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Biochemistry

Transduction of chemical into electrical energy*

(cyclic chemical reactions/acetylcholine cycle/bioelectricity/nerve excitability)

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ABSTRACT **The paper recalls some fundamental notions, developed by Otto Meyerhof, which were used in the analysis of the transduction of chemical into mechanical energy during muscular contraction. These notions formed the basis of the approach to the analysis of the transduction of chemical into electrical energy, i.e., the very principle underlying nerve and muscle excitability and bioelectricity. Instrumental for this purpose was the use, since 1937, of electric organs of fish, a tissue highly specialized for bioelectrogenesis.**

In a lecture: "Zur Energetik der Zellvorgaenge" at the Philosophical Society in Kiel, 1913, Otto Meyerhof (1) discussed the problem of how the chemical energy introduced into the organism as food stuff is finally used for cellular functions. Meyerhof recognized many fundamental principles in biology. These ideas, partially derived from his experimental observations, guided him in his life work. Since Meyerhof's basic notions deeply influenced Ochoa's and also my scientific thinking—in fact that of all biochemistry of this century—it may be appropriate to recall first some of these basic concepts. They have become such an integral part of biochemical and biological thinking, that the younger generation is frequently unaware of their origin.

In order to analyze the problem of cellular energy transformations into function, in which Meyerhof was so passionately interested, he selected muscular contraction. The transduction of chemical into mechanical energy was taken as an example only, not because he was particularly interested in this special function, but because in this case the two aspects, chemical and mechanical phenomena, were in the range of measurements with the primitive methods available at this time. Among the basic notions for the understanding of cellular mechanisms, resulting from his ideas and his work, the following may be recalled: (*i*) the importance of bioenergetics in general and the fact that chemical energy transformations occur in sequences of chemical reactions (as we know today, this is possible because of the structural organization of the chemical systems); (*ii*) the cyclic character of cell reactions recognized in the early 1920s in glycolysis: only a small fraction of the endproduct (lactic acid) is irreversibly used for oxidation; the resulting energy is used for the resynthesis of glycogen; (*iii*) in view of the vital role of enzymes in all cellular functions, Meyerhof extracted and partially purified enzymes from muscles and thereby opened the way to the establishment of the glycolytic cycle; and (*iv*) the paramount importance of high-energy phosphate derivatives in intermediary metabolism. In 1926 he demonstrated, with Suranyi, the high enthalpy of phosphocreatine hydrolysis and within a few years that of several other phosphate derivatives. Among them was ATP, which he and Lohmann postulated, in 1934, to provide the primary energy for muscular contraction (2).

These four key notions also formed the basis for the analysis of the transduction of chemical into electrical energy, i.e., the mechanism underlying nerve excitability and bioelectricity. Instrumental for this work was the use, since 1937, of the electric organ of electric fish, a material generating bioelectricity with a degree of specialization apparently unique in nature. Only 3% of the organ is formed by proteins, 92% by water. The electric organ parallels the usefulness of muscle for the analysis of the problem of transduction during muscular contraction. During the last 15 years two other factors were crucial for the advances achieved in a molecular interpretation of bioelectricity: (*i*) the spectacular progress of protein and macromolecular chemistry and (*ii*) the vast amount of information accumulated about biomembranes by a combination of techniques, particularly electron microscopy with biochemistry. As a result, cell membranes turned out to be the site of many vital cellular functions. They are not, as was proposed in 1960, essentially a bimolecular leaflet of phospholipids, surrounded on the inside and outside by some proteins; in fact, a great variety of proteins make up more than two-thirds of the mass of most biomembranes (Fig. 1) (3, 4). More than 30 different proteins have been isolated from some membranes. Proteins account readily for the great diversity, specificity, and efficiency of membrane functions.

