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PROLONGED ACTION OF TUFTSIN ON PENICILLIN-INDUCED EPILEPSY

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UDC 616.853-092.9-085.31:547.96-036.8-7:616.831-073.97

KEY WORDS: peptide; tuftsin; epilepsy; brain structures; behavior; electroencephalogram.

In intact animals the effect of the tetrapeptide tuftsin (Thr-Lys-Pro-Arg) extends to nonspecific immunity, motor activity, and memory [1, 4, 6, 8]. In stress situations it possesses a stress-protective and antidepressant action [2, 3, 6], but in depressions it has an antidepressive effect. Meanwhile, the potential value of tuftsin in other forms of CNS pathology, including the epileptic state, has not yet been elucidated. This is an urgent problem because of the increasing interest of clinicians in this peptide, for if administered intranasally its effect is predominantly on processes in the CNS [5].

In the investigation described below the effect of a single dose of tuftsin on the EEG recorded from brain structures and on behavior of animals with experimental epilepsy, induced by large doses of penicillin, was studied. Particular attention was directed to the delayed effect of administration of a single dose of the peptide: observations on the EEG and behavior of the animals were made during the period of 3-4 days after administration of tuftsin.

EXPERIMENTAL METHOD

The experimental conditions envisaged two versions of tuftsin administration: against the background of frank epileptiform activity, associated with the action of penicillin, and before penicillin was given, i.e., its preventive action. Experiments were carried out on rats (180-200 g) with electrodes implanted into all parts of the cerebral

Brain Research Institute, Russian Academy of Medical Sciences, Moscow. Institute of Biological Research, Belgrade, Yugloslavia. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 114, No. 7, pp. 56-58, July, 1992. Original article submitted November 25, 1991.

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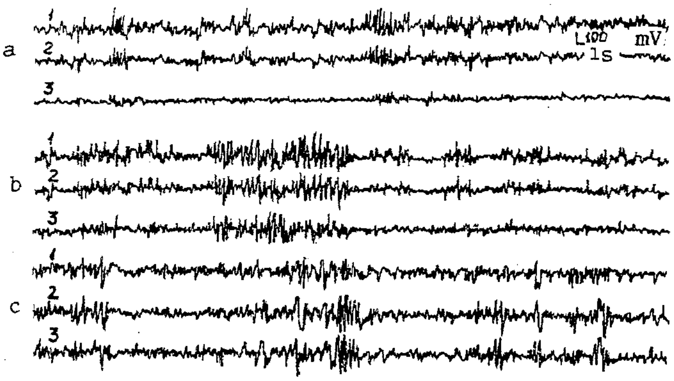

Fig. 1. EEG of brain structures of rat No. 1. 1) Right parietal cortex, 2) left parietal cortex, 3) motor cortex; a) after injection of penicillin, b) after injection of penicillin + tuftsin, c) after injection of tuftsin + penicillin.

cortex and the caudate nuclei. The experimental animals were divided into groups: 1) a control group of animals, receiving physiological saline intraperitoneally; 2) a group of experimental animals receiving penicillin (crystalline preparation of potassium benzylpenicillin, from "Galenika," Yugoslavia), in a dose of 100,000 U/kg body weight; 3) animals previously receiving penicillin, and having well-marked epileptiform activity and myoclonic seizures of the snout and neck, received 0.3 μg/kg tuftsin intraperitoneally; 4) animals receiving tuftsin 15-20 min after injection of penicillin, i.e., before the appearance of epileptiform activity and of myoclonic seizures. Administration of penicillin and tuftsin was accompanied by monitoring of the animals' EEG and behavior.

Behavior of the rats was studied in an open field, using an "Opto-Varimex" apparatus (Columbus, USA), and it was assessed in the form of movement time (MT), distance travelled (DT), i.e., motor activity, duration of stereotyped movements (ST), and their number (BSM). The last two parameters were regarded as characteristic of the rats' emotional state. The number of "rears" (VIB) was regarded as an indicator of the animals' investigative activity. The significance of changes in behavior in the open field was estimated by Wilcoxon's test. The behavior of the animals, and also the EEG were studied on the day of administration of tuftsin or other preparations and during the next 2-3 days.

EXPERIMENTAL RESULTS

Without embarking on a detailed description of the epileptiform discharges arising in the conscious rats after injection of penicillin, attention nevertheless must be paid to the fact that discharges themselves, by frequency of their appearance per unit time, duration of each discharge, and spike amplitude, were largely dependent on the animal's initial state [9].

In these experiments epileptiform cyclic discharges had a duration of 0.1-0.5 sec, they were in sequence with intervals varying from 8 to 20 sec, and they lasted 4-5 h (Fig. 1a), after which generalized epileptic seizures were observed, often leading to the animals' death. On the appearance of seizures the cyclic discharges were accompanied by myoclonic contractions of muscles of the animals' snout and neck. The particular features of the animals' behavior in this situation were a statistically significant reduction of investigative, emotional, and motor activity (Figs. 2b, 3b, and 4b).

