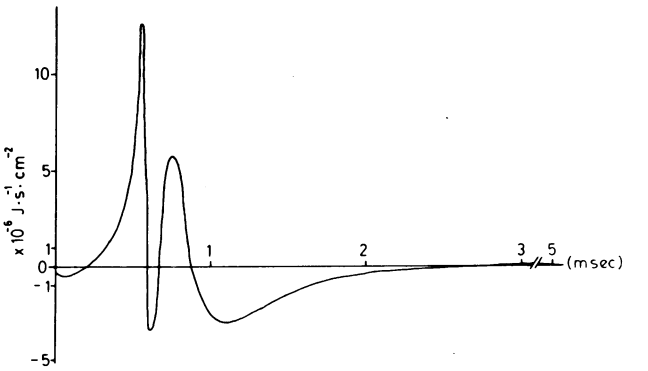

FIG. 2. Energy changes associated with the discharging and recharging of an axonal membrane capacitor during an *AP*.

axolemma during the *AP* lead to a power dissipation:

$$\Phi_{\text{cap}} = I_c \cdot E = -C \cdot E \cdot \frac{\partial V}{\partial t}.$$

The − sign accounts for the fact that a decrease in transmembrane potential corresponds to a positive (i.e., evolved) heat.

The computed values of Φ_{cap} are represented in Fig. 2. The sign alternations are due to the fact that the overshoot of the transmembrane potential above zero and its return to the resting value of −60 mV are uphill processes. In computing Φ_{cap}, the membrane capacity C was considered as not changing during the *AP* (16). Values up to 50% increase have been reported, however (17). Even if we take into account such a change, this does not modify appreciably the global heat balance. As V returns to its resting value after the passage of the impulse, the integral of Φ_{cap} over the whole duration of *AP* is zero, but it consists of a heat evolution of 4.12×10^{-2} μcal·g^{-1} in the first 1 msec, followed by an equal heat reabsorption. Nevertheless, it should be observed that capacitive heat is an order of magnitude smaller than the ionic heat production which is solely positive. Owing to this fact, the consideration of only these two types of phenomena cannot be expected to lead to even qualitative understanding of the initial heat of activity.

Energy changes within the active membrane

After failing to explain the initial heat of activity by the condenser or the ionic mixing theory, Howarth *et al.* (18) proposed the existence of a certain kind of "membrane heat" related to structural changes in the membrane during depolarization and repolarization, but these remained unidentified. As Abbott and Howarth (5) later admitted "this is only a convenient way of thinking . . . without relating the heat to some measurable entity."

In terms of our molecular model of action potential (11), it is quite natural to equate the membrane heat to the enthalpy variation associated with transitions undergone by the Na and K gateways during the impulse. Each membrane protomer involved in these transitions passes back and forth over an energy barrier whose detailed profile could be established if the energetics of each reaction step were known. Until such knowledge is available, only a global image of the transition can be considered. We assume that the resting state corresponds to a higher free energy level than the active one. The fact that a spontaneous transition toward the active state, thus implying a self-excitation of the axon, does not occur already suggests that a higher energy transition state (i.e., an activated complex) must be reached. The activation energy for passing from the resting to the transition state is provided by the association of AcCh

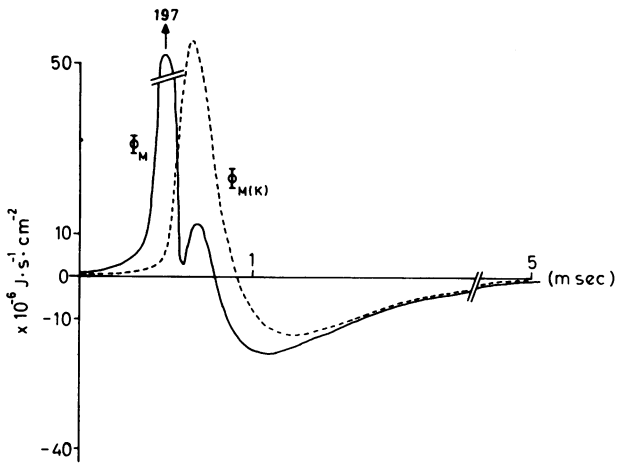

FIG. 3. Energy changes associated with the transitions of K gateways in the membrane [$\Phi_{M(K)}$] and to both Na and K gateways (Φ_M) during an *AP*.

