ENHANCEMENT OF ACTIVITY OF AN EPILEPTOGENIC FOCUS IN THE FROG HIPPOCAMPUS BY KYNURENINS AND ITS DEPRESSION BY SEROTONIN

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Experiments on frogs with an epileptogenic focus produced by injection of 1000 units penicillin (0.4 μl) into the primordial hippocampus showed that preliminary injection of two kynurenins — quinolinic acid (QA, 0.1 μg) and kynurenin itself (K, 1 μg) — into the region of the focus or their injection into an already functioning epileptogenic focus led to an increase in the frequency of interictal epileptiform discharges and of electrographic correlates of fits on the EEG. Anthranilic acid (AA, 5 μg) had no effect on activity of the focus whereas serotonin (S, 1 μg) and 5-methoxytryptamine (1 μg) substantially depressed it. The provoking action of the kynurenins on epileptically predisposed brain neurons, it is suggested, may play an important role in the pathogenesis of epilepsy.

KEY WORDS: epilepsy; hippocampus; kynurenins; serotonin.

Until recently the only source of enzymic (tryptophan-pyrrolase, tryptophan-2,3-dioxygenase) formation of kynurenins from tryptophan was considered to be the liver. The problem of penetration of kynurenins into the brain or of their formation in the brain has not been raised [2], although many and varied neurotropic effects of kynurenins have been discovered [9]. In recent years tryptophan-pyrrolase activity has been discovered in the brain [5] and a kynurenin synthesized in brain tissue has been found [7]. Between 80 and 90% of tryptophan molecules, incidentally, follow the pathway of kynurenin formation.

Only two papers have so far been published [10,11] in which data are given on the ability of kynurenins to increase the excitability of the CNS through their direct central action.

The object of the present investigation was to study the effect of three kynurenins (D,L-kynurenin — K, quinolinic acid — QA, anthranilic acid — AA) on the activity of an epileptogenic focus in the frog hippocampus, i.e., on neurons with pathologically enhanced excitability, and to compare it with the effect of serotonin (S),* another important tryptophan metabolite, and of the S-mimetic 5-methoxytryptamine (mexamine, M).

The frog was chosen as the test object, first, because experiments on these animals for the stimulation of epilepsy and with injection of drugs into brain structures are technically easier to carry out than on warm-blooded animals and, second, S is the leading monoamine mediator in the frog brain [3] and it is to be expected that all processes connected with activation and inhibition of S-ergic systems would be manifested more clearly.

EXPERIMENTAL METHOD

Experiments were carried out in winter and spring on 92 frogs (Rana temporaria), chiefly males, weighing 35-40 g and living in the Leningrad Region. The frogs were anesthetized with 25% urethane solution (5 mg/g), the cranial bones were removed, and the hemispheres of the forebrain were exposed. Chemical and bipolar electrodes, glued together, were used for monopolar and bipolar recording of activity from the left and right primordial hippocampus. This ensemble was so designed that, when fixed in the electrode holder of an SÉZh-2 universal stereotaxic instrument the chemical electrode was inserted into the left and the bipolar electrode simultaneously into the symmetrical point of the right hippocampus. The chemical electrode, inserted into the left hippocampus, consisted of a cannula-needle (diameter 0.4 mm), insulated with transparent plastic, for injection of solutions into the brain, which also served as one of the two electrodes forming a

* Kynurenins have been shown to be antagonists of many of the neurotropic effects of serotonin [2].

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TABLE 1. Changes in Activity of Epileptogenic Focus in Hippocampus under Influence of Drugs Injected into Focus after Stabilization of Pathological Activity 50-60 min after Beginning of Experiment

Drug injected and dose, μg	No. of experiments	Interictal discharges (mean number/minute of recording EEG from 60th to 120th minute	Fits (number during 10 min of observation from 60th to 120th min of experiment)
Control	15	6,9‡	3,0‡
QA—0,1	6	25,0‡	5,6‡
K—1,0*	6	18,9‡	5,3‡
S—1,0*	6	1,58‡	0,2‡
M—1,0*	6	2,38‡	0,2‡
AA—5,0†	2	7,1‡	3,1‡

*Dose of 0.1 μg did not affect activity of focus in two experiments.
†Dose of 0.1 and 1 μg (each of two experiments) did not change activity of epileptogenic focus.
‡ $P < 0.001$.

