PARTICIPATION OF THE NUCLEIC METABOLISM IN THE FORMATION OF THE ELECTRICAL PROPERTIES OF THE APICAL DENDRITES OF THE CEREBRAL CORTEX

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M. B. Shtark

Laboratory of Electrophysiology (Head—Candidate of Medical Sciences, M. B. Shtark, Scientific Consultant—Professor F. N. Serkov) of the Psychoneurological Institute (Director A. G. Leshchenko), Odessa

(Presented by Academician V. N. Chernigovskii)
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The role of nucleic metabolism, in particular, the transformations of RNA in the specific activity of the neuron, is a vital problem of modern neurophysiology and neurochemistry. The increase in the intensity of nucleoprotein synthesis during the activity of the central neurons and their processes plays a great role in carrying out the conditioned reflex activity, the function of memory, learning, etc. [1, 4, 8].

In view of this, we attempted to study the interrelationship of the nucleic metabolism in the soma and the dendritic processes of the central neurons with their functional activity (bioelectric phenomena).

EXPERIMENTAL PROCEDURE

The experiments were conducted on 20 cats, narcotized with nembutal (50 mg/kg). Two openings 10 mm in diameter were trepanned above symmetrical portions of the hemispheres (the anterior third of the suprasylvian gyrus). A third of the meninx was removed, and to each portion of the exposed cortex was applied a pair of platinum stimulating electrodes (distance between the electrodes 1 mm). On both portions, pick-up wicks, moistened with warm Ringer's solution, were set up 4-8 mm from the stimulating electrodes. An indifferent electrode was fastened in the nasal bone (a scheme of the experiment is depicted in Fig. 1, J). Stimulation of the cortex was accomplished with the electronic stimulator with radiofrequency output, designed by an engineer of the laboratory, N. V. Nyrova, guaranteeing the delivery of single and paired rectangular stimuli, with durations from 100 to 200 μsec, amplitude up to 30 V. The dendritic potentials arising upon stimulation beneath the pick-up electrode on the surface of the cerebral cortex were delivered to the input of a UBP-1 biopotential amplifier, and after amplification were recorded on the screen of an ÉNO-1 oscillograph by the method of superposition on stationary motion picture film. The starting of the expected unrolling of the oscillograph was strictly synchronized with the delivery of the irritating stimulus. The upper deflection of the beam corresponded to the appearance of negativity beneath the active electrode.

After recording of the "background" and the obtaining of a stable value of the dendritic potentials (DP) drawn off from the symmetrical portions of the hemispheres, filter paper moistened with a 0.1-0.5% solution of ribonuclease was applied to the cortex of one of them, or a crystal of this enzyme was applied. The DP was recorded both from the "control" hemisphere and from the portion upon which the enzyme acted. At the end of the recording of the changes in the DP that arose after 5-30 min in connection with the local influence of ribonuclease, both portions of the cortex—"control" and "experimental"—were excised with a sharp surgical needle [11], then fixed with chilled formalin or Carnoy-Brodskii fluid and treated by the histochemical methods of Brachet-Unna and Einarson-Krog to determine RNA in the neurons and processes of the cerebral cortex. Then the preparations were photographed and determined photometrically on a microphotometer of the MF-2 type, and the results of the photometric measurement were treated statistically.

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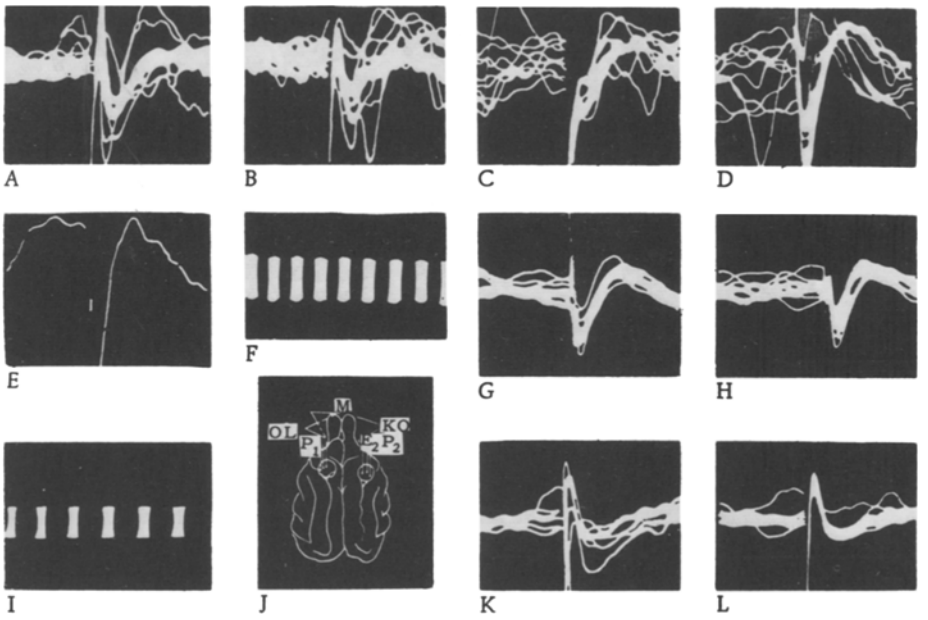

