CHARACTER OF FORMATION OF A GENERATOR OF PATHOLOGICALLY ENHANCED EXCITATION (EPILEPTIC FOCUS) IN THE RAT SENSOMOTOR CORTEX DURING REPETITIVE ELECTRICAL STIMULATION

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The appearance of a generator of pathologically enhanced excitation (GPEE) is a typical pathological process in the brain which lies at the basis of various nervous disorders [1]. The study of the conditions, mechanisms of onset, and particular features of activity of different GPEE is therefore very important both for the general pathology of the nervous system and for special neurology.

A typical expression of GPEE in the cerebral cortex is an epileptic focus, which can be induced by various chemical epileptogens applied locally. However, each of these epileptogens makes its own specific contribution to the mechanisms of onset of the epileptic focus [11]. The aim of the investigation described below was accordingly to study the formation and activity of a GPEE (epileptic focus) in the cerebral cortex under the influence of direct electrical stimulation (ES) and of natural synaptic (transcallosal) stimulation of the homotopic region of the contralateral cerebral cortex (a so-called mirror focus) coupled with it. A special feature of the ES applied was that it consisted of several consecutive series of repetitive electrical stimulations [9]. This type of repetitive ES (RES) is close to that used in kindling [6] and in order to obtain long-term posttetanic potentiation (LTP) [4]. However, effects arising under such circumstances differ from the effects of kindling and LTP and have a number of distinguishing features.

The appearance of a prolonged after-discharge (AD) served as the criterion of GPEE formation, with the characteristic feature of a self-supported activity [1].

EXPERIMENTAL METHOD

Experiments were carried out on 150 noninbred male rats weighing 280-300 g. Under superficial ether anesthesia the animal's head was fixed in a stereotaxic apparatus and the cranial bones were trephined in the region of the right and left sensomotor cortex (A = 1 mm from the bregma, L = 1.5 mm from the median raphe), leaving the dura mater intact. The animals were then immobilized with suxamethonium, which was injected intraperitoneally in a dose of 5 mg/kg, and artificial ventilation was applied. Repetitive electrical stimulation (RES) of a region of the cortex of the right cerebral hemisphere was carried out through the intact dura by means of a coaxial steel electrode with external diameter of 2 mm and internal of 1 mm. In the zone of RES and in the symmetrical region of the opposite hemisphere silver ball electrodes were applied to the dura and the electrocorticogram (ECoG) was recorded on a polygraph ("Nihon Kohden," Japan). In some experiments the ECoG also was recorded in the occipital cortex of the left and right hemispheres.

The experiments began 20-30 min after application of the electrodes with determination of the threshold value to induce direct and transcallosal responses (DR and TCR respectively), recorded in the above-mentioned zones of the cortex. For this purpose the strength of stimulation (unipolar square pulse 0.1 msec in duration) was increased in steps of 0.5 V until DR, and subsequently TCR were twice the magnitude of the spontaneous ECoG. This stimulus strength was taken as the threshold. RES with twice the threshold strength for evocation of TCR was used in the experiments.

