EFFECT OF ACTIVATION AND DESTRUCTION OF THE SUPERIOR COLLICULI ON SEIZURE ACTIVITY IN RATS

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One of the mechanisms of suppression of activity of the epileptic system, which is a combination of hyperactive brain formations and the pathogenetic mechanism of the epileptic syndrome, is activation of the functionally antagonistic (antiepileptic) system [4]. Among the structures of the latter are included formations of the cerebellum [6], caudate nuclei [10], caudal reticular nucleus of the pons [5, 9], etc. Activation of the superior colliculi (SC) has been shown to depress seizure activity [11, 12]. The aim of this investigation was to study the mechanism of this effect on a model of a generalized epileptic syndrome induced by systemic injection of picrotoxin under conditions of hyperactivation of both SC. SC were activated by creating a generator of pathologically enhanced excitation (GPEE) in them [4] with the aid of penicillin and bicuculline The effect of destruction of SC on seizure activity also was investigated.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats (96 animals altogether) weighing 180-250 g. Each group included not less than seven animals. To create a GPEE in SC the sodium salt of benzylpenicillin (5-15 MU) and bicuculline acetate (25-50 ng), which were injected bilaterally under open ether anesthesia, taking coordinates from the atlas [13] (AP = −6.3; L = 2.0; H = 4.5), by means of a "Top" microinjector (India) in a volume of 1.0 μl and at the rate of 0.5 μl/min. Animals of the control group received an injection of 1.0 μl of 0.9% sodium chloride solution into SC under similar conditions. The testing injection of picrotoxin (2.0 mg/kg, intraperitoneally) was given 15-60 min after microinjection of the preparation into SC. Seizure reactions in the rats were observed in a chamber (30 × 45 × 20 cm) for 45 min after injection of picrotoxin. The intensity of the seizures was expressed in points, on the 5-point scale adopted previously [7]. The latent period of the first seizures and the number of animals with generalized seizure reactions also were determined. In a separate group (15 rats), to investigate the electrical activity of the brain, under hexobarbital anesthesia (100 mg/kg, intraperitoneally) constantan electrodes 0.15 mm in diameter were implanted in the formations of the ventral hippocampus, frontal and parietal cortex, rostral parts of the caudate nuclei, reticular part of the substantia nigra, cortex of the cerebellar vermis, and SC. The reference electrode was fixed in the nasal bones. Electrical activity was recorded by means of a 16-channel EEG 16S electroencephalograph (Hungary). Bilateral damage to SC was caused by the method in [8] by means of kainic acid ("Sigma," USA; 1.0 μg in 1.0 μl phosphate buffer solution, pH 7.4), which was injected under pentobarbital anesthesia (35 mg/kg, intraperitoneally) 30 days before observation. Animals of the control group received an injection of 1.0 μl of phosphate buffer solution, pH 7.4, into SC under similar conditions. The seizure threshold relative to picrotoxin (ED_{50}) was determined by probit analysis [1]. Picrotoxin kindling was carried out by giving a single daily intraperitoneal injection

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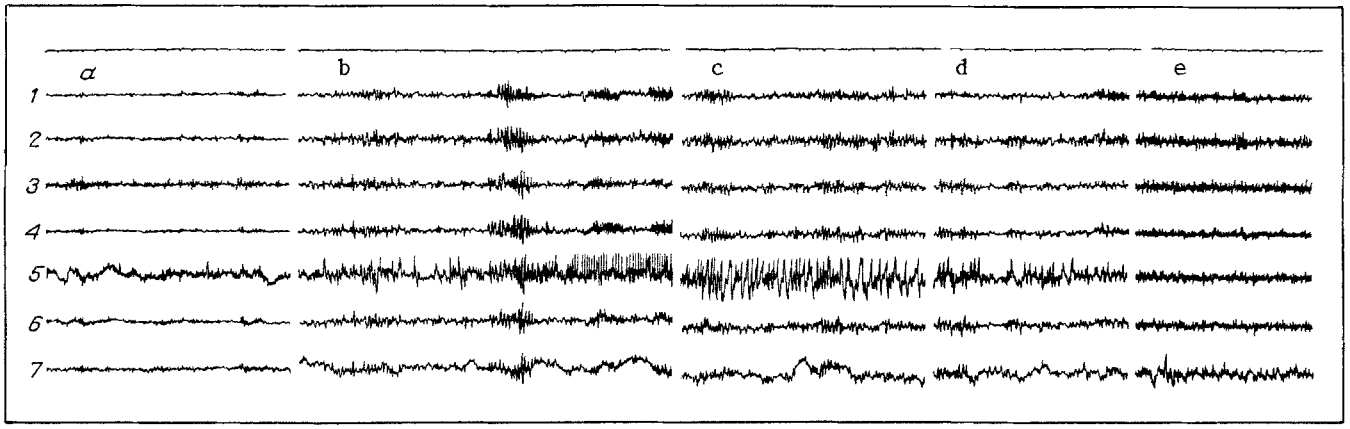