Fig. 1. Dynamics of the dendritic potentials after local application of a crystal of ribonuclease (5 μg) in the cerebral cortex. A) before application; B) 10 sec after application; C) after 4 min; D) after 8 min; E) after 8 min 20 sec; F) calibration for A-E; G,H) after 30 and 34 min; I) calibration for G, H—250 μV and 20 msec; J) scheme of experiment—P_1, P_2—stimulating couple for "experimental" and "control" hemispheres; E_1, E_2) pick-up and indifferent electrodes; K) dendritic potentials at the symmetrical point of the "control hemisphere before the application of ribonuclease on the "experimental" hemisphere; L) after application. Voltage of stimulating current 6.5 V, duration of stimulus 250 μsec. Upward deviation of the beam corresponds to the appearance of negativity beneath the active electrode.

EXPERIMENTAL RESULTS

In our experiments, the DP were represented by a negative-positive complex, arising after a latent period equal to 0.5-1.5 msec. The amplitude of the negative oscillation, as is well known, is related to the postsynaptic activation of the apical dendrites [31]; it comprised 250-600 μV, while its duration was 10-20 msec. The amplitude of the positive components of the DP, related to the electronic influence of the apical dendrites on the soma of the pyramid neurons, was equal to 100-350 μV, with a duration of 20-40 msec. The reliability of the amplitude, frequency, and phase values of the DP were monitored by 10 to 20 frame-by-frame superpositions of the potentials (Figs. 1, 2).

The application of solutions and pure crystalline ribonuclease produced distinct and regular changes in the basic parameters of the DP.

These effects were definitely dependent upon the concentration of the enzyme and the area of its contact with the cortical elements.

A gradual drop in the amplitude of the negative component of the DP, arising 5-8 min after the application of the enzyme, was noted (Fig. 1B; Fig. 2C-E). Then the positive oscillation of the DP was substantially deepened; the entire complex was shifted to the isoline after 20-25 min. The latent period of the induced potential also increased somewhat. We observed the described changes in the DP in all our experiments.

The second fact that we noted was the inversion of the sign of the DP, the appearance of a primary positive oscillation beneath the pick-up electrode 8-20 min after application of the enzyme. This hyperpolarization effect of ribonuclease is comparatively stable; it may be recorded without any significant changes for a long period of time—up to 150 min after application of the substance. With the passage of time, the amplitude of the primary hyperpolarization wave was sharply increased, reaching 600-800 μV; i.e., sometimes exceeding the value of the primary negative oscillation of the initial DP (see Fig. 1, D-E; Fig. 2,G-J). In certain experiments, this oscillation

