ANTICONVULSANT PROPERTIES OF PEPTIDE $ACTH_{4-7}$Pro-Gly-Pro REVEALED DURING AMYGDALOID KINDLING AND AUDIOGENIC EPILEPSY IN RATS

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Among the wide range of centrally acting agents, the effect of regulatory peptides on epileptic processes has received less study [3] than the role of mediators and receptors for them. There is evidence that opioid peptides are involved [7, 12], but as regards ACTH fragments and their analogs the results have been contradictory [9, 11]. Because of the special features of the pharmacologic action of ACTH fragments [1, 2], we suggested that the protected analog $ACTH_{4-7}$Pro-Gly-Pro (PGP), which has prolonged action [1], could affect the formation of seizure activity of the brain.

In the investigation described below anticonvulsant properties of $ACTH_{4-7}$PGP were revealed in two experimental models: reflex audiogenic epilepsy [4] and the phenomenon of amygdaloid kindling, induced by electrical stimulation of one amygdaloid nucleus in the rat brain, and connected with changes in the noradrenergic [6, 10] and cholinergic [5] neurotransmitter systems.

EXPERIMENTAL METHOD

Amygdaloid kindling was studied in noninbred male albino rats weighing 200-250 g for a maximum of 39 days (18 rats in the experimental and 10 in the control groups). For stimulation, bipolar nichrome electrodes were implanted into the amygdala, taking stereotaxic coordinates from the atlas of Fifkova and Maršala. To record epileptogenic activity, electrodes were implanted into the contralateral amygdala, the dorsal hippocampus, and the cortex of the frontal lobe. All operations were performed under pentobarbital anesthesia. Stimulation of the amygdala began after 2 days and epileptogenic activity was recorded in unrestrained animals. The initial strength of stimulation was 200 μA. Square pulses 1 msec in duration and with a following frequency of 100 Hz were used. With generalization of the seizure activity the duration of the volleys of after-discharges (AD) increased (from 10.4 ± 1.6 to 23.4 ± 6.7 sec by the 4th day); the threshold of evocation of AD was lowered. At this stage of kindling, when single stimulation of the amygdala evoked a behavioral kindling response and generalization of AD, 50 μg/kg of $ACTH_{4-7}$PGP (synthesized at the Institute of Molecular Genetics, Academy of Sciences of the USSR) was injected intraperitoneally. Rats of the control group, receiving the same schedule of stimulation, were given an injection of physiological saline. The action of the peptide was compared with that of known blockers of epileptic activity: pentobarbital (20 mg/kg) and calcium valproate (from "Germed," East Germany; 100 mg/kg intraperitoneally). At the end of the experiment the location of the electrode tips was verified morphologically. The effect of $ACTH_{4-7}$PGP on audiogenic epilepsy was studied in 27 female Krushinskii–Molodkina (KM) rats, genetically predisposed to audiogenic epilepsy, weighing 250 g. The control and experimental animals were subjected (at intervals of 1 day) to acoustic stimulation with a strength of 112 dB. The response was evaluated in points on Krushinskii's scale [4]. $ACTH_{4-7}$PGP in a dose of 50 μg/kg was injected intraperitoneally 2 h before acoustic stimulation, or at the same time on days without acoustic stimulation.

