IONIC MECHANISMS OF AUTORHYTHMIC ACTIVITY (INVESTIGATIONS ON MATHEMATICAL MODELS OF EXCITABLE MEMBRANES)

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This investigation was carried out on mathematical models of membranes of the giant axon and node of Ranvier of the frog. Autorhythmic activity appears only when the following relationship exists between parameters of ionic permeability: 1) the entering ionic current at rest begins to exceed the departing current by an amount sufficient to produce the development of regenerative depolarization of the membrane; and 2) during development of the spike the departing current (potassium current, leak current) increases to values sufficient to produce full repolarization (or hyperpolarization) of the membrane. The first condition can be satisfied in the giant axon by increasing sodium conduction (g_{Na}) and also by decreasing potassium conduction (g_K) during the resting potential. An excess of sodium permeability (P_{Na}) is an essential condition in the node of Ranvier. The spike frequency depends both on the degree of initial change in ionic permeabilities (conductances) of the membrane and (to a greater degree) on the time constant of potassium permeability (τ_n). This is because the gradient of increase of interspike depolarization (the "pacemaker potential") is highly dependent on the rate of decrease of g_K (or P_K) after the end of the action potential. If the excess of g_{Na} is very great or the decrease in g_K is too severe, the rhythmic discharge becomes decremental in character, for in both cases the process of membrane repolarization, necessary to abolish the inactivation, is incomplete.

Analysis of the ionic mechanisms of autorhythmic activity is an important problem in modern physiology and medicine. Unfortunately, in the experimental study of this problem on living objects considerable difficulties are encountered, because existing methods of investigation cannot be used to record changes in ionic permeabilities and currents actually during the generation of action potentials. This can be done only on mathematical models of excitable membranes constructed from the results of experiments in which the potential was recorded, and thus providing good reproduction of all the principal phenomena of bioelectrical activity of the corresponding structures [2, 15].

The main results obtained by a study of the conditions and mechanisms of generation of autorhythmic activity on models of the giant axon of the squid [9] and node of Ranvier of amphibians [7] are described briefly in this paper.

EXPERIMENTAL

Systems of equations suggested by Hodgkin and Huxley [9] and Frankenhaeuser and Huxley [7] were used. Integration was carried out by the Runge-Kutta method. The M-220 computer performed the calculations. Of the various possible changes in parameters of ionic permeability leading to generation of the

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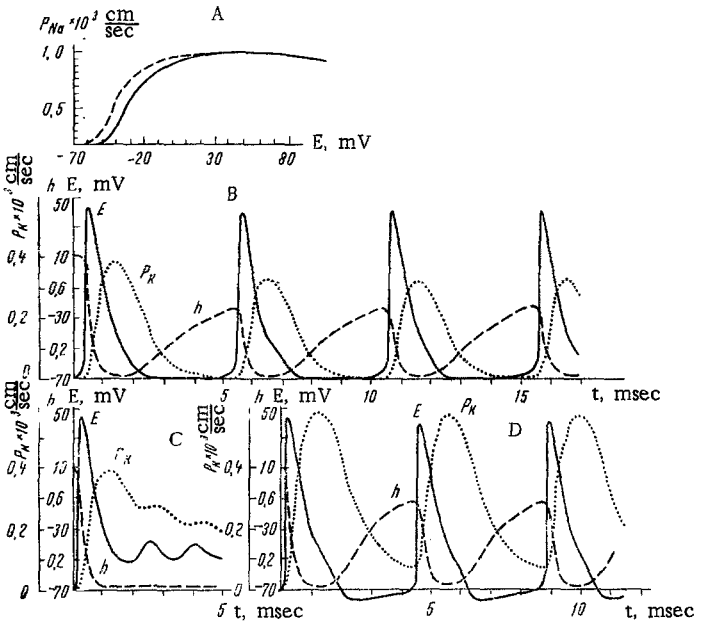

Fig. 1. Autorhythmic activity of model of membrane of node of Ranvier due to increase in sodium permeability, P_{Na}. A) Dependence of P_{Na} on fixed value of potential on inner side of membrane, E (continuous line), and of change in potential (broken line) leading to increase in P_{Na} during resting potential (E = −70 mV); B) autorhythmic activity during displacement of sodium characteristic (curve P_{Na} against E) by 12 mV. Displacement due to corresponding changes in constant B in equations (12, 13) taken from Frankenhaeuser and Huxley [7]. Changes in E, in potassium permeability (P_K), and variable inactivation (h) are shown; C) extinction of discharge after shift of characteristic curve by 14 mV; D) recovery of rhythmic activity with simultaneous increase of P_{Na} and P_K (displacement of sodium and potassium characteristic curves to be left through 15 mV).

nerve impulse [1], only those were chosen which had been observed by different workers under experimental conditions.