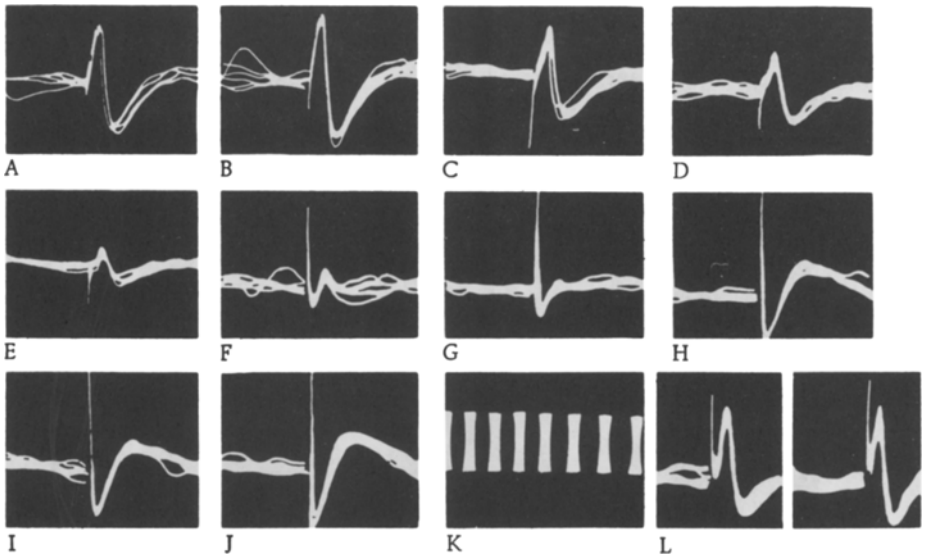

Fig. 2. Dynamics of the dendritic potentials after local application of a 0.5% solution of crystalline ribonuclease in the cerebral cortex: A) before application of the enzyme; B) after 30 sec; C) 4 min; D) 5 min; E) 7 min; F) 10 min; G) 16 min; H) 19 min; I) 6 min; J) 54 min after application; K) 20 msec, 250 μV; L) dendritic potentials at the symmetrical point of the "control" hemisphere before the application of the ribonuclease on the symmetrical point of the "experimental" hemisphere; M) after application. Voltage of the stimulating current 6 V, duration of the stimulus 200 μsec. Conditions of current pick-up—see text and Fig. 1.

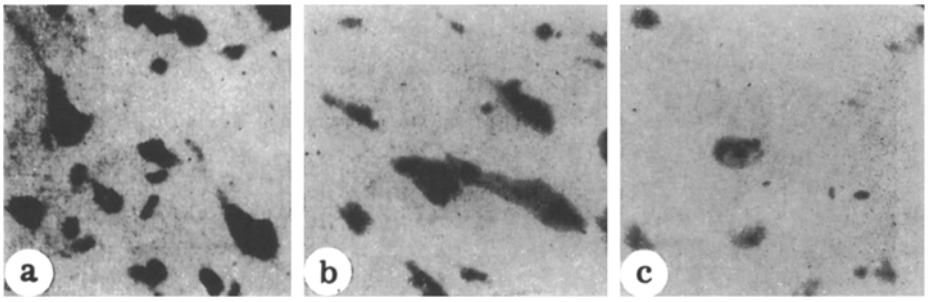

Fig. 3. Distribution of RNA in the pyramid neutrons and their dendrites at the site of pick-up of the dendritic potentials (layer IV): a) before application; b) after 15 min; c) 34 min after application of 0.5% ribonuclease solution. Staining according to Einarson-Krog. Ocular 10, objective 20.

reached to the maximum amplitude (700-800 μV) 25-30 min after the application of ribonuclease; it was not removed by the application to the cerebral cortex of poisons that stimulate the postsynaptic membrane (strychnine nitrate) and did not disappear for a long time after the surface of the cortex of the "experimental" hemisphere was rinsed with warm Ringer solution.

As a rule, the primarily inhibited action of ribonuclease described was recorded over a substantial territory of the cortex (up to 10 mm^2) between the pick-up and stimulating electrodes. The application of weak (0.1%) solutions of the enzymes converts this effect to a strictly local effect, limited only to the region of application of the substance beneath the pick-up electrode. The changes in the parameters of the DP in relation to the application of crystalline ribonuclease are presented in the table.

The dendritic potentials recorded upon stimulation of the cortex of the symmetrical control hemisphere retain strictly stable parameters (see Fig. 1K, L; Fig. 2L, M).

Dynamics of the Amplitude, Duration, and Phase of the Basic Components of the Dendritic Potentials after Application of 0.5% Ribonuclease Solution

Expt. No.	Conditions of experiment	Sign of potential of component		Amplitude of component		Duration of component	
		First	Second	First	Second	First	Second
				in μV		in msec	
1	Before application of ribonuclease	−	+	400	325	12	48
2	The same	−	+	420	340	12	48
3	" "	−	+	410	330	12	48
4	" "	−	+	320	300	12	48
5	After application of ribonuclease	−	+	30	250	12	22
6	The same	−	+	200	300	24	56
7	" "	+	−	350	310	24	58
8	" "	+	−	400	330	24	58
9	" "	+	−	510	460	24	58
10	" "	+	−	650	520	24	60
11	" "	+	−	600	500	26	56
12	" "	+	−	550	420	24	54
13	" "	+	−	480	400	24	50
14	" "	+	−	460	380	20	48
15	" "	+	−	450	360	13	48

The results of cyto- and histochemical investigations showed that the application of ribonuclease produces a rather rapid decrease in the RNA concentration (5-15-fold), both in the cytoplasm of the cellular body and in the dendrites of the pyramid neutrons. It is distinctly evident that a drop in the RNA content is observed first of all in the protoplasm of the dendrites of the pyramid neutrons, and then in the protoplasm of the cellular bodies (Fig. 3B, C). We very rarely observed a restoration of the initial RNA concentration, and only in those observations in which weak concentrations of the enzyme were used, and the local effect of conversion of the DP was preserved for a long time. In histochemical investigations of the cortical tissue along the vertical, it was established that the enzyme penetrates into the brain to a depth of no less than 100 μ and in most experiments penetrates the basic cellular groups of the third to fourth layers. Consequently, in any case the penetration of ribonuclease deep into the brain guarantees its interaction with the bulk of the dendrites and the bodies of the pyramid neutrons of the cortex in the zone of recording of the DP.

