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Physiology of Excitable Membranes

Editor
J. SALÁNKI

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Physiology of Excitable Membranes

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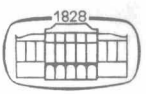

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FOREWORD

This volume is one of the series published by Akadémiai Kiadó, the Publishing House of the Hungarian Academy of Sciences in coedition with Pergamon Press, containing the proceedings of the symposia of the 28th International Congress of Physiology held in Budapest between 13 and 19 July, 1980. In view of the diversity of the material and the "taxonomic" difficulties encountered whenever an attempt is made to put the various subdisciplines and major themes of modern physiology into the semblance of some systematic order, the organizers of the Congress had to settle for 14 sections and for 127 symposia, with a considerable number of free communications presented either orally or as posters.

The Congress could boast of an unusually bright galaxy of top names among the invited lecturers and participants and, naturally, the ideal would have been to include all the invited lectures and symposia papers into the volumes. We are most grateful for all the material received and truly regret that a fraction of the manuscripts were not submitted in time. We were forced to set rigid deadlines, and top priority was given to speedy publication even at the price of sacrifices and compromises. It will be for the readers to judge whether or not such an editorial policy is justifiable, for we strongly believe that the value of congress proceedings declines proportionally with the gap between the time of the meeting and the date of publication. For the same reason, instead of giving exact transcriptions of the discussions, we had to rely on the introductions of the Symposia Chairmen who knew the material beforehand and on their concluding remarks summing up the highlights of the discussions.

Evidently, such publications cannot and should not be compared with papers that have gone through the ordinary scrupulous editorial process of the international periodicals with their strict reviewing policy and high rejection rates or suggestions for major changes. However, it may be refreshing to read these more spontaneous presentations written without having to watch the "shibboleths" of the scientific establishment.

September 1, 1980

J. Szentágothai

President of the
Hungarian Academy of Sciences

PREFACE

This Volume contains a plenary lecture and most of the papers presented at five symposia of the Section "General Cell Physiology" at the 28th International Congress of Physiological Sciences. Cell physiology has become an extremely wide field of biological sciences, and it was impossible to cover its entire spectrum in the program. A number of important, fast developing subjects were, therefore, selected for discussion concerning the general aspects of both non-excitable cells and neuronal membranes. Papers of this Volume deal with some recent results on excitation, namely on ionic channels, on CA-currents, on optical changes during excitation, on presynaptic modulation, and on transmission in autonomic ganglia.

The chairmen of the Symposia acted not only as organizers of their topics by selecting the invited speakers, but most of them have also made a contribution to the proceedings by compiling an introduction and concluding remarks to the respective Symposia. Furthermore, they were also active as co-editors of the Volume for which I wish to express my sincere gratitude.

I wish to thank Dr. T. Kiss, Secretary of the Section, and Mrs. Maria Kiss for their careful and enthusiastic work during the Congress and for their help in compiling this Volume.

J. Salánki

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IONIC MECHANISMS OF EXCITABILITY OF NERVE CELLS

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METHODS OF INVESTIGATION

Experiments carried out during the last decades on isolated perfused squid axons have definitely shown that the mechanism of the electric excitability is based on transmembrane ionic currents produced by discrete macromolecular complexes - ionic channels - capable to pass selectively certain types of ions. The ionic channels contain sensors which can rapidly open or close the ion-conducting pathway in response to changes in the intramembranal electric field. Obtaining of these basic results was possible because of several important technical achievements: an effective control of the physico-chemical conditions at the external and internal sides of the surface membrane and reliable membrane potential clamping for measuring the transmembrane ionic currents. For a quite long time these technical conditions were reached only on giant axons, although it was of a great interest to develop similar possibilities for other objects, in particular for the nerve cell itself.

In 1975 a technique for controlled replacement of the ionic content of the cytoplasm of isolated nerve cells was developed in our laboratory (Krishtal, Pidoplichko, 1975). The main idea of this technique is a production of a permanent hole in the cell membrane. The isolated cell is placed in saline solution corresponding to extracellular medium. The hole (10-30 μm wide) contacts the solution representing the desired intracellular medium. The replacement of intracellular ions

by ions of the latter solution goes on very fast; in fact this technique can be described as intracellular dialysis.

