CHANGES IN SENSOMOTOR CORTICAL PEPTIDE LEVELS IN THE RAT BRAIN ON THE APPEARANCE OF POSTSTIMULUS PAROXYSMAL DISCHARGES

G. N. Kryzhanovskii, V. K. Lutsenko, N. N. Khlebnikova, T. V. Goryacheva, and M. Yu. Karganov

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Repetitive electrical stimulation (ES) of the sensomotor cortex of the rat brain by low-frequency bursts of stimuli leads to the formation of a generator of pathologically enhanced excitation (GPEE) in the cortex [1], whose activity is manifested as paroxysmal after-discharges (AD) of the spike–wave type [4]. One possible mechanism of the developing predisposition to generation of paroxysmal AD by the brain may be changes in the activity and state of the pro- and antiepileptic systems. During kindling, the onset of a state of increased predisposition to seizures correlates with the appearance of proepileptogenic substances of peptide nature in the brain [5], and changes in brain levels of Met-enkephalin [16] and somatostatin (SS) [8]. Met- and Leu-enkephalin, when injected intraventricularly, themselves induce epileptiform discharges in the EEG [14, 15]. On the other hand, there is evidence of the antiepileptic effects of the endogenous opioid system [6]. It is becoming more and more evident that different CNS peptides have different roles in epileptogenesis [6], and conclusions can be drawn only with respect to concrete forms and stages of appearance of seizures.

The aim of this investigation was to study the role of peptides in the genesis of epileptiform activity of this type in the cerebral cortex, namely paroxysmal AD of the spike–wave type, caused by repetitive ES of the sensomotor cortex [4], and to determine whether endogenous peptide pro- and anticonvulsants can appear in the brain of rats with a GPEE, together with accompanying changes in Met-enkephalin, Leu-enkephalin, and SS levels.

EXPERIMENTAL METHOD

Noninbred male rats weighing 280-300 g were used. The method of determination of thresholds for evoking direct and transcallosal responses and the method of creating a GPEE and recording the ECoG were described previously [4]. Tissue samples (4-6 mg) of the sensomotor cortex from the zone of ES (ZES) in the right hemisphere and the zone of synaptic stimulation (ZSS) of the left hemisphere were removed by means of a cold metal spoon and kept at −20°C. If a GPEE was present, the brain tissue was removed 1-2 min after the end of the 3rd prolonged (30 sec) AD. Tissue samples from ten rats were pooled (for the left and right hemispheres separately), and the tissue was homogenized and extracted with methanol [2]. Portions (0.8 ml) of methanol extracts of sensomotor cortex from ZES and ZSS were dried on a rotary evaporator. In a separate series of experiments, concentrations of Met-enkephalin, Leu-enkephalin, and SS were determined in the extract by radioimmunoassay (kits from "Incstar," USA). In this case, hot 1 M acetic acid was used as the extracting medium [3].

The effect of cerebral cortical extracts from rats (intact and with a GPEE) on the dynamics of AD formation in the recipients was studied on 104 noninbred male rats weighing 250-350 g. Lyophilized samples were dissolved in 0.9% NaCl solution so that 5 μl of solution contained 40 μg of "protein."

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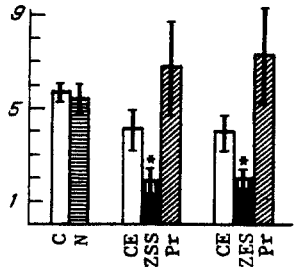

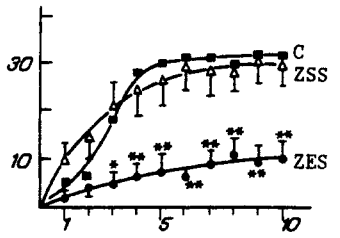

Fig. 1 Fig. 2

Fig. 1. Effect of donors' brain extracts on "latency" of appearance of AD in recipients' sensomotor cortex. Ordinate, number of series of electrical stimulations needed to induce first AD. C) Control (n = 65); N) naloxone (n = 7); ZES and ZSS) tissue extracts from sensomotor cortex from zone of electrical stimulation and zone of synaptic stimulation of rats with GPEE (n = 18 and n = 20 respectively); Pr) extracts from above-mentioned brain regions after proteolysis (n = 5 and n = 5); CE) extracts from homologous regions of cortex of intact animals (n = 16 and n = 16). *) Significant ($p < 0.01$) reduction of "latency" of appearance of AD compared with control (C).

Fig. 2. Effect of tissue extracts from zone of electrical stimulation (ZES) and zone of synaptic stimulation (ZSS) of brain of rats with GPEE on duration of paroxysmal AD in sensomotor cortex of recipient animals. Ordinate, duration of AD (sec); abscissa, number of bursts of electrical pulses. C) Dependence of duration of AD on number of series of electrical stimulation in control (n = 150); ZES and ZSS) the same after application of extracts of sensomotor cortex from corresponding brain zone to recipient's cortex (n = 18 and n = 20 respectively). Starting with the third series of electrical stimulation, curve for ZES differs significantly from control: *$p < 0.05$; **$p < 0.01$.