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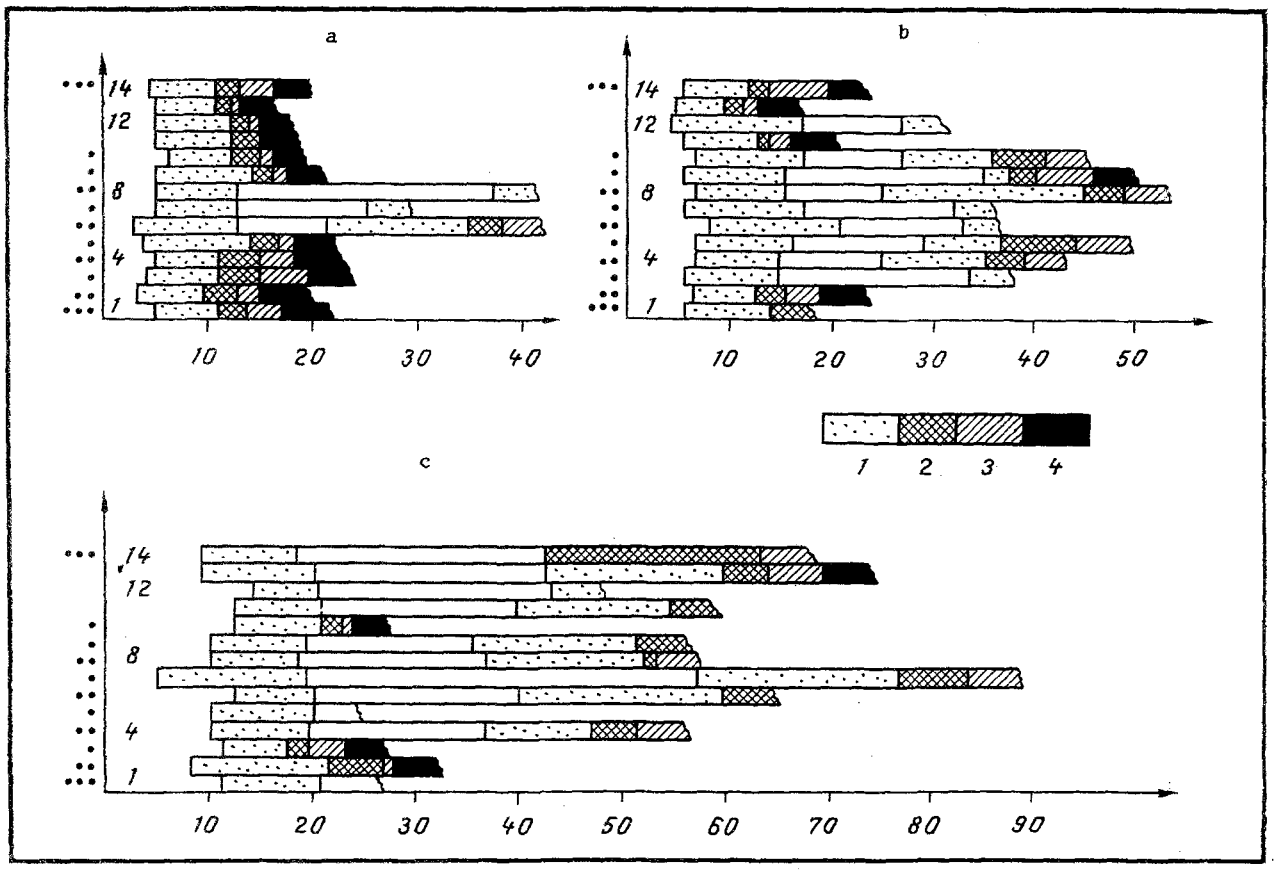

Fig. 1. Effect of $ACTH_{4-7}PGP$ on duration of stages of audiogenic seizure in KM rats. Abscissa, days of experiments; ordinate, time, sec. Dots indicate injection (intraperitoneal) of peptide in a dose of 50 μg/kg. Interval of 3 days between 12th and 13th experiments. Stages named in accordance with Krushinskii's classification [4]: 1) motor excitation, running, jumping; 2) development of stuporose state with falling on abdomen; 3) clonic seizures with falling on the side; 4) tonic seizure, transient breath holding. a, b, c) Individual animals. Acoustic stimulation stopped on the appearance of stage 2.