The main technical problem in the realization of this idea is the reliable separation of the "working" part of the cell membrane containing the extracellular solution from the pathways supplying the intracellular one. This problem was solved by fixing the cell in a conical pore of a plastic membrane separating two perfusion compartments. The use of plastic membrane is of a great importance for an effective adhesion of the cell surface to the surface of the pore (because of absence of surface charges on it); neutral glues can be used in addition.

In 1978 two modifications of this idea have been proposed using glass micropipettes for fixation of isolated cells (Lee et al., 1978; Takahashi, Yoshii, 1978); in one of them a special treatment of the glass for neutralizing its fixed charges was employed.

The use of plastic membrane has an important advantage because it is easy to make in it a pore of necessary shape and diameter for cell fixation; on this basis a transition from cell dialysis to cell perfusion has been achieved. For this purpose two holes are made on opposite sides of the cell membrane and connected to separate perfusion systems. In the presence of hydrostatic pressure difference between these systems a regulated flow of solution through the cell can be produced (Krishtal, 1978).

The use of dialyzed and perfused cells creates ideal electrical conditions for clamping the membrane potential and measuring ionic currents under controlled changes of transmembrane ionic gradients. It enabled us to separate and characterize the specific ionic currents evoked in the somatic neuronal membrane of several invertebrate and vertebrate species; several more complicated technical problems have also been solved, such as recording of asymmetric displacement currents connected to the activation of ionic channels, recording of stochastic fluctuations of ionic currents and evaluation of the activity of single channels.

MAIN COMPONENTS OF THE IONIC CURRENT IN A SOMATIC MEMBRANE

The switch-off of chosen ionic gradients (by replacing the corresponding permeable ions with impermeable ones) opens the way for the separation of the individual components from the overall transmembrane ionic current and the precise measurement of these components.

Invertebrate neurones. Figure I represents schematically the individual components of the ionic current evoked in the electrically-excitable somatic membrane, based on measurements on nerve cells from different gastropodal molluscs.

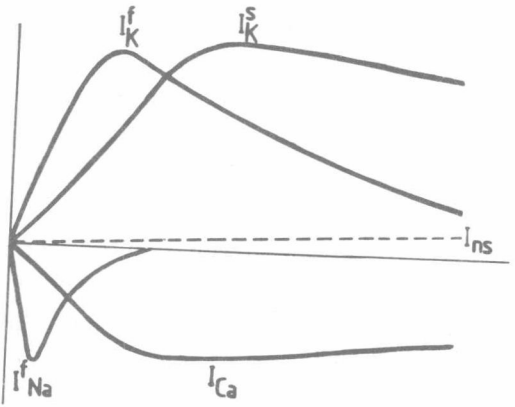

Fig. I. Diagram of separate ionic currents in the somatic membrane of mollusc neurones

The characteristic feature of inward currents in all investigated neurones is the presence, besides the sodium current, of a considerable specific calcium current (Kos -

tyuk et al., 1975). The constant property of the latter is a more slow development in time. The quantitative description of the activation kinetics of the calcium current can be satisfactory done within a modified Hodgkin-Huxley model using the square power of the variable m; the activation kinetics of the sodium current needs for its description the cubic power of this variable (Kostyuk, Krishtal, 1977a; Kostyuk et al., 1979a). The inactivation of the calcium current goes on extremely slow comparing to that of the sodium current; it has a complicated time-course.

The potential-dependence of the activation of the calcium current is less steep than that of sodium current. The corresponding current-voltage characteristics is non-linear, it approaches exponentially the potential axis at high depolarizing potential shifts. No reversal of the calcium current can be obtained even under artificial increase in the intracellular free calcium concentration. To the contrary, already a small increase in intracellular calcium (up to 10^{-7} M) blocks the calcium conductance (Kostyuk, Krishtal, 1977b). The apparent reversal of calcium current described by some authors (Adams, Gage, 1979) is in fact an artefact due to the activation at strong depolarization of low-specific outward current channels which can pass not only potassium ions but even such replacing ions as Tris (see below).