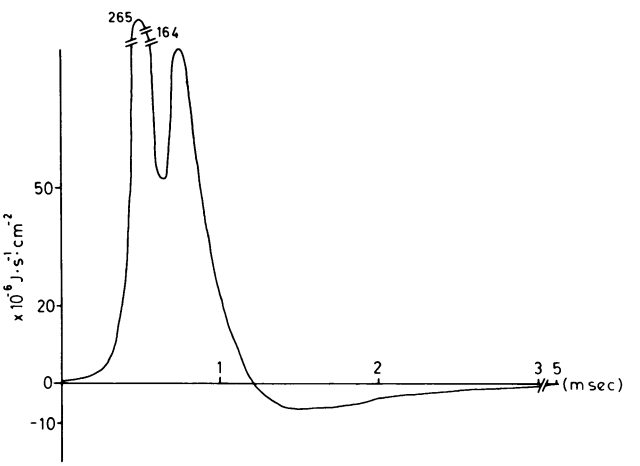

FIG. 4. Overall computed energy changes in squid giant axon during an *AP*.

with the receptor and the resulting effect on Na gateways (11).

Owing to the fact that the spread of excitation implies, as a first event, the transconformation of the Na gateways, the conduction velocity of the nerve impulse should be proportional to the rate of attainment of the transition state. It then follows that an arrhenius analysis of the temperature dependence of conduction velocity will give the activation enthalpy for the passage from the resting to the transition state. For the squid giant axon with the data in ref. 19, an apparent activation enthalpy of 4.5 kcal·mol^{-1} is obtained. As for the difference between the transition and active states, it can be, and actually was, obtained by applying the activated complex theory to the kinetics of conductance changes. The calculations lead to values around 15 kcal·mol^{-1} (20, 21). It then appears quite tenable to accept that the net enthalpy variation of ionophores passing from the closed to the open state is $\Delta H \simeq 10$ kcal·mol^{-1}.

This value means that each protomer undergoing the transition will release (and then absorb) an energy:

$$q = \frac{\Delta H}{N_A} = 1.66 \times 10^{-20} \text{ cal}$$

or

$$6.94 \times 10^{-20} \text{ J}$$

in which N_A is Avogadro's number. In keeping with our model (11), in which a cooperativity of four AcCh molecules for the Na gateway and of four Ca^{2+} for the K gateway was assumed, we make the critical hypothesis that each Na gateway is an oligomeric structure formed by four protomers undergoing the transition. The same holds true for the K channels. Therefore, the instantaneous energy changes are:

$$\Phi_M = 4q \frac{\partial N_{Na}}{\partial t} + \frac{\partial N_K}{\partial t}$$

in which $\partial N_{Na}/\partial t$ and $\partial N_K/\partial t$ are the numbers of Na and K channels undergoing the transition per unit time. Because the ionic conductances are at all times proportional to the number of open channels, it follows that:

$$\frac{\partial N_{Na}}{\partial t} = \frac{(N_{Na})_{total}}{(g_{Na})_{max}} \cdot \frac{\partial g_{Na}}{\partial t}.$$

Here $(N_{Na})_{total}$ is the whole number of Na channels per unit of surface area and $(g_{Na})_{max}$ is the maximum value of Na conductance attained during the impulse. An analogous expression holds for the K channels.

There is no direct estimate of the number of K channels but it appears to be roughly the same as for Na, which is 483 μm^{-2} (22).

In the case of the squid giant axon, for which we have already computed the ionic and capacitive energy dissipations, the membrane contribution to these changes is:

$$\Phi_M = \left\{0.680 \frac{\partial g_{Na}}{\partial t} + 1.546 \frac{\partial g_K}{\partial t}\right\} \times 10^{-6} \text{ J} \cdot \text{s}^{-1} \cdot \text{cm}^{-2}.$$

The time course of Φ_M as well as the energy dissipation of the K gateway during the *AP* are represented in Fig. 3. By integrating the values of Φ_M over the time of an impulse, one finds that before the end of the first msec there is an outburst of 0.026 μJ·cm^{-2}, or $\simeq 0.5$ μcal·g^{-1}, followed by an equal reabsorption of heat during the closing of the channels in the nxt 2 msec.

Energy changes during the *AP* in other nerve fibers

The above calculations provide a complete image of energy changes during an *AP* in the giant axon of the squid. All the results were obtained by carrying further the computer simulations of the kinetic equations describing our molecular model. We are now able to predict the heat changes associated with the propagation of an impulse in the squid giant axon. They are represented in Fig. 4 as the sum of Φ_{ionic}, Φ_{cap}, and Φ_M. Notice that the rapidity of these thermal changes, together with their small values, makes it difficult to obtain direct microcalorimetric measurements. Such measurements exist only for smaller nonmyelinated fibers such as those from the nerve of the walking leg of the crab *Maia squinado* and from rabbit vagus nerve.