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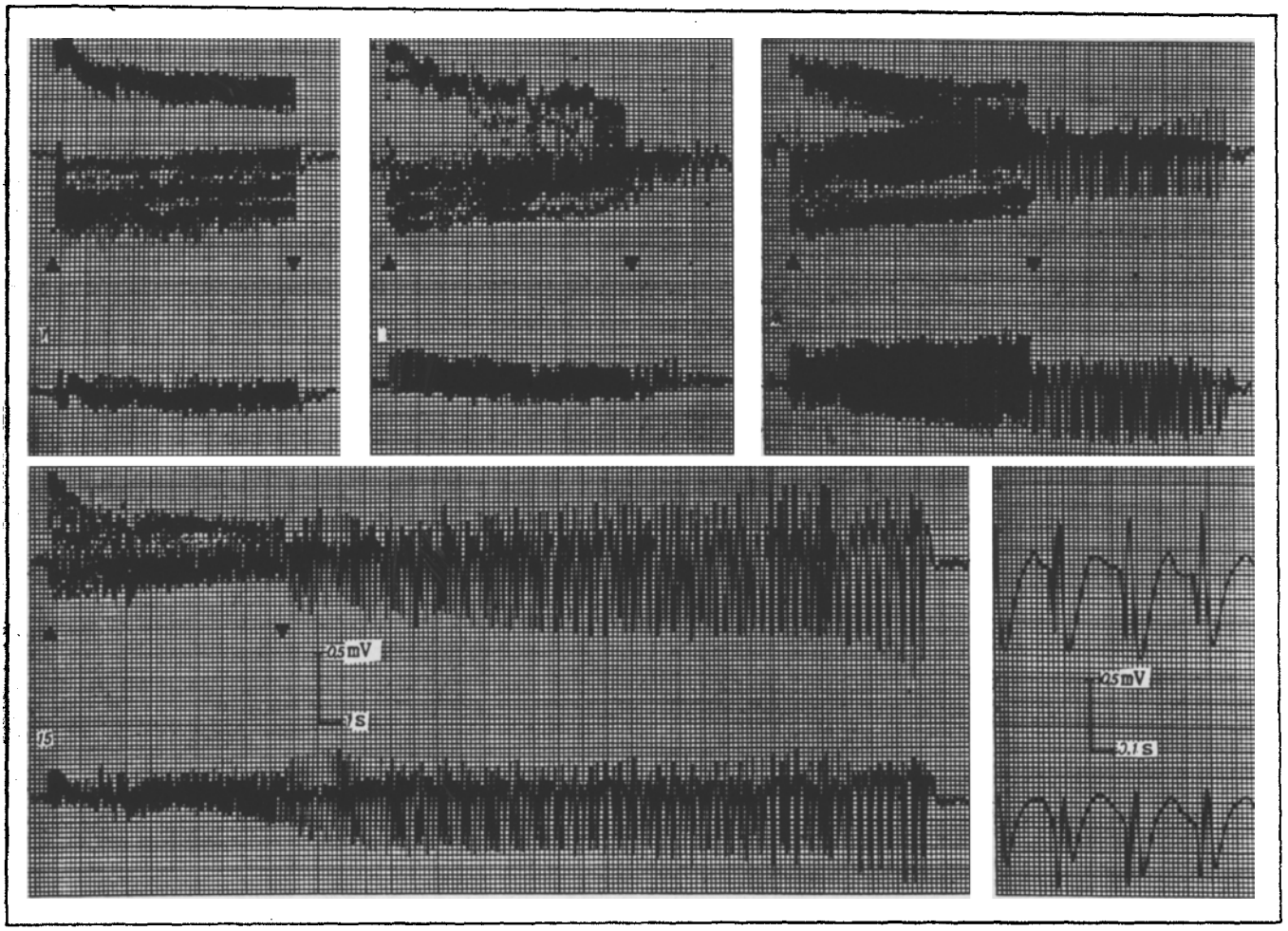

Fig. 1. Dynamics of AD formation in rat sensomotor cortex during repeated RES. Top trace denotes ECoG in stimulated region of right hemisphere; bottom trace shows ECoG in symmetrical zone of left hemisphere. Arrows pointing upward and downward mark beginning and end of RES. 1, 5, 9, and 15) Serial numbers of RES series.

The first series of RES was applied 15 min after determination of the threshold strength to evoke TCR (T) and had the following characteristics: intensity of a single stimulus 2T, duration 0.1 msec, frequency 8 Hz, total duration of RES 10 sec. Each subsequent series of RES began 10 min after restoration of the spontaneous ECoG.

On traces of the ECoG the amplitude of DR, TCR, and the spike-wave complex (maximal amplitude of the negative-positive wave), and the duration of AD (from the beginning of the first spike-wave complex to the end of the last) were measured. During analysis of the results, standard statistical methods (Student's t test, Fisher's F test) and computer analysis of the character of distribution of durations of AD (IBM PC, "Statgraf" program, USA).

EXPERIMENTAL RESULTS

A single electrical stimulation of the sensomotor cortex evoked both PR and TCR; to produce a TCR, a stronger (by 75%) stimulation was needed. During a single series of RES changes in the amplitude of both PR and TCR were observed. The trend of the changes in these potentials could differ (compare changes in PR and TCR during the ninth series of RES in Fig. 1). No regular post-tetanic potentiation or inhibition of PR and TCR could be discovered on repetition of RES.

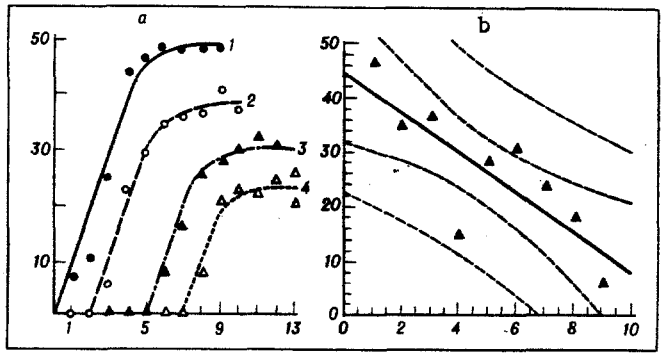

Fig. 2. Changes in duration of AD during successive RES of sensomotor cortex. Abscissa, serial number of RES; ordinate, duration of AD (in sec). a) Dependence of duration of AD on time of appearance of first AD. Curves indicate increase in duration of AD in subgroups of animals with appearance of the first AD after the 1st (1, n = 25), 3rd (2, n = 18), 6th (3, n = 10), and 8th (4, n = 10) RES. After AD began to appear their duration increased progressively in all groups during 4-5 RES and then stabilized. A similar relationship was observed in rats with the appearance of AD after the 2nd (n = 20), 4th (n = 15), 5th (n = 18), 7th (n = 14), and 9th (n = 20) RES, b) negative correlation between duration of stable AD and time of appearance of first AD. Filled triangles denote mean duration of stable AD for subgroups indicated above. Coefficient of correlation —0.82. Broken lines denote confidence interval.

