PHYSIOLOGY

CHANGES IN UNIT ACTIVITY OF HIPPOCAMPAL AREA CA3 DUE TO THE NEUROIMMUNOMODULATOR NEUROTROPIN

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UDC 612.826

KEY WORDS: hippocampal area CA3; unit activity; neurotropin

The attempt to discover the concrete neurophysiological and neurochemical mechanisms lying at the basis of learning and memory is reflected in the study of the role of neuroimmunomodulation in the organization of different forms of goal-directed behavior in animals [7]. However, the study of regulatory neuroimmunomodulating processes during the formation and realization of integral behavioral acts is impossible without elucidation of their role in the neuronal mechanisms maintaining different levels of integrative activity of the brain and of the body as whole.

The aim of this investigation was to study the role of the neuroimmunomodulator neurotropin (NT) in the organization of unit activity in area CA3 of the hippocampus (HP) in animals after food deprivation for 24 h.

EXPERIMENTAL METHOD

Experiments were carried out on 6 unrestrained male chinchilla rabbits weighing 2.5-3.5 kg. As a preliminary step steel cannulas were inserted into the region of the lateral ventricles of the brain (AP 0.5, L 4) contralaterally relative to the side of recording of unit activity. A standardized extract of NT (Institute of Bioactive Research, Nippon Zoki, Osaka, Japan), containing conjugated polysaccharides and possessing a wide biological spectrum of action [6], was used for intraventricular injection. By means of a microinjector the preparation was injected in portions of 10 μl, so that it was possible to investigate the time course of single unit activity depending on the change in NT concentration. The experimental rabbits were divided into three groups (2 animals in each group) depending on the volume of NT injected: group 1) 10-50 μl, group 2) 10-80 μl, group 3) 10-140 μl. A single neuron was studied in each of the six experimental animals, with a consecutive stepwise increase in concentration of NT. Discharge activity of neurons along the track of the microelectrode was then recorded. Unit activity in area CA3 of HP (AP 4-4.5, L 6.5-7, H 4-6) was recorded by means of glass microelectrodes with a tip 1 μ in diameter, filled with 3 M NaCl solution. To insert the microelectrodes into the brain tissue a micromanipulator of original design was used, one which could be fixed to the skull of the experimental animal. A set of electrophysiological apparatus, adapted for working with unrestrained rabbits, was used in the experiments. Unit activity was recorded on magnetic tape of a 4-channel measuring tape recorder (7003, Brüel and Kjaer, Denmark). Activity of 40 neurons in HP before injection of NT and 27 neurons after injection was recorded. The signals were analyzed by computer (Apple II, USA). The program of analysis for each neuron included calculation of the mean (μ) interspike interval (ISI), the dispersion of the mean ISI (σ). the coefficient of variation (CV), and also the mean discharge frequency (ρ). A method of constructing histograms of percentage distribution of ISI with piecewise-irregular and logarithmic scales, also was used [3, 4]. Serial correlation graphs of ISI [4] and a modified method of calculating coefficients of dynamic irregularity of ISI were used as additional methods of statistical analysis [2].

EXPERIMENTAL RESULTS

Animals tested initially after food deprivation for 24 h were divided into four groups in accordance with the distribution of their ISI on histograms reflecting organization of their spike trains (Table 1). The first three groups

P. K. Anokhin Research Institute of Normal Physiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR K. V. Sudakov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 113, No. 3, pp. 227-230,March, 1992. Original article submitted July 25, 1991.