Injection of tuftsin against this background led to an increase in frequency of the epileptiform cyclic discharges and to lengthening of the duration of each discharge to 2.5 sec (Fig. 1b). Meanwhile these cyclic discharges did not develop into generalized discharges. On this experimental day, with respect to the behavioral parameters used, the animals after receiving the injection of tuftsin (Figs. 2c, 3c, 4c) were virtually indistinguishable from those receiving penicillin alone. Next day (when epileptiform activity was less marked), the investigative activity and emotional manifestations of the animals receiving penicillin alone closely resembled their level in the control animals, or only their motor activity was depressed (Figs. 2b, 3b, 4b).

Fig. 2

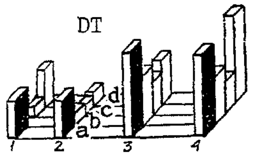

Fig. 3

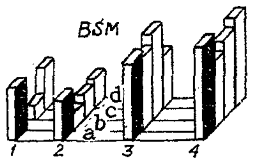

Fig. 4

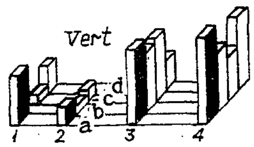

Fig. 2. Dynamics of parameters of animals' motor activity (DT – distance traveled). Variants of combination of preparations: a) control group (physiological saline, shaded black), b) penicillin, c) penicillin + tuftsin, d) tuftsin + penicillin, 1st and 2nd injections consecutively. Days of observation: 1st, 2nd, and 3rd. Scale – 10% in 27.5 mm. Units of measurement – ratio of results of a particular experiment to results obtained in intact animals, expressed in percent.

Fig. 3. Dynamics of emotional state of animals (BSM – number of stereotyped movements). Legend, scale, and units of measurement the same as in Fig. 2.

Fig. 4. Dynamics of animals' investigative activity (VERT denotes number of vertical rears). Legend, scale, and units of measurement the same as in Fig. 2.

On the 3rd day, the motor and investigative activity of the two groups of animals was depressed, but the emotional parameters were close to those in the control (Figs. 2b, c, 3b, c, 4b, c). Thus in the two variants of the experiments examined changes in the animals' behavior took place during a period of 3 days, they were undulating in character, and most marked on the 3rd day.

Consequently, injection of tuftsin into these animals did not change the trend of the pathological processes developing after injection of penicillin.

In the second variant of the experiments tuftsin was injected before the appearance of EEG signs of the action of penicillin. Under these conditions a different picture was observed: lengthening of the latent period of the appearance of the first epileptiform discharge (to 60-65 min). After the appearance of the first epileptiform discharge they followed one another more frequently (the intervals between discharges were shortened to 4-10 sec). The duration of each cyclic epileptiform discharge reached 2.5 sec (Fig. 1b). Meanwhile, in this variant of tuftsin administration also, these patients did not develop any fatal seizures even after 5-6 h. As regards the behavioral parameters in the open field, on the day of injection of the preparations in the order indicated, motor and investigative activity were significantly depressed, but the emotional parameters were not significantly lowered (Figs. 2d, 3d, 4d). Next day, all behavioral parameters of these animals were depressed (Figs. 2d, 3d, and 4d). On the 3rd day they returned closer to the control level. Thus in these animals, unlike those under the influence of penicillin alone, more significant changes of behavior took place on the 2nd day after injection of the preparations, but on the 3rd day all parameters were restored, a result which was not observed after injection of penicillin alone.

The results show that in penicillin-induced epilepsy the effect of tuftsin both on the EEG and on the behavior or rats depends on the order of administration of the preparations. When tuftsin was injection after epileptiform activity and myoclonic seizures had already developed, the epileptiform activity was intensified, but was not converted into generalized convulsions. The behavioral parameters on the day of tuftsin administration remained the same as before tuftsin was given (i.e., during the action of penicillin alone). On subsequent days either the motor activity only or all behavioral parameters of both groups of rats were depressed – the effect was undulating in character.

After preventive injection of tuftsin and its prolonged action a different picture was observed: on the 2nd day all forms of behavioral activity were depressed, although penicillin alone inhibited only motor activity; on the 3rd day all behavioral parameters had returned closer to the control level, although penicillin alone (without tuftsin) caused changes in all forms of behavioral activity. Under these circumstances, on the 1st day of the preventive action of tuftsin, epileptiform discharges with a longer latent period appeared, but there were no generalized convulsions (the latter occurred also when tuftsin was injected after epileptiform activity had already developed).

For tuftsin to exhibit a corrective effect against penicillin-induced epilepsy, its period of action must be sufficiently long, but the mode of administration of the peptide also is important for this effect – the most favorable result was given by the preventive use of tuftsin.

Probably tuftsin should be used over a long period of time and for preventive purposes.

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