Protein was determined by Bradford's method [7]. In some experiments the solutions thus obtained were incubated before use with pronase ("Calbiochem," USA; 0.1 mg/ml), as described previously [3]. The test solutions of tissue extracts or physiological saline, in a volume of 5 μl, were applied to the surface of the hemisphere from a syringe after puncture of the dura mater. The electrophysiological experiment began 20-25 min after application of the solutions. The results of the electrophysiological studies were recorded in the recipients in precisely the same way as in donors [4]. When the effect of naloxone on the characteristics of AD was investigated, naloxone ("Du Pont," USA) was injected intraperitoneally in a dose of 1 mg/kg 20 min before the beginning of the first series of ES. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Application of a solution of cortical extracts from ZES or ZSS of rats with a GPEE to the surface of the ipsilateral cortex of the recipient sharply reduced the latent period of the first paroxysmal AD. In this case, evocation of an AD required a series of ES 2-3 times smaller than in the control (Fig. 1). Extracts of brain tissue from healthy animals had a similar action, although it was much weaker (differences from the control not significant, see Fig. 1).

To determine the possible contribution of the peptide to the proconvulsive action of the brain extract from rats with GPEE, the extracts were subjected to the action of peptidases (pronase), which degrade peptides. After proteolysis, brain extracts from rats with a GPEE completely lost their proconvulsive activity (Fig. 1).

TABLE 1. Changes in Neuropeptide Concentrations in Sensomotor Cortex of Rats with GPEE Compared with Control

Name of peptide	Number of animals	Hemisphere			
		stimulated	p	opposite	p
Leu-enkephalin	9	44,5±15,3	—	23,1±10,3*	0,01
Met-enkephalin	10	118,5±42,2	—	91,4±14,0	—
Somatostatin (SS)	19	106,0±14,7	—	101,0±14,1#	0,05

Legend. All results shown as percentages of concentrations of Leu- and Met-enkephalin and SS in corresponding region in control (taken as 100%); *) significant decrease compared with control, #) significant interhemispheric difference. Number of animals in control groups: 9, 9, and 15 (for Leu- and Met-enkephalin and SS respectively).

By contrast with the virtually identical proconvulsive effect of extracts from the two hemispheres of rats with GPEE on the latency of AD in the recipients, the duration of AD was modulated differently by the extracts. The tissue extract from ZES of rats with a GPEE caused shortening of AD throughout the period of study of the characteristics of AD (Fig. 2). Conversely, the extract from ZSS, when applied to the ipsilateral hemisphere of the recipient, had no significant effect on the duration of AD (Fig. 2).

Tissue extracts from the left and right hemispheres of healthy animals contained about equal amounts of Met- and Leu-enkephalin and SS. In animals with a GPEE no significant changes in the Met-enkephalin concentration could be found either in the electrically stimulated right, or the synaptically activated left hemisphere (Table 1). In the case of SS, a significant ($p < 0.05$) but very small degree of interhemispheric asymmetry (about 5%) was found. In rats with a GPEE the Leu-enkephalin concentration fell sharply in both hemispheres, and in the synaptically activated left hemisphere the result was highly significant ($p < 0.01$).

In a separate series of experiments the effect of naloxone, a blocker of opiate receptors on the latency of appearance of paroxysmal AD was investigated. No effect of naloxone on the latency of AD could be detected.

Inactivation of the active principle of brain extracts from rats with a GPEE after proteolysis, the method of extraction of the tissue to ensure extractions of peptides and not of proteins, and the observed thermostability are evidence in support of the peptide nature of proconvulsive factors.

The combination of the proepileptic action of the extract from ZES, with its ability to shorten the duration of the paroxysmal AD in recipients, can be explained by the presence of several peptides in the extract, with different effects on cortical excitability.

Meanwhile it must be recalled that individual peptides can have a dual effect. For example, opioid peptides possess both a proconvulsive action and ability to inhibit spike activity. The first action is the result of presynaptic depression of inhibition, i.e., disinhibition [15, 11], whereas the second is a direct postsynaptic action [7]. It is possible that peptides extractable from the hyperactivated cerebral cortex can also cause depression of inhibitory processes in the region of their application to the cortex.

The proconvulsive action of extracts of both cerebral hemispheres of rats with a GPEE cannot be explained by a change in the concentrations of Met- and Leu-enkephalin and SS in the extracts. The SS concentration in the extracts rose, but not significantly, that of Met-enkephalin was unchanged, and the Leu-enkephalin level fell (Table 1). The fall in the Leu-enkephalin level in the brain of rats with a GPEE is in agreement with proof of the considerable release of endogenous opioids during seizure activity [7]. The absence of any effect of naloxone on the latency of appearance of AD leads us to question the contribution of other opioid peptides, not studied in this investigation, in the mechanism of AD in the sensomotor cortex of the rat brain. Judging by the results of investigations with naloxone, the opioid peptidergic system likewise is not involved in the genesis of paroxysmal activity due to hereditary factors in some animals [8, 9], and in focal epilepsy in man [10].

In animals with a GPEE in the right, electrically stimulated, hemisphere the concentration of peptide factors with a strong antiepileptic action also is increased. Even a single application of cortical extract from ZES leads to marked shortening of AD throughout the period of observation, whereas the tissue extracts from ZSS does not induce this type of action.

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