TABLE 2. Changes in Activity of Epileptogenic Focus in Hippocampus after Preliminary Injection of Tryptophan Metabolites into Region of Focus (5 min before injection of penicillin)

Drug injected and dose, μg	No. of experiments	Interictal discharge (mean number per minute of recording EEG)		Fits (mean number during 10 min of experiment)	
		from 0 to 120th min of experiment	P	from 0 to 120th min of experiment	P
Control	15	12,5	—	2,8	—
QA—0,1	6	26,0	<0,001	3,8	<0,001
K—1,0	6	29,8	<0,001	4,7	<0,001
S—1,0	6	0	<0,001	0	<0,001
M—1,0	6	3,2	<0,001	0	<0,001
AA—5,0	2	13,0	>0,001	2,9	>0,001

bipolar chemical electrode. The second of the two electrodes consisted of a nichrome wire, 0.10 mm in diameter, insulated with transparent plastic and glued to the cannula. The bipolar electrode inserted into the right hippocampus of the frogs differed from the chemical electrode only in the fact that, instead of a cannula-needle, it incorporated a nichrome wire 0.18 mm in diameter. The reference electrode was a silver disc inserted into the mouth.

The chemical and bipolar electrodes were inserted into the brain to a depth of 0.5-0.6 mm, according to the coordinates of an atlas of the frog's brain [8], to reach the greatest concentration of hippocampal pyramidal cells. Hippocampal potentials were recorded on an ÉÉGU-16-0.2 16-channel electroencephalograph.

An epileptogenic focus was created in the hippocampus of the frogs by injection of 0.4 μl of a solution of the potassium salt of penicillin containing 1000 units of the antibiotic through the chemical electrode by means of a micromanipulator. The results of preliminary experiments showed that this dose of penicillin is the smallest to produce an epileptogenic focus in the hippocampus of frogs in all experiments.

For 120 min after injection of the penicillin the EEG was recorded for subsequent counting of single interictal discharges (bi-triphasic spikes or pointed waves) per minute of EEG recording during each 10-min interval of the experiment and the mean number of electrographic correlates of fits (a continuous prolonged paroxysmal discharge on the EEG), also during each 10-min interval of the experiment.

The drugs were injected into the location of the epileptogenic focus either 5 min before or 60 min after injection of penicillin into the hippocampus. The activity of the focus became stabilized 50-60 min after its formation. All solutions of drugs were injected into the hippocampus in a volume of 0.4 μl. The following

doses of the drugs were used: K 0.1 and 1 μg (0.25% solution, pH 2.8), QA 0.1 μg (0.025% solution, pH 5.1), AA 0.1, 1, and 5 μg (1.25% solution, pH 5.0), S 0.1 and 1 μg (0.25% solution, pH 3.7), and M 0.1 and 1 μg (0.25% solution, pH 5.8). The solutions of the drugs were made up in bidistilled water. In experiments on control frogs, bidistilled water (pH 6.9) was injected into the region of the epileptogenic focus, and changing the pH of the water over the range from 3.0 to 6.0 in different experiments did not affect the dynamics of epileptogenesis in the control animals.

The results were subjected to statistical analysis by means of the Wilcoxon — Mann — Whitney U-criterion.

EXPERIMENTAL RESULTS

The results given in Table 1 show that injection of K or QA into the region of an actively functioning, stabilized epileptogenic focus led to an increase in the frequency of the interparoxysmal epileptiform discharges of the EEG and in the number of fits in the frogs. Conversely, S and M considerably reduced the pathological activity of the focus. AA, in the doses tested, did not change the activity.

Preliminary injection of kynurenins S and M into the hippocampus (Tables 1 and 2) caused the same changes in activity of the epileptogenic focus in that structure as their injection into an already functioning focus described previously: QA and K provoked whereas S and M inhibited its pathological activity.

Under experimental conditions evidence was thus obtained of a direct effect of tryptophan metabolites of the kynurenin series on excitability of CNS neurons. It must be emphasized that the kynurenins differed in this respect: AA, under the conditions used, did not affect the excitability of the epileptically predisposed neurons whereas QA was about 10 times as active as K.

These experiments also showed that antagonism between kynurenins and S in their neurotropic effects [2] also extends to the effect of these tryptophan metabolites on activity of an epileptogenic focus.

The possibility cannot be ruled out that the provocative action of kynurenins on epileptically predisposed neurons may play a definite role in the pathogenesis of epilepsy, for increased excretion of kynurenins [4, 6], unconnected with any vitamin B_6 deficiency [6], has been found in patients with epilepsy.

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