The excitable membrane that surrounds nerve and muscle cells has the special ability to change, with high speed and precision, the permeability to ions, the carriers of bioelectricity. The great heat production during the rising phase and the heat absorption during the falling phase of the action potential leave no alternative to the assumption of chemical reactions controlling the permeability cycle. The electrodiffu-

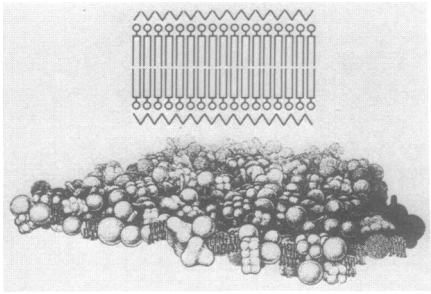

FIG. 1. Membrane models. Upper part: "unit membrane" of Robertson (3); lower part presents the hypothetical model of the inner mitochondrial membrane, proposed by Sjoestrand and Barajas in 1970 (4). The model comprises only those enzymes and coenzymes which were experimentally established to be present in this structure.

Abbreviation: AcCh, acetylcholine.

* *This paper is dedicated to Severo Ochoa in admiration of his brilliant contributions and leadership in biochemistry, and as an expression of my personal affection; it is based on a lecture presented at a Symposium in Madrid in September 1975 in honor of his 70th birthday.*

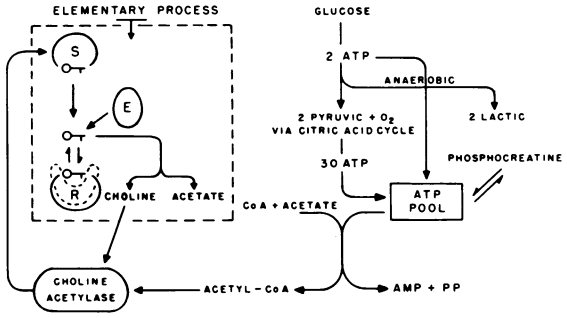

FIG. 2. AcCh-cycle and sequence of energy transformations. On the left side are the four proteins of the cycle and on the right side the sequence of energy transformations (6).

sion formalism, the basis of the Hodgkin-Huxley theory that dominated electrophysiology for three decades, is inadequate for explaining the mechanism of bioelectricity. Classical thermodynamics is only approximately applicable to biomembranes; because they are anisotropic systems, nonequilibrium thermodynamics must be used.

The biochemical approach to bioelectricity, based on the notions mentioned before and, more specifically, on the analysis of the properties and functions of the proteins associated with acetylcholine (AcCh), lead to the development of the AcCh-cycle (Fig. 2). This cycle was shown in 1953 in a Harvey Lecture when Ochoa was President of the Society (6). At that time the protein nature of the receptor and its conformational change induced by AcCh were postulates; today these are experimentally established facts. The further developments brought the AcCh-cycle to the present form shown in Fig. 3 (7). The figure indicates schematically the transduction of chemical into electrical energy. The citric and glycolytic cycles provide the energy (ATP) for the potential source of electrical energy, the ionic concentration gradients. These gradients are a common feature of almost all living cells, i.e., they are not limited specifically to the excitable cells. However, the use of this potential source of energy for generating electrical currents is the specific function of the AcCh-cycle, which controls the rapid ion movements by action on the ion permeation zone, the gateway (see Fig. 4).

In the resting, stationary, state the membrane potential ($\Delta\psi_r$) reflects the dynamic balance between active transport and AcCh synthesis and of the various ions asymmetrically distributed across the membrane. Fluctuations of the membrane potential such as, e.g., seen in the so-called miniature end plate potentials and other similar phenomena, are presumably caused by fluctuations in the local AcCh concentration, maintained at a stationary level during the continuous translocation of AcCh through the cycle. The rate and amount of AcCh activated is greatly increased during activity (7). It is suggestive to propose that a change in the membrane electric field causes a conformational change in the storage protein, thereby releasing AcCh. Electric fields, in intensity comparable to the depolarization voltage (15–20 kV/cm or 15–20 mV/100 Å), have been found to induce conformational changes in biopolymers (8, 9).