EXPERIMENTAL RESULTS

In the resting state, ionic currents flowing through a membrane (from the cell and into it) balance each other so that their albegraic sum is equal to zero. Calculations have shown that if this equilibrium is disturbed in any way in favor of the ingoing sodium current (I_{Na}), the membrane is converted from a stable resting state into a state of autorhythmic activity.

In investigations on a model of the node of Ranvier, an excess of the ingoing current over the outgoing was obtained by increasing the sodium permeability of the membrane (P_{Na}).

The curve of the peak value of P_{Na} of a function of the potential on the inner side of the membrane (E) is shown in Fig. 1A. Subsequently, for brevity, this curve will be described as the "sodium characteristic." The effect of displacing this characteristic curve along the voltage axis toward lower values of E by 12 mV (simulation of the effect in the model of a deficiency of Ca^{++} ions in the medium on the sodium canals) is shown in Fig. 1B.

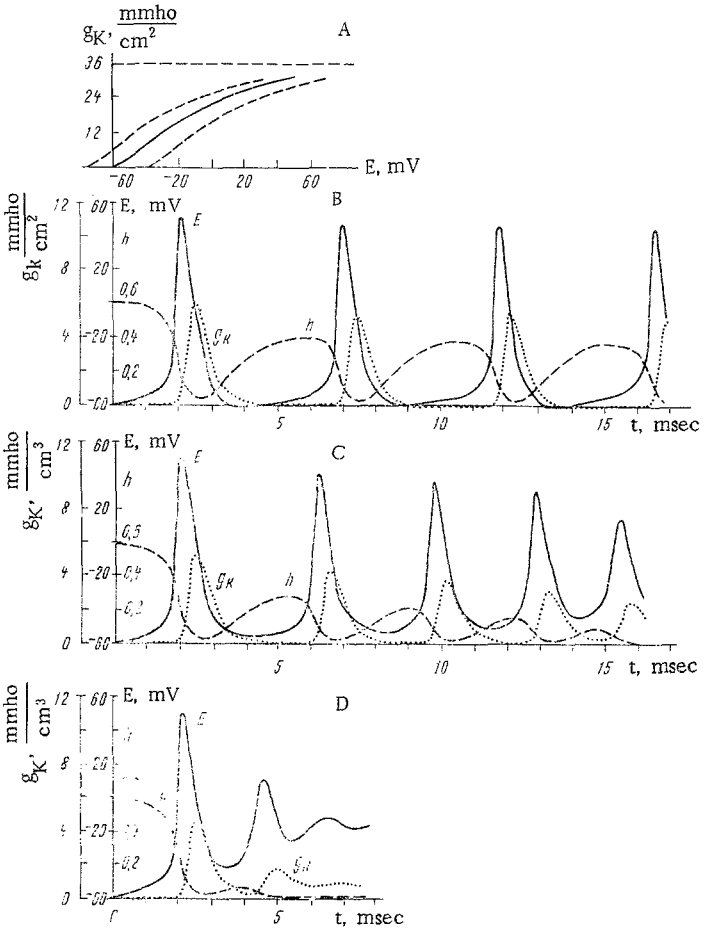

Fig. 2. Autorhythmic activity in models of membrane of squid giant axon during decrease in potassium conduction (g_K). A) Potassium characteristic curve in "normal" membrane (continuous line) and its displacement to the left (increase in g_K) and to the right (decrease in g_K) on account of changes in V in equations (11, 12) given by Hodgkin and Huxley [9]. Resting potential E = −60 mV; B) rhythmic discharge following shift of characteristic curve to right by 30 mV; C) extinction of discharge during shift of characteristic curve by 35 mV; D) the same after shift of characteristic curve by 40 mV.