The selectivity of sodium channels in the somatic membrane is similar to that of axonal sodium channels (cf. Hille, 1972). The selectivity of calcium channels can be described by the sequence Ba > Sr > Ca > Mg; the relative permeability for these ions (as judged from the maximum current values) is 2.8 : 2.6 : 1.0 : 0.2 (Doroshenko et al., 1978). A characteristic feature of the functioning of calcium channels is the presence of effective binding of the penetrating ions in the region of both the external and internal channel mouth; the apparent dissociation constants for the external binding site are: K_{Ca} = 5.4 mM; K_{Sr} = 10 mM; K_{Ba} = 15 mM (Akaike et al., 1978; Valeyev, 1979). The presence of binding is the reason for effective competitive blocking of the

calcium current by other divalent cations. The dissociation constants for the blocking cations (measured in the presence of 4 mM Sr^{2+} are: $K_{Ni} = K_{Co} = 0.74$ mM; $K_{Mn} = 0.36$ mM and $K_{Cd} = 0.07$ mM (Krishtal, 1976; Ponomaryov et al., 1979). Effective binding of the penetrating ions on both sides of the channel indicates that the energy profile of the ion inside the channel can be approximated by two energy wells separated by an energy barrier; the penetrating ions have to fill up these wells in succession. The free energy levels at different points of the channel profile have been computed on the basis of data about concentration and potential dependence of the ionic current using diagrammatic technique. Because of the deepness of the internal energy well, the calcium channel in fact can pass ions only in the inward direction and in virtual absence of internal calcium ions. Even small increase in intracellular calcium fills up this well and blocks the channel (Kostyuk et al., 1980).

Computer summation of membrane currents evoked by equal number of equal hyper- and depolarizing potential shifts is an effective way to subtract all symmetric events and to reveal in pure form the asymmetric ones. Using this approach, an asymmetric intramembranal displacement of charges has been detected upon membrane depolarization after blocking the sodium and calcium ionic currents (Adams, Gage, 1976; Kostyuk et al., 1977). This "displacement current" has a complicated time-course. It can be separated into 2 components - F-sensitive and F-resistant - having different time-course by intracellular administration of fluoride ions (which destroy irreversibly the calcium conductance). The decay of both of them is exponential. A detailed comparison of the potential-dependence and kinetics of both these components with the characteristics of the sodium and calcium conductances leads to the conclusion that they reflect the functioning of the gating mechanisms of the corresponding ionic channels which respond directly to the changes of the intramembranal electric field. The kinetics and potential-dependence of the F-sensitive asymmetric displacement current

fit well to the displacement of the m-particles in the Hodgkin-Huxley model (2 particles for each channel having the effective valency of 3 -Kostyuk et al., 1979b).

Knowing the total amount of charges transferred by the "gating" current across unity membrane surface and the number and effective valency of the gating particles, the density of the corresponding channels and their unitary conductance can be calculated. The use of this approach for the calcium channels is complicated by the rapid decline of calcium conductance in the course of cell perfusion not followed by parallel decline in the amount of displaced charges. The probable mechanism of this peculiar channel inactivation will be discussed below. For this reason the measurements have to be made in the very beginning of cell perfusion. For neurones of Helix pomatia having maximum displaced charges of 1500 - 2000 $e^-/\mu m^2$ the density of calcium channels must be about 250-300 per μm^2 and the current through each channel 0.02 $\pm$ 0.01 pA.