During repeated RES self-maintained AD appeared, consisting of a series of spike-wave complexes, whose amplitude and frequency increased during repetition of RES (Fig. 1). In most cases the amplitude of the spike-wave complexes was less in the left than in the directly stimulated right hemisphere. In five rats AD in the left hemisphere stopped sooner, and in four they lasted longer than in the primary focus on the right. Wide spread of AD over the cerebral cortex was not observed: in the occipital cortex AD in the form of a spike-wave complex could not be recorded on either the left or the right side.

After the appearance of the first AD, the duration of which did not exceed 1-2 sec in 60% of rats studied, an increase in the duration of AD was observed during four or five consecutive series, after which RES began to evoke an AD of about equal duration (stable AD, Fig. 2a). Computer analysis revealed an asymmetrical distribution of duration of stable AD in the group of animals studied (gamma-distribution), with significant predominance of AD with a duration of the order of 10 sec (Fig. 3).

Comparison of the curves showing dependence of the duration of stable AD on the number of series of RES in animals of different groups shows that they all were S-shaped, and differed in the number of series of RES required to evoke the first AD and the duration of stable AD. Negative correlation was found between the two last values (Fig. 2b, coefficient of correlation —0.82, $p < 0.05$). This means that the easier it was to evoke AD in the cortex by means of RES, the longer the duration of the stable AD arising in the course of repeated RES.

Repetitive stimulation of brain structures, depending on the conditions of stimulation, can evoke prolonged post-tetanic potentiation [3, 4], and also a steady-state increase in predisposition to seizures (kindling) [6]. In the present investigation repeated RES did not lead to any significant potentiation of the amplitudes of PR and TCR in the rat sensomotor cortex. Potentiation of evoked responses has been described in the visual cortex [4], but not to short (as in the present investigation) repeated RES (10 sec), but after long, continuous stimulation for 1 h. The pattern of onset of long self-maintained AD thus revealed points to the formation of a GPEE under the influence of periodic repetitive ES. Significant changes in predisposition to seizures discovered in the rats 24 h after a series of RES (the data are given in a subsequent publication) enable these changes to be regarded as a manifestation of synaptic readjustments of the neurons and their connections in the structure of the GPEE.

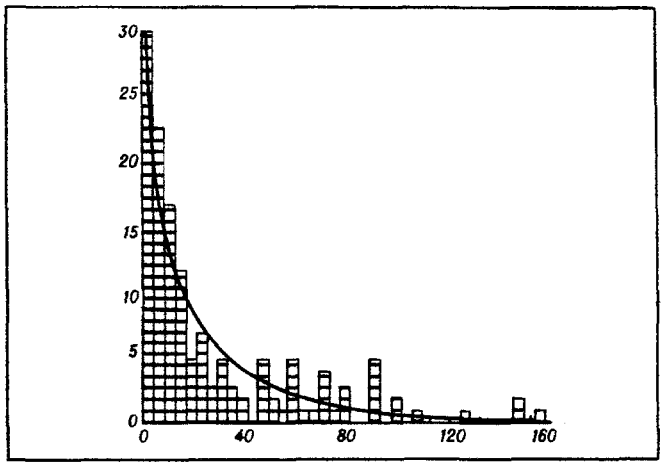

Fig. 3. Histogram of distribution of durations of stable AD in sensomotor cortex of 150 noninbred male rats. Abscissa, duration of AD (in sec); ordinate, number of animals with AD of a particular duration. Long AD (duration over 30 sec) were considered stable if their duration remained unchanged during 3-5 repetitions of RES. Short AD remained unchanged for 20 RES. Data shown for AD appearing after 7th series of RES. Curve superposed on histogram corresponds to gamma distribution.

The restricted spread of the spike-wave complexes over the cortex indicates a cortical origin of the activity recorded in the case when rhythmic activity was generated by neurons of the nonspecific thalamic nuclei, and activity of the spike-wave type can be derived from widely separated regions of the cerebral cortex [2, 8]. Analysis of seizure activity showed that the primary GPEE was located in the region of stimulation, and that a secondary dependent GPEE appeared in the synaptically stimulated region. The fact that although AD induced synaptically in the homotopic region of the sensomotor cortex of the opposite hemisphere corresponded to AD in the stimulated region, they nevertheless differed from the latter in a number of features, pointing to the role of intrinsic mechanisms of regulation in the generation of responses to synaptic stimulation from the primary GPEE. The GPEE was evidently formed by neurons mainly in cortical layers IV-V, which have a lower threshold of epileptization [5].