0007-4888/92/0003-0285$12.50

TABLE 1. Statistical Parameters of Spike Activity of 40 Area CA3 Neurons of the Rabbit Hippocampus after Food Deprivation for 24 h

Distribution of ISI	Number of neurons	Region of values of dominant ISI on histograms, msec		Mean ISI, msec (μ)	Dispersion (τ)	Coefficient of variation, per cent (CV)	Mean firing rate, spikes/sec (ρ)	Mean coefficient of dynamic irregularity, %	Coefficient of serial correlation
		piecemeal	logarithmic scale						
Bimodal	7	1—20; 100—400	1,5—15; 100—400	40—200	50—572	124—321	2—25	43—63	0,01—0,2
	1	10—20; 1000	15—25 1000	558	1478	265	2	43	0,01
Trimodal	1	1—5; 300—400; 1000	2,5—6; 250—400; 1000	481	1169	243	2	62	0,1
Monomodal	1	5—20	10—15	33	56	158	30	39	0,2
Polymodal	23	5—400	2,5—400	34—199	43—447	97—261	5—29	28—65	0,01—0,1
	7	5—1000	2,5—1000	127—247	339—646	179—349	4—8	25—49	0,01—0,1

TABLE 2. Statistical Parameters of Firing Pattern of 27 Area CA3 Neurons of Rabbit Hippocampus after Intraventricular Injection of Neurotropin

Distribution of ISI	Number of neurons	Region of values of dominant ISI on histograms, msec		Mean ISI, msec (μ)	Dispersion (τ)	Coefficient of variation, per cent (CV)	Mean firing rate, spikes/sec (ρ)	Mean coefficient of dynamic irregularity, %	Coefficient of serial correlation
		piecemeal	logarithmic scale						
Monomodal (50 μl NT)	3	1—20	2,5—15	9—15	3—43	38—298	69—113	10—39	0,2—0,7
	4	20—40	10—40	23—39	19—94	81—342	26—43	18—27	0,1—0,3
	2	20—60	15—60	48—50	57—69	138—190	4—20	33—60	0,1—0,3
Trimodal (80 μl NT)	1	60—100	60—100	112	412	367	9	11	0,1
	5	1—5; 300—400 1000	1,5—6; 250—400; 1000	266—881	498—2671	187—303	1—4	35—76	0,01—0,2
Polymodal (140 μl NT)	13	1—600	1,5—600	67—271	59—662	82—327	1—15	33—84	0,01—0,2

Legend. Volume of NT injected given between parentheses.

consisted of neurons with a clearly defined bimodal, trimodal, or monomodal distribution of their ISI. The last, and numerically the largest group, consisted of cells with "regular" (within certain limits of values of the interval spectrum) distribution of ISI, and without any clear dominance of any particular intervals. This group of neurons was conventionally described as "polymodal." The basic statistical parameters of discharge activity are given in Table 1 for each group of cells, within the range from minimal to maximal values. However, individual values of these parameters are given for three single neurons in Table 1. The reason is that one neuron in group 1 (with dominant ISI in the region of 20 and 1000 msec) was distinguished as a separate subgroup, and the second and third groups each contained only 1 neuron. As was shown previously [5], a state of hunger in animals is reflected in the discharge pattern of the overwhelming majority of neurons in different brain formations, in the form of a bimodal and trimodal distribution of ISI within regions of values of 5-20, 100-200, and over 1000 msec. In the present investigation neurons with this kind of ISI distribution accounted for only 20% (8 of the 40 cells tested). In our opinion these quantitative differences arose because in previous experiments animals previously trained in food-getting skills under the conditions of the experimental chamber were used. Interaction of motivational and reinforcing excitation at the brain neuron level, lying at the basis of acquisition of individual experience by the animal, is evidently the decisive component in the integration of elements of the system [1]. This orderly interaction is achieved through selection of the necessary degrees of freedom of the neurons, i.e., the organization of their discharge activity, expressed as dominance of particular ISI in spike trains. Analysis of histograms with a logarithmic scale of ISI distribution, which we have used more recently, enables the range of values of dominant intervals in the discharge pattern of the neurons, characterizing a state of hunger, to be extended, with the following limits: 1.5-25, 100-400, and over 1000