Ca^{2+} ions have been assumed for a century to be essential for nerve and muscle excitability. The specific role of Ca^{2+} ions in the control of the ion permeation zone by their release from the receptor protein caused by the conformational change was postulated several years ago (see, e.g., ref. 10), when evidence accumulated for the strong and special effects of Ca^{2+} ions on the conformation of proteins and macromolecular structures in general, such as, e.g., in muscular contraction. Recently, Neumann and Chang† have obtained experimental evidence for conformational changes induced by AcCh in homogeneous receptor protein, obtained by affinity chromatography (11). The isolated receptor proteins have a high binding capacity for Ca^{2+} ions, and it has been shown that the association of AcCh with the receptor protein causes release of Ca^{2+} ions from the macromolecule.

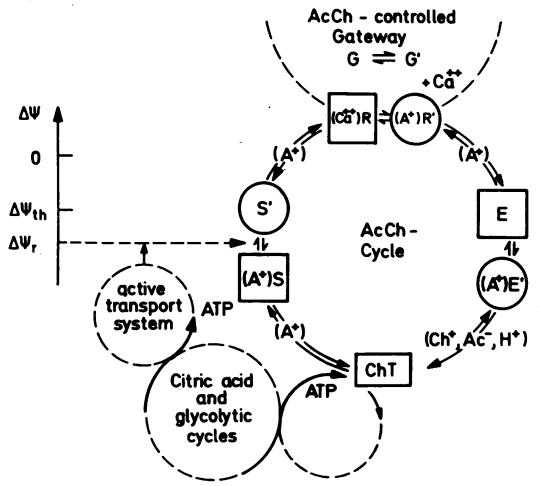

FIG. 3. Scheme of the transduction of chemical into electrical energy; role of the AcCh-cycle (7). The glycolytic and citric acid cycles provide the energy, ATP, both for maintaining the resting (stationary) membrane potential ($\Delta\psi_r$), caused by the asymmetric distribution of various ions across the membrane, and for a steady supply flux of AcCh to the storage protein (S). ($\Delta\psi_{th}$, threshold potential that evokes an action potential.) The AcCh-cycle effects the cyclic chemical control of stationary membrane potentials and transient potential changes. The binding site of the storage protein (S) for AcCh is assumed to be dependent on the membrane potential, $\Delta\psi_r$, and is thereby coupled to the active site transport system (and the citric and glycolytic cycles). When changes in the membrane electric field release AcCh, it is transduced to the receptor protein (R) to which Ca^{2+} ions are bound. AcCh induces a conformational change of the receptor that releases Ca^{2+} ions; they act on the ion permeation zone, the gateway, G (see Fig. 4). The Ca^{2+} ions induce conformational changes of phospholipids or lipo- or glycoproteins in the gateway, probably producing an aqueous zone of a few angstroms, thereby permitting the rapid Na^+ ion influx leading to the rising phase of the action potential (G open). In the meantime, AcCh has been transduced to the enzyme AcCh-esterase (E), which hydrolyzes AcCh into choline (Ch^+), acetate (Ac^-), and protons (H^+). The removal of AcCh from the receptor permits the return of the receptor to its resting conformation, binding Ca^{2+} and thereby restoring the barrier for the rapid ion movements (G' closed). The hydrolysis of AcCh, the virtually irreversible step of the cycle, is followed by the supply of energy, ATP, to the enzyme choline *O*-acetyltransferase (ChT), which acetylates choline and assures the steady supply of AcCh to the storage protein.

The control of the rapid ion movements during electrical activity is thus proposed to be performed by a series of reactions of the AcCh-cycle. AcCh acts as a signal that is greatly amplified by these reactions: per molecule of AcCh activated, 15,000 to 30,000 ions (or more) move across the membrane in both directions. Just as Meyerhof referred to ATP as the specific operative substance in muscular contraction, AcCh has the equivalent specific function in bioelectricity,

† E. Neumann and H. W. Chang (1976), submitted for publication.