Another way to study the functioning of single channels is an analysis of ionic current fluctuations which one may observe if number of channels participating in the response is considerably decreased. To use this possibility a technique was developed for sucking a micropatch of the membrane of an isolated perfused cell into a small pore in the plastic membrane. A very effective electric insulation of this patch from the rest of the membrane was achieved. To increase the share of each channel in the total ionic current, the most effectively penetrating ions were used. All this enabled the recording of current fluctuations in the order of 10^{-11}A, quite sufficient for statistical analysis (Kostyuk et al., 1980a). A study of the potential-dependence of the mean square deviation of the fluctuations has shown an increase of the latter with the increase of depolarization (e.g. with an increase of the total ionic conductance), reaching maximum at $\langle I \rangle / I_{max} = 0.5$, and then again decaying. This is in accordance with the suggestion that such fluctuations are produced by randomly opening and closing single

channels.

In study of calcium channels recordings of current fluctuations were made with extracellular medium containing I30mM of Ba^{2+}. The single channel current calculated from the equation

$$i = \frac{\sigma^2}{\langle I \rangle \left(1 - \frac{\langle I \rangle}{I_{max}}\right)}$$

was 0.20 ± 0.02 pA (Kostyuk et al., I980a). If the relative conductance of a single channel for Ca^{2+} and Ba^{2+} is the same as for the total current, then the calcium current through a single channel should be about 0.I pA, and its conductance 0.5 pS. These values are almost one order less than those for a single sodium channel (cf. Conti et al., I976). The obtained values are little dependent both on testing potential (since the equilibrium potential is very high) and on the level of inactivation. These observations are in accordance with the suggestion that the channel might have only 2 functional states (open or closed). In the open state it can pass at most 6.10^5 barium or 3.10^5 calcium ions per sec.

To obtain fluctuation spectra the autocorrelation function of the noise was obtained and then subjected to Fourrier-transformation. Practically the spectra of several realizations were recorded during depolarization, averaged and then subtracted from those recorded at the holding potential level. The spectra could be satisfactory approximated by the Lorentz function characteristics for the processes with a single relaxation time-constant. The cut-off frequency was little dependent on the testing potential; the mean value of the relaxation time-constant for the calcium channel determined from this value is 0.7 ± 0.2 ms (Kostyuk et al., I980a).

Of special interest is the fact that the relaxation-time of calcium channel determined from the spectral characteristics of the current fluctuations, is almost independent on the testing potential. This finding is not in agreement with the predictions of the model that the displacement of the gating particle directly transfers the channel from a non-con-

ducting state into a conducting one. Probably, the displacement of such particles by an electric field is a necessary but insufficient condition for this transition; it only creates the conditions for the realization of the next step (opening $\rightleftharpoons$ closing). Such a scheme would account for the change in the ratio between the ionic and the gating current of calcium channels in the course of prolonged perfusion of the cell (which was mentioned above). During such a perfusion some factors are washed out of the cell necessary just for the fulfilment of the last, potential-dependent step.

The characteristic property of the outward (potassium) currents in the somatic membrane is its separation into a fast and a delayed component (I_K^f and I_K^s) differing in activation and inactivation kinetics and potential-dependence of steady-state inactivation. These components have been studied in detail in nonperfused mollusc neurones (Neher, 1971; Connor, Stevens, 1971a; Kostyuk et al., 1975b). An important functional characteristic of I_K^f is a strong steady-state inactivation already at resting potential level and its removal by hyperpolarization; due to this property the corresponding channels play an important role in the generation of autorhythmic membrane potential waves. The measurement of equilibrium potentials for both potassium currents indicate that their channel selectivity is similar; if the potassium gradient is switched-off by replacement of the Tris ions for K^+, these currents disappeare completely. At the same time, after such replacement a potential-dependent outward current still remains due to a flux of replacing ions through less-specific ionic channels (I_{ns}). It differs from the fast and delayed potassium currents by exponential rise, absence of inactivation and resistance to the action of tetraethylammonium (TEA). An increase in intracellular Ca^{2+} concentration potentiates this current. All its characteristics are similar to those of the TEA-resistant component of potassium current, which is also potentiated by intracellular Ca^{2+} (Heyer, Lux, 1976; Thompson, 1977) and produced by less selective ionic channels (Doroshenko et al., 1979). Probably, these channels form the ba-