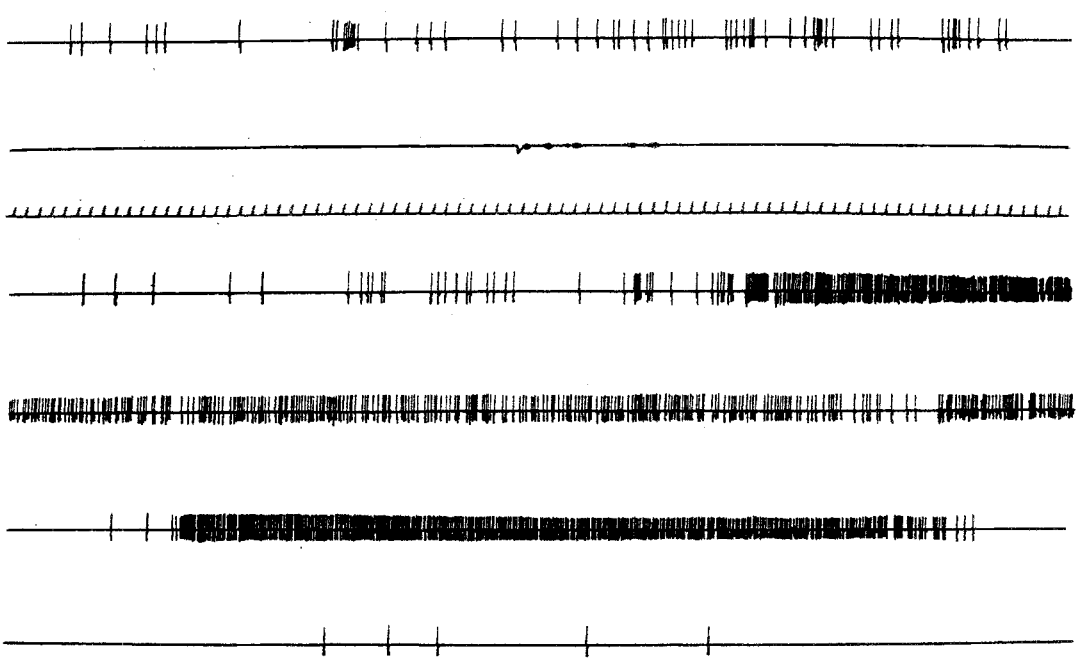

Fig. 1. Dynamics of firing pattern of a neuron in hippocampal area CA3 after intraventricular injection of NT. From top to bottom: initial spike discharge, marker of ending of injection of NT (50 μl), time marker (100 msec), spike discharge reflecting long-term regularization in firing pattern and appearance of bursting activity in response to increase in volume of NT to 80 μl (bottom trace).

msec. However, in animals with no previous experience of achieving the required result under experimental conditions, a state of hunger (after food deprivation for 24 h) does not lead to release from excessive degrees of freedom in the firing pattern of 75% (30 of 40 tested) of neurons in area CA3 of HP (Table 1), preventing their combining into a wider set of excitations of the whole functional system, which could be expressed in the animal's concrete goal-directed behavior.

The process of integration of all forms of excitation reaching the neuron is the key process by which the nerve cell becomes incorporated into extensive sets of interneuronal connections, and its individual place is defined in the crucial mechanisms of the functional system as a whole [1]. In this connection both the mechanisms whereby the neuron processes and recodes incoming into outgoing signals and the principles governing their manifestation in response to changes in the dynamic situation, expressed through reorganization of the firing pattern, assume particular importance. After intraventricular injection of NT dose-dependent changes were found in the spike-interval characteristics of 27 neurons in area CA3 of HP. Reorganization of their spike trains was observed when NT was given in volume of 10 μl, but became most marked when the volume of NT injected was increased to 50 μl. A single injection of NT in a volume of 50 μl caused, after an interval of 40-45 sec, reorganization of the initial firing pattern of a single HP neuron, expressed as the appearance of an initial, prolonged (17 sec) phase of regularization of spike generation followed by alternation of long (from 3 to 31 sec) periods of regularization and shorter (1 to 5 sec) periods of total inhibition of the discharge (Fig. 1). The rhythm of alternation of prolonged regularization and inhibition of the discharges sometimes was disturbed by the appearance of spike activity resembling the spontaneous pattern, but as a whole it was stable in character, and if the experimental conditions remained unchanged, it persisted throughout (until 2 h of observation) the time of recording of a single neuron. Quantitative analysis of the distribution of ISI of different duration indicates that it is basically monomodal (Fig. 2). The increase (compared with values for spontaneous activity) in values of coefficients of serial correlation and the decrease in values of coefficients of dynamic irregularity (Fig. 2) points to a high degree of dependence of adjacent ISI and of orderliness of the spike train of a neuron under the influence of NT. Injection of an additional volume of NT (10-30 μl over and above 50 μl) led to disappearance of long-term regulation of spikes in the firing pattern of the neuron and to the appearance of groups of high-frequency bursts of spikes (Fig. 1, bottom trace). The distribution of ISI on the histograms became trimodal in