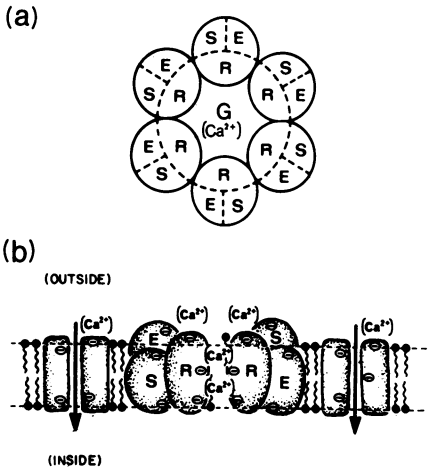

FIG. 4. Scheme of the AcCh-controlled gateway (G). (a) Basic excitation unit containing in this example six SRE-assemblies, viewed perpendicular to the membrane surface. S, AcCh-storage site; R, AcCh-receptor protein; E, AcCh-esterase. (b) Cross section through a basic excitation unit flanked by two units that model ion passages for K^+ ions; the arrows represent the local electrical field vectors due to partial permselectivity to K^+ ions in the resting stationary state. The minus signs ⊖ symbolize negatively charged groups of membrane components (5).

in fact apparently also in the control of rapid ion movements in other membranes (such as, e.g., red blood cells, placenta, and others).

A concept of great importance for the understanding of details of nerve excitability and bioelectricity has been suggested by Eberhard Neumann: the notion of a Basic Excitation Unit. According to this concept, the rapid ion permeation zone (gateway) is not controlled by one single protein assembly, but is surrounded by several such assemblies (Fig. 4) (12). The concept of the basic excitation unit has enabled Neumann to elaborate during the last 3 years an integral model of bioelectricity in which many electrical parameters established during the last decades are interpreted in molecular terms (5). For example, the action potential is caused by the simultaneous release of a critical cooperative number of AcCh molecules (Fig. 5), which are capable of producing

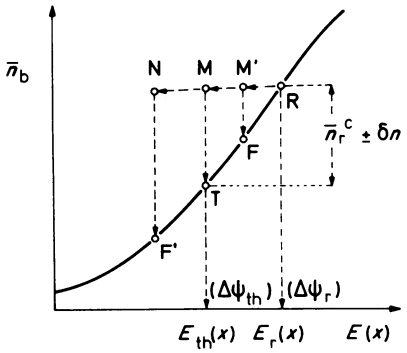

FIG. 5. Model representation of the field-dependent stationary states for AcCh storage. The mean number, $\bar{n}_b$, of AcCh ions bound to the storage site at the membrane site x of the release reaction, as a function of the electric field $E(x)$ (at constant pressure, temperature, and ionic strength). The intervals M'F, MT, and NF' correspond to the maximum number of AcCh ions released, $\bar{n}_r$, for three different depolarization steps: a subthreshold change from the resting state R to F, a threshold step R to T releasing the threshold or critical number $\bar{n}_r^c$ ($\pm$ δn fluctuation), and a suprathreshold step R to F' with $\bar{n}_r > \bar{n}_r^c$ (ref. 5).

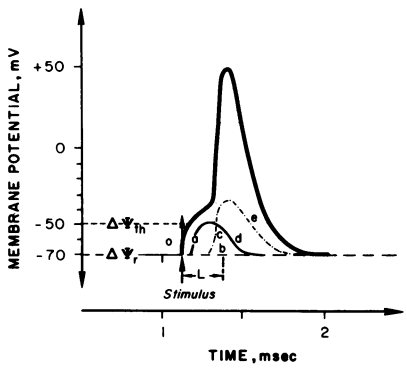

FIG. 6. Schematic representation of the time course of chemical reactions in relation to the action potential (o). Potential change owing to the change of the membrane potential in rest ($\Delta\psi_r$), to the threshold potential ($\Delta\psi_{th}$). (a) and (d), time course of AcCh release and hydrolysis, respectively; (b) and (c), time course of Ca^{2+} release from receptor and of gateway opening; (e), time course of Ca^{2+} reuptake and conformational relaxation of the receptor, leading to closure of the gateway (11).

the all-or-none response; below this number only local potential changes are produced. Fig. 6 shows the electrical response as a function of the various chemical processes (12).