The regular drop in the amplitude of the negative potential described is evidently due to changes in the properties of the postsynaptic membrane of the dendrites and protoplasm of the processes. It would be simplest to attribute it to a quantitative deficiency of RNA in the dendrites and soma of the pyramids, which is confirmed histochemically. On the basis of the data of Sholl [12], it may be considered that the greatest amount of the enzyme interacts first of all with the protoplasm of the dendritic processes, since the latter take up 90% of the active surface of the neurons of the cerebral cortex and contain substantially greater amounts of RNA per unit surface than the perikaryon. This also follows from photometric data: the amount of RNA decreases first in the protoplasm of the dendrites, and then in the perikaryon of the neurons.

In addition, the drop in the amplitude of the DP is related to the concentration of the enzyme solution, since the lower the latter, the less the duration of the primary hyperpolarizing effect. All this convinces us that it is linked with a reorganization of the protoplasmic RNA metabolism and a drop in the excitability of the postsynaptic membrane of the dendrites. It is known that RNA synthesis is closely related to the number of synaptic stimulations that reach the neurons; transitory sensory stimulation produces a 40-60% increase in the RNA concentration in the

cytoplasm [7, 9]. If under our conditions, simulating a rather protracted synaptic bombardment (by electrical stimulation), we did not observe any definite increase in the RNA concentration in the dendrites and soma of the pyramids, after its decrease caused by the application of ribonuclease, we may presume the existence of "metabolic" factors lying at the basis of the described changes in the DP. It may also be assumed that the drop in the amplitude of the negative component of the DP during the first minutes after application of ribonuclease is related to a parabiotic effect. However, such an explanation can scarcely be complete, if we consider that in addition to the drop in the amplitude of the DP, when ribonuclease is applied, phase changes also take place in the structure of the dendritic potentials.

At the present time, without having recourse to the well-known hypothesis of D. Purpura and H. Grundfest [6, 10] of heterogeneity of the synaptic organization of the apical dendrites of the cerebral cortex, we shall assume that following treatment of this fact. Evidently, the enzyme, acting on the cytoplasmic RNA, inhibits its polymerization, which gives rise to a disturbance of the active transportation of potassium ions, and hence a state of ionic asymmetry between the extra- and intracellular media, as well as a change in the potassium-sodium gradient, [1, 5, 13]. These changes are the direct result of the specific action of the enzyme and determine the synaptic hyperpolarizing effect.

LITERATURE CITED

1. Kh. S. Koshtoyants, Problems of the Enzymology of the Processes of Excitation and Inhibition and the Evolution of the Function of the Nervous System [in Russian] (Moscow, 1963).
2. V. V. Portugalov, In the book: Structure and Function of the Nervous System (Moscow, 1962), p. 95.
3. A. I. Roitbak, Bioelectric Phenomena in the Cerebral Cortex [in Russian] (Tbilisi, 1955), Part 1.
4. M. H. Briggs and G. B. Kitto, Psychol. Rev., 1962, Vol. 69, p. 537.
5. M. Dixon and E. Webb, Enzymes [Russian translation] (Moscow, 1961).
6. H. Grundfest, Electroenceph. clin. Neurophysiol, 1958, Suppl. 10, p. 88.
7. C. A. Hamberger and H. Hydrn, Acta oto-laryng. (Stockh.) 1950, Suppl. 75, p. 53.
8. H. Hyden, In the book: Functional Morphology of the Cell [Russian translation] (Moscow, 1963), p. 185.
9. H. Hyden, Z. mikr.-anat. Forsch., 1943, Bd. 54, S. 96.
10. D. P. Purpura, M. Girado, and H. Grundfest, Z. mikr.-anat. Forsch., 1960, Vol. 12, p. 95.
11. J. Sentagotai, In the book: Structure and Function of the Nervous System [Russian translation] (Moscow, 1962), p. 6.
12. D. A. Sholl, Organization of the Cerebral Cortex (London, 1956), p. 143.
13. J. F. Sutcliffe, Biol. Rev., 1959, Vol. 34, p. 159.