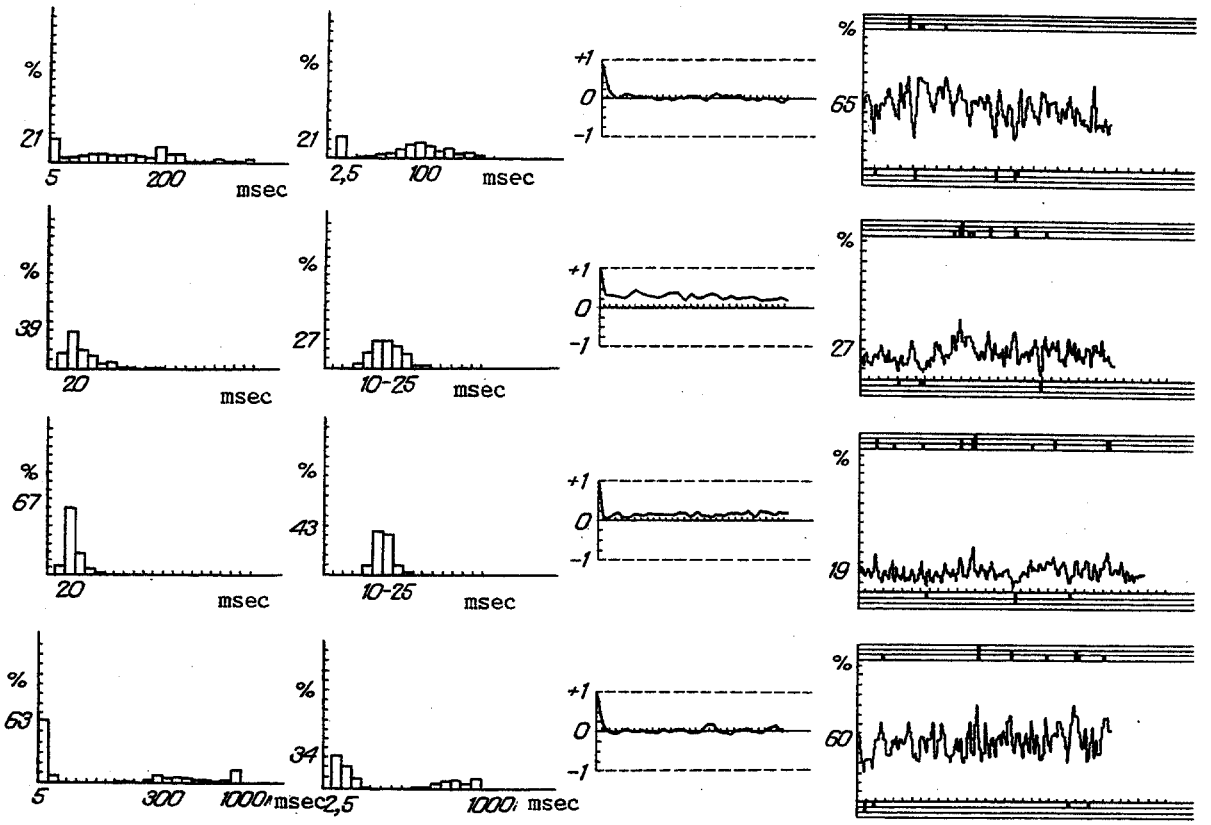

Fig. 2. Distribution of ISI of hippocampal area CA3 neuron in response to intraventricular injection of NT. From left to right: histograms with intermittent-irregular and logarithmic scales of ISI distribution, coefficient of serial correlation, graph of dynamic irregularity of spike activity (ordinate – mean dynamic coefficient of irregularity of firing pattern, in per cent; abscissa, different levels of significance of deviation from mean coefficient: $p < 0.01$, $p < 0.05$, and $p < 0.1$). From top to bottom: values correspond to initial activity of neuron, appearance of long-term regularization of discharge (injection of NT in a dose of 50 μl), and appearance of bursting activity with an increase in volume of NT up to 80 μl (bottom row).