It can be seen that an increase in P_{Na} at values of E close to the resting potential led to depolarization. This, in turn, caused a further increase in P_{Na} and, consequently, in I_{Na}, thus increasing the depolarization still more, and so on. The final result of this regenerative process was the development of the first action potential (AP). During its development, the almost complete inactivation of P_{Na} took place (decrease in the value of h, characterizing the proportion of sodium canals free from inactivation) and an increase in potassium permeability (P_K). Both these processes contributed toward repolarization of the membrane, creating the conditions for reactivation of the sodium system (an increase in h) and recovery of the low P_K. However, the decrease in P_K with the abolition of inactivation again led to an excess of I_{Na} over I_K and the leak current I_l (in the node of Ranvier, I_l makes a significant contribution to the total outgoing current) and, consequently, to fresh depolarization of the membrane. When this depolarization reached the necessary level, a new AP appeared. Its amplitude, however, was slightly lower than that of the preceding spike, because at the time of generation of the second AP the initial value of h had not yet been restored. After the second AP all these events were repeated and a third AP developed, and so on. The minimal shift of the sodium characteristic curve sufficient to cause the generation of rhythmic activity was about 10.5 mV. With a shift of 10.2 mV only subthreshold depolarization (local response) with a maximum of 1.4 mV took place at time 2.03 msec.

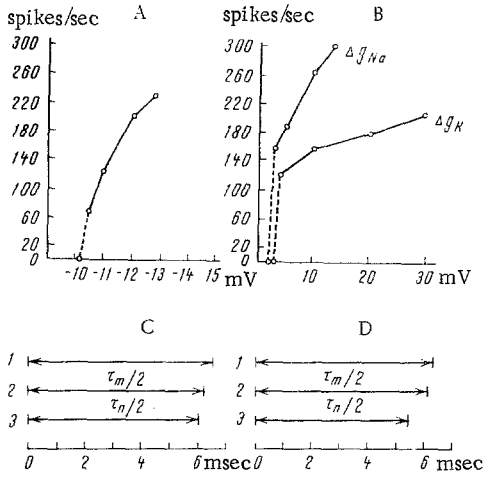

Fig. 3. Dependence of frequency of rhythmic discharge on constants of ionic permeability of membrane. A) Model of node of Ranvier: frequency (ordinate) as a function of degree of increase in P_{Na} because of shift in sodium characteristic curve (in mV; abscissa); B) model of giant axon: frequency (ordinate) as a function of degree of increase in g_{Na} (curve ΔG_{Na}) or decrease in g_K (curve Δg_K) because of a shift of the sodium (to the left) and potassium (to the right) characteristic curves (in mV; abscissa); C) interspike interval during rhythmic activity produced by shift of sodium characteristic curve by 3 mV (1) and its modification by shortening of τ_m(2) and τ_n(3) by half; D) interspike interval in rhythmic discharge due to decrease in potassium permeability (shift of curve of g_K versus E by 10 mV to the right) (1) and its modification by shortening of τ_m (2) and τ_n(3) by half.

The rate of development of prespike depolarization and, consequently, the spike frequency increased with an increase in shift of the sodium characteristic curve only up to a certain limit. Beyond this limit, the rhythmic discharge became decremental in character (Fig. 1C), because with an excessive increase in P_{Na}, the ingoing current at the end of the spike could not be completely compensated for by the outgoing ionic current ($I_K + I_l$). As a result, repolarization of the membrane was incomplete and the next rise of potential began at an excessively low value of h (Fig. 1C).

This extinction of autorhythmic activity could easily be abolished by increasing P_K at the same time as P_{Na} is increased. The effect of a simultaneous shift of the sodium and potassium (P_K versus E) characteristic curve by 15 mV toward an increase in both P_{Na} and P_K during the resting potential is illustrated in Fig. 1D. The increase in P_K and, correspondingly, in the outgoing I_K facilitated recovery of the membrane potential: after the end of the AP, after-hyperpolarization actually developed. As a result, despite the considerable increase in P_{Na}, no extinction of activity took place: by the beginning of the next spike, the value of h had risen to 71.5% of its initial value, quite sufficient for generation of a normal AP.

Similar results were obtained using a model of the squid giant axon. In this case, however, autorhythmic activity was found to be generated in the membrane of this structure not only by a primary increase in sodium conduction (g_{Na}), but also as the result of a decrease in potassium conduction of the membrane (g_K).

The curve of g_K versus E (potassium characteristic curve) and the effect of displacing it through 30 mV toward higher values of E are shown in Fig. 2A, B.