The character of dependence of the duration of AD on the number of series of RES indicates that a cumulative process, characterized by a threshold and saturation, is involved in the formation of AD. The most likely candidate for the role of cellular mechanism triggering seizure activity of neurons is the entry of Ca^{2+} ions into them [7]. However, this cannot explain synchronization of activity of the neuron population and the prolonged retention of seizure activity in the cortex purely on account of cellular mechanisms. An important role in synchronization processes is played by interneuronal interaction: synaptic positive feedback [10] and a number of extrasynaptic factors (lowering of the extracellular calcium level, elevation of the extracellular potassium level, etc. [12, 13]). The whole complex of these mechanisms determines the particular features of activity of the GPEE and, in particular, its self-supporting activity.

LITERATURE CITED

1. G. N. Kryzhanovskii, Determinant Structures in Pathology of the Nervous System [in Russian], Moscow (1980).
2. S. Ochs, Principles of Neurophysiology [Russian translation], Moscow (1969).
3. A. Baranyi and M. Szente, Brain Res., **423**, 378 (1987).
4. R. L. Berry, T. J. Teyler, and H. Taizhen, Brain Res., **481**, 221 (1989).
5. B. W. Connors, Nature, **310**, 685 (1984).
6. G. V. Goddard, D. C. McIntyre, and C. K. Leech, Exp. Neurol., **25**, 295 (1969).
7. U. Heinemann and B. Hamon, Exp. Brain Res., **65**, 1 (1986).
8. H. H. Jasper, Handbook of Physiology: Neurophysiology, Vol. 2, Washington (1960), p. 1307.
9. J. Mares, P. Benes, P. Mares, et al., Physiol. Bohemoslov., **32**, 30 (1983).

10. R. Miles, P. K. S. Wong, and R. D. Traub, Neuroscience, **12**, 1179 (1984).
11. D. A. Prince and D. Farell, Neurology (Minneapolis), **19**, 309 (1969).
12. C. P. Taylor and F. T. Dudec, J. Neurophysiol., **52**, 143 (1984).
13. Y. Yaari, A. Konnerth, and U. Heinemann, J. Neurophysiol., **56**, 439 (1986).

LIPID PEROXIDATION IN THE KIDNEYS OF RATS WITH NEPHRITIS CAUSED BY NEPHROTOXIC SERUM AND WITH PROTEINURIA INDUCED BY ALBUMIN LOADING

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Evidence has now been obtained to demonstrate the important role of free oxygen radicals (FOR) in the pathogenesis of some experimental models of immune damage to the kidneys: nephrotoxic nephritis (NTN) [11], Heymann's passive nephritis [12], and rejection of a transplanted kidney [10]. It has been shown that FOR can be produced by both polymorphs and monocytes infiltrating the glomerulus at different stages of immune damage [7, 11] and by activated mesangial cells of the glomerulus [5]. One of the main mechanisms of realization of the damaging action of FOR is their ability to initiate lipid peroxidation (LPO) of cell membranes.

It was accordingly decided to investigate LPO in kidney tissue in one form of immune injury, namely NTN induced by antirenal serum. Since NTN is characterized by considerable proteinuria, to assess its effect on LPO processes in the kidneys their activity was determined in rats with proteinuria induced by a short but massive load of exogenous protein, when no visible immune reactions can be found in the kidney tissue [9].

EXPERIMENTAL METHOD

Experiments were carried out on 61 male Wistar rats weighing initially 150-200 g. Of the total number of rats 20 had NTN, nine had proteinuria induced by injection of large quantities of protein, and 32 rats served as the control. NTN was induced by injecting nephrotoxic serum (NTS) into the femoral vein in a dose of 0.8 ml/100 g body weight on 1 or 2 consecutive days. NTS was obtained by immunizing a rabbit with the glomeruli of a rat kidney [3]. Protein loading and consequent proteinuria were produced by intraperitoneal injection of human albumin, dissolved in physiological saline, in a dose of 1.5-2 g protein per rat weighing 200 g, on 3 consecutive days [9].

In the experiments of series I activity of LPO processes was studied in the kidneys at different stages of development of NTN: 30 min and 3 h after a single injection of NTS (six and three rats respectively), and on the 4th and 16th days after the first of two injections of NTS (six and five rats respectively). The control consisted of 25 rats receiving normal rabbit serum instead of NTS.

In the experiments of series II LPO activity was determined in the kidneys of nine rats after three daily injections of protein, and in seven control rats receiving physiological saline intraperitoneally.

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