character, with dominance of intervals within the regions of values 1.5-5, 250-400, and over 1000 msec (Fig. 2, bottom row). An increase in the volume of NT injected by a further 20-50 μl caused disintegration of the firing pattern of the neuron, manifested as the disordered polymodal character of ISI distribution on the histograms, and was accompanied by a change in the basic parameters of activity. This same disordered state of the spike trains persisted until the end of the experiment in all neurons recorded along the track of the microelectrode, which constituted a group of cells with polymodal ISI distribution (Table 2). Comparison of the data given in Tables 1 and 2 shows that NT, depending on its dose, causes reorganization of the firing pattern of neurons in area CA3 of HP in animals subjected to food deprivation for 24 h. This conclusion is confirmed by a study of the dynamics of spike activity of single neurons during a stepwise increase in the volume of NT injected (Figs. 1 and 2). Thus the neuroimmunomodulating action of NT (within a certain concentration range) at the level of cells in area CA3 of HP is manifested as a change in their plasticity, in the form of long-term regularization of spike generation with short periods of inhibition, and in the form of highly structured burstlike activity. Metabolic reactions evoked by NT evidently involve both mechanisms of induction of intercellular interaction and mechanisms determining the integrative activity of a single neuron and, consequently, capable of modifying the adaptive powers of the nervous system.

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EFFECT OF PARATHYROID HORMONE ON $^{45}Ca^{2+}$ ACCUMULATION NEUROSECRETORY CELLS AND ON BLOOD VASOPRESSIN LEVELS AFTER PARATHYROIDECTOMY AND INJECTION OF PARATHYROID EXTRACT

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UDC 612.447:616.831.4

KEY WORDS: parathyroid glands; calcium; parathyroid hormone; hypothalamo-hypophyseal system; vasopressin

Despite the important role of calcium ions in secretory processes the role of the Ca-regulating hormonal system in activity of the hypothalamohypophyseal neurosecretory complex still remains largely unstudied. Changes in functional activity of the supraoptic nucleus (SON) under conditions of specific parathyroprival hypocalcemia, discovered by the writers previously [1], suggests that under these circumstances the synthesis of vasopressin (VP) and its release into the blood stream are disturbed.

The aim of this investigation was to study the parathyroid hormone-dependent accumulation of $^{45}Ca^{2+}$ in hypothalamic neurosecretory cells and to determine the blood BP level after parathyroidectomy and administration of parathyroid extract.

EXPERIMENTAL METHOD

Experiments were carried out on 55 male albino rats weighing 180-200 g, divided into four groups: 1) intact (control), 2) parathyroidectomized by electrical coagulation 5 days before the experiment, 3) animals receiving parathyroid extract intramuscularly in a dose of 0.5U/100 g body weight daily for 7 days, 4) animals receiving the same dose of parathyroid extract but in a single injection. The animals of group 4 were decapitated 30 min after injection of the hormone. The total serum calcium concentration was determined spectrophotometrically, and ionized calcium was determined on a Kone-Microlit ion-selective analyzer (Finland), inorganic phosphorus by means of the Bio-La-Test set of reagents (Czechoslovakia), and VP by radioimmunoassay using kits from "Buhlmann Laboratories" (Switzerland). To study parathyroid hormone-dependent entry of $^{45}Ca^{2+}$ into neurosecretory cells, the anterior hypothalamic region was separated, weighed,

Department of Normal Physiology, Erevan Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR G. N. Kryzhanovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 113, No. 3, pp. 230-232, March, 1992. Original article submitted September 17, 1991.