The decrease in g_K resulting from this displacement had the effect that I_{Na} began to exceed I_K during the resting potential. As a result of this, regenerative depolarization began and ended with the generation of the first AP. The subsequent course of events was similar in principle with that occurring during generation of the autorhythmic discharge produced by an increase in sodium conduction. The only difference was that during a primary decrease in g_K the rate of increase of initial depolarization preceeding the first AP was slower and g_K rose to a lower value during development of the spike, so that after-hyperpolarization, which is characteristic of the membrane of the squid giant axon, was weakened or disappeared.

With an increase in the shift of the potassium characteristic curve the duration of interspike intervals was reduced (Fig. 3B), but after shifts of the order of 35-40 mV (Fig. 2C, D) extinction of rhythmic activity similar to that found in the presence of an excessively great increase in the sodium permeability of the membrane was observed (Fig. 1C). The cause of this extinction is clear: an inadequate increase in g_K during development of the AP caused the outgoing ionic current to be too weak to produce normal repolarization of the membrane; on the other hand, the residual depolarization prevented abolition of inactivation (an increase in h). Under these circumstances, the smaller the spike the lower the level of increase of g_K, and the weaker the recovery of h (Fig. 2C).

It was stated above that the spike frequency in the autorhythmic discharge depended on the degree of increase of sodium or decrease of potassium conduction of the membrane (Fig. 3A, B). Meanwhile, the time constants of the sodium and potassium permeabilities have a very significant effect on the duration of interspike intervals. Special analysis showed that, other conditions being equal, a shortening of the time constant

of activation of potassium permeability (τ_n) has a slightly greater effect on the spike frequency than an equal shortening of the time constant of sodium permeability (τ_m). This can easily be understood if it is remembered that the chief cause of interspike depolarization (the "pacemaker potential") is a decrease in g_K (or P_K). Accordingly, the shorter τ_n, the faster the increase in P_K and also its subsequent decrease and, consequently, the shorter the interval before generation of the next AP (Fig. 3C, D).

The frequency of the autorhythmic discharge rose significantly when a weak depolarizing current was applied to the membrane.

Hence, there are at least two essential conditions under which the membrane of nerve fibers can change from the stable resting state into a state of self-maintained "spontaneous" rhythmic activity. The first condition is that during the resting potential the entering ionic current must exceed the leaving current. The importance of this condition was previously stressed by Noble [11, 12] in a theoretical analysis of the autorhythmic activity of the membrane of Purkinje fibers. The present investigation shows that, first, this excess of one over the other must be large enough or otherwise only a local response develops, and second, it can be brought about either by an increase in P_{Na} or by a decrease in P_K during the resting potential. The second condition of automatic activity, which is extremely important, is the appearance of an adequately strong outgoing current during the AP, capable of ensuring full repolarization, or even hyperpolarization of the membrane which is necessary for abolishing inactivation of the sodium canals. Unless this condition is observed, the autorhythmic discharge will be decremental in character.

Under experimental conditions autorhythmic activity arises in nerve and muscle fibers usually during a decrease in the Ca^{++} ion concentration in the medium [6, 7]. The reason for this phenomenon will become clear if it is remembered that a decrease in the extracellular Ca concentration displaces both the sodium and the potassium characteristic curves of the membrane toward an increase in P_{Na} and P_K during the resting potential [4, 6, 8]. However, since this shift is accompanied by an increase in the sodium inactivation of the membrane to such an extent that the entering I_{Na} is weakened [2, 6, 8], rhythmic activity in a medium with reduced Ca concentration does not always arise or is ill-defined.

In single nodes of Ranvier autorhythmic activity could be obtained only when a decrease in the Ca concentration in the extracellular medium was combined with application of tetraethylammonium, which blocks potassium canals [2], to the membrane. The analysis of this phenomenon in model experiments is not yet complete.

The appearance of autorhythmic activity during a decrease in the potassium conduction of the membrane is a very interesting fact (Fig. 2). There is reason to consider that this same mechanism lies at the basis of the effects of Ba^{++} ions, which cause rhythmic activity in myocardial cells [3, 13], neurons of Helix pomatia [10], and smooth-muscle fibers [15]. The potassium canals of the membrane in these cells evidently possess very high sensitivity to the blocking action of Ba^{++}. That these ions do in fact reduce g_K is demonstrated by data showing an increase resistance of the membrane [14] and also its depolarization in a solution containing Ba^{++} ions [3, 14].

It will be clear from this description how extremely important it is to seek agents with a specific action on particular ion canals of different excitable membranes. The successful solution of this problem will really pave the way for the development of methods of controlling the autorhythmic activity of excitable tissues.

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