As to the factual basis of the concept of the role of AcCh, I would like to make only two brief comments. AcCh-esterase (acetylcholine hydrolase; EC 3.1.1.7) is present in the excitable membrane throughout the animal kingdom; no exception has ever been found. It is present in all parts of the membrane (conducting and synaptic). Fig. 7 shows the localization of the enzyme by the dense reaction products in the excitable membrane of an axon of the dorsal roots of frog; Fig. 8, in a synaptic junction of an intercostal muscle of a mouse: one sees the enzyme localization, the dense end products in the membrane of the nerve terminal as well as in the deep invaginations of the postsynaptic membrane (13, 14). The concentration of the enzyme is extraordinarily high. In the excitable membrane of a single cell (electroplax) of electric eel, 10^{11} molecules (100 billion) of enzyme are present. The number of receptor molecules is of the same order of magnitude. However, neither the localization nor the concentration of the enzyme forms the evidence for the concept presented. It is based upon a great variety of different types of experiments. An essential part of the evidence comes from the effects of powerful and specific inhibitors of either the AcCh-receptor or AcCh-esterase on electrical activity, when applied to appropriate preparations and/or under appropriate experimental conditions. Many apparent

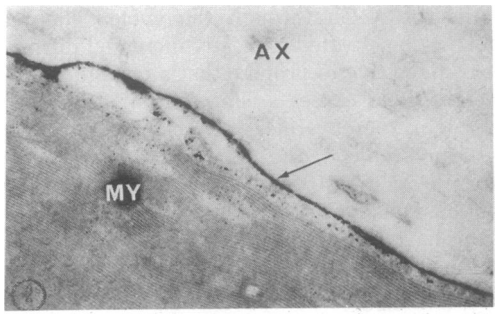

FIG. 7. Myelinated (MY) ventral root axon (AX) of frog. The dense end product is present in the excitable (plasma) membrane (arrow). For details, see ref. 13.

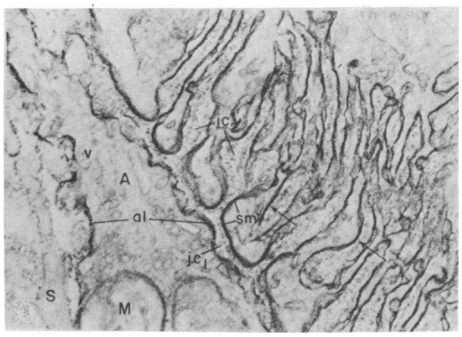

FIG. 8. Electron micrograph showing the histochemical localization of AcCh-esterase at the motor end plate of mouse intercostal muscle. The dense end product is found in the membrane of the axon terminal (A) and the membrane covering the deep invaginations of the postsynaptic membrane. For details see ref. 14.

contradictions of the early period have been fully explained during the last few years, due to new information and due to the greatly improved and refined techniques and instruments that have become available during the last 15 years (7). Recently, a model of bioelectricity, based on an AcCh-cycle and a Ca^{2+}-cycle, has been proposed by Dubois and Schoffeniels (15, 16).

The analysis that has led toward an understanding of the transduction of chemical into electrical energy has been briefly summarized here. It is, as already mentioned, based on the notions developed by Meyerhof. The concept is supported by a vast number of solid biochemical and biophysical data. Some of the evidence is suggestive, although not yet conclusive. There are some postulates that still require experimental tests. However, every concept or theory is, according to a statement of J. J. Thomson, not a creed, but a tool designed to stimulate further research. In a recent lecture, Werner Heisenberg quotes a remark of Albert Einstein: "Whether you find something or not depends on your theory; the theory decides your observations." The notions and theories that were used in the studies of the chemical basis of the mechanism of bioelectricity have proved their value; they have permitted the accumulation of much pertinent information. This information has permitted the elaboration of the first tentative model of nerve excitability and bioelectricity integrating the biochemical and the biophysical data. We must be aware, however, of the extraordinary complexity of living cells: we are in all fields of biological sciences at the beginning. But molecular biology offers the most exciting and fascinating challenge for generations to come and the best hope for penetrating into some mysteries of life.

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