EXPERIMENTAL RESULTS

Experiments of KM rats showed that $ACTH_{4-7}PGP$ effectively abolished certain elements of the seizure response if the peptide was injected either the day before or 2 h before provocation of the seizure. After a long interval (9 months) the first acoustic stimulation was given and the identical number of rats (30%) achieving a seizure response of 4 points was observed in the experimental and control groups. Immediately after injection of the peptide, rats of the experimental group responded in 100% of cases to acoustic stimulation with a reaction assessed at 4 points, but in the control group a clonicotonic seizure was observed in only one rat. Thus, the first application of $ACTH_{4-7}PGP$ could actually potentiate the seizure response. Further daily administration of the peptide led to a gradual reduction of the audiogenic seizure response. This effect appeared cumulative until the 4th or 5th acoustic stimulation, against the background of the 6th-10th injections of the peptide. Although the latent period of stage 1 was unchanged its duration was increased by 2-3 times; moreover, the initial motor activity of the rats was replaced by immobility and no seizure developed (Fig. 1). The same response was observed in five of the nine rats. In the remaining animals the anticonvulsant action of the peptide was expressed as abolition of the 4th stage or its delayed appearance. Withdrawal of the peptide, during continued acoustic stimulation, led to the appearance of a seizure in the rats, rated at 4 points. Resumption of $ACTH_{4-7}PGP$ administration (3 doses each of 50 μg/kg) led to lengthening of the latent period of the 3rd and 4th stages of the seizure response compared with those obtained during withdrawal of the preparation.

In the group of control animals acoustic stimulation led to the appearance of stage 4 and shortening of the latent periods of stages 3 and 4 in eight of the 10 rats. It can be

concluded from these results that $ACTH_{4-7}PGP$ has an anticonvulsant action which is phasic in character, depends on the individual sensitivity of the animals, and when given in a course of injections it changes the structure of the audiogenic fit by lengthening the latent periods and blocking the severest stages of the seizure.

Involvement of $ACTH_{4-7}PGP$ in the regulation of the seizure state of the brain also was demonstrated on the amygdaloid kindling model. The results demonstrated a significant decrease in the duration of AD in response to stimulation of the amygdala between 30 and 60 min after injection of the peptide ($p < 0.01$). Allowing for individual differences in the formation of kindling, the effect of the peptide also was evaluated in terms of delay of the actual kindling process. Complete blocking of AD also was found under the influence of the peptide for a period of 20-100 min, but this effect was observed only after 2 or 3 injections of $ACTH_{4-7}PGP$, and when thresholds of stimulation of the amygdala were low. These observations are in agreement with effects obtained on KM rats as regards the fact that $ACTH_{4-7}PGP$ can participate in the regulation of the seizure response, by delaying and inhibiting it. However, the peptide had no effect if the animal developed a strong motor seizure. Reducing the dose to 25 μg/kg in our experiments was ineffective. To compare effects in kindling experiments, calcium valproate was injected, inducing transient blocking of AD, stereotyped movements, and motor seizures.

It can also be concluded indirectly on the basis of data showing delayed extinction of changes in the human EEG in response to repetitive acoustic and photic stimulation [8] that ACTH fragments may have a possible anticonvulsant action. Contradictory data were obtained in experiments on animals. It has been shown that the ACTH/MSH analog OPC 2766, if injected intraperitoneally, depresses seizure activity in the amygdala evoked by kindling [9]. Conversely, after intraventricular injection of $ACTH_{4-10,1-10,4-9}$ analogs into rabbits enhancement of seizure discharges was found in the hippocampus, although fragments such as $ACTH_{1-4,4-11,1-13,1-17,1-24}$ were ineffective [11]. Systemic injection of $ACTH_{4-7}PGP$, as our data showed, weakens the seizure state, but the effect depends on the degree of development of the seizure process in the limbic structures of the brain.

It can thus be concluded that $ACTH_{4-7}PGP$ has an anticonvulsant action, but not on all components of seizure activity, both during kindling and during audiogenic epilepsy. Repeated administration of the peptide is effective.

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