In these cases the contributions of capacitive and mostly of what we termed membrane heat become dominant. With the same values for the membrane capacity (1 μF/cm^2) and for the enthalpy change per membrane protomer (6.94×10^{-20} J) and with the number of Na gateways as 50 μm^{-2} and 75 μm^{-2} for crab and rabbit vagus nerves (23) whose specific membrane surface areas are 5×10^3 cm^2·g^{-1} and 6×10^3 cm^2·g^{-1}, the integrated energy changes per impulse are listed in Table 1. The values for squid giant axon are also included for comparison.

Discussion and conclusions

The main feature of our analysis may be summarized by saying that we do not ascribe all of the initial heat of activity to only one phenomenon, as most often was tried, but we approach all

Table 1. Energy changes (μcal·g^{-1}·imp^{-1}) in conducting membranes: Comparison between calculated and experimental values

	Ionic heat	Capacitive heat		Membrane heat		Observed*	
	+	+	−	+	−	+	−
Squid giant axon	0.95	0.04		0.5		—	—
M. squinado nerve	5.7†	2.7		3.3		14	12
Rabbit vagus nerve	9.5†	3.15		6.0		24.5	22.2

+, Heat production; −, heat absorption.
* From (3, 14). As pointed out by the authors, these values may be overestimated.
† Estimated from number of ionophores and specific surface area.

the obvious energy changes during the impulse. It is possible that there are still other processes not considered here, but we think that the main contributions are those we computed. As for the structural changes within the membrane, instead of ascribing them to the dielectric of membrane capacitor, we assume that they are related to the conformational transitions of the Na and K gateways—that is, to membrane proteins.

A good quantitative agreement between the computed heat changes and the microcalorimetric measurements appears when comparing the experimental data with the calculated values (Table 1). Apart from this general agreement, the main experimental observations are described and explained in our treatment. For instance, the close association of the evolution of heat with the rising phase of the *AP* and absorption of heat with the falling phase clearly appear from Fig. 4, showing that all the positive heat is restricted to the first 1 msec of the *AP*. Because the values plotted in Fig. 4 are for the giant axons, the relative weight of membrane and capacitive heats (which provide the negative heat contributions) is lower ($\simeq$36%) but this is markedly increased for smaller fibers as shown in Table 1.

The most distinctive feature of our treatment is the proposal that, together with the recharging of the membrane capacitor, the closing of the ionic channels explains the heat absorbtion. In order to return from the conducting to the poorly conducting conformation, the gateways cannot use the thermal energy previously delivered, as so often stated.

The increase in thermal energy produced by the positive phase of the initial heat is given by $\Delta E = k\Delta T$ in which k is the Boltzmann constant and ΔT is of the order of 10^{-6} K (18). Therefore, $\Delta E \simeq 10^{-28}$ J—i.e., many orders of magnitude lower than the quanta necessary for inducing the transconformation of one protomer, which is 6.94 10^{-20} J as calculated above. Until there is a precise molecular interpretation of how the energy arising from the ionic gradients could be transduced into a conformation change, we prefer to disregard such a nonoperational interpretation.

As proposed previously (11), we assume that the release of AcCh from the Na gateways is mainly due to a displacement of the equilibrium as a result of a change in the affinity of the receptor that could result from the change in electric field or from an allosteric effect of Ca^{2+} and/or K^+. We know that Ca^{2+} prevents the binding of AcCh to its receptor (13, 14).

Although AcCh hydrolysis in itself is an endothermic process (−0.3 kcal·mol^{-1}), the buffering action of the medium *in vivo*, due presumably to imidazole and amino groups on protein side chains, has protonation heats of 7 to 12 kcal·mol^{-1} (24). We thus assume that AcCh hydrolysis is delayed and rather explains part of the recovery heat. This should amount to a heat production of 0.25, 1.60, and 2.706μcal·g^{-1}·imp^{-1} for squid axon, crab nerve, and rabbit vagus nerve, respectively, if one takes into consideration the values given above for the number of Na ionophores and the specific membrane surface areas.

In our proposal, the closing of the ionic channels explains part of the heat reabsorption. It must therefore be accompanied by an increase in the entropy of the system. Accordingly, the entropy of the conducting state should be lower than that in the resting state, a conclusion in agreement with the optical measurements (25).

Finally, our treatment of *AP* energetics suggests experiments of pharmacological dissection of the initial heat of activity. For instance, one would expect that tetrodotoxin, which blocks Na^+ currents, will eliminate the contribution of Na^+ to Φ_{ionic} so that in Fig. 1 only the dotted curve will obtain. Similarly, an agent blocking the transition of the Na gateways will lead to the replacement of the solid curve in Fig. 3 by the dotted line and to the corresponding modification of the overall curve of energy dissipation.

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