Fig. 1. Changes in electrical activity of brain structures following injection of penicillin into SC. a) Initial background; b) 6.5 min after injection of penicillin (10 MU in 1.0 μl); c, d, e) 9, 30, and 50 min respectively after a. All derivations (1-6) except 4 from left cerebral hemisphere: 1) frontal and 2) parietal cortex; 3 and 4) ventral hippocampus; 5) SC; 6) reticular part of substantia nigra; 7) cortex of cerebellar vermis. Time marker 1 sec; calibration, 100 μV.

of picrotoxin ("Sigma," USA) in a dose of 0.8 mg/kg into animals of the experimental group and in a dose of 1.1 mg/kg into animals of the control group. At the end of the experiments the locations of the electrodes and of the areas of destruction of SC were confirmed histologically. The results were subjected to statistical analysis by variance and nonparametric methods [2, 3].

EXPERIMENTAL RESULTS

Changes in the EEG of the Rats during Creation of the GPEE in SC. In the first series of experiments, the changes in the EEG were studied in rats in response to microinjections of penicillin and bicuculline into SC. The results of injection of penicillin (10 MU) are shown in Fig. 1. Before injection of penicillin, desynchronized activity was observed on the EEG of the actively conscious animal, with the predominant amplitude of discharges of 80-120 μV, against the background of which single slow-wave potentials with an amplitude of up to 250 μV were recorded (Fig. 1a). Spike discharges, from 200 to 500 μV in amplitude and with a frequency of 5-12/sec (see Fig. 1b, zone 5) began to appear in the zone of injection 5-15 min after microinjection of penicillin. Meanwhile in other structures spindle-shaped grouped discharges appeared with a frequency of 8-10 μsec and an amplitude of 250 μV (Fig. 1b, zones 1-4, 6, and 7). During the next 3-7 min there was an increase in amplitude of the spike discharges in SC to 0.8-1.5 mV (see Fig. 1c, zone 5). Periods of similar hypersynchronized activity lasted from 10-20 sec to 1.0-1.5 min, and the frequency of their appearance varied from 1-2/min to 1-2/15 min. Discharges of theta-activity with an amplitude of 100-150 μV were recorded in other parts of the brain (Fig. 1c, zones 1-4, 6, and 7). A decrease in the frequency and amplitude of the spike discharges, with the appearance of pointed and slow waves up to 300 μV in amplitude and with a frequency of 2-5/sec was observed 25-45 min after injection of penicillin (Fig. 1d, zone 5). During the next 7-25 min synchronized activity in the zone of injection of the convulsant disappeared (Fig. 1e, zone 5). A separate series of experiments showed that injection of bicuculline (25 ng) into SC causes similar changes in electrical activity, consisting of the appearance of hypersynchronized activity in SC with a frequency of 7-15/sec and with an amplitude of the separate discharges of between 350 and 840 μV.

Effect of GPEE in SC on Picrotoxin-Induced Seizure Activity. After bilateral microinjection of penicillin (10 MU) systemic injection of picrotoxin into SC led to the appearance of the first seizure reactions, whose latent period was significantly longer than that in animals of the control group. With an increase in the dose of penicillin an increase in the latent period of the first seizures was observed. In 20 of the 35 animals of the experimental group, 25-33 min after injection of picrotoxin, myoclonic contractions of the muscles of the snout were observed, with individual paroxysmal spasms and single clonic contractions of muscle groups of the limbs and trunk. The latent period of the seizures was significantly longer than that of animals of the control group, and the parameters of the severity of the seizure reactions were significantly below those observed in the control (Table 1).

TABLE 1. Effect of Intracollicular Injection of Penicillin and Bicuculline on Seizure Activity Induced by Systemic Injection of Picrotoxin (M ± m)

Experimental conditions	Latent period of first seizures, min	Average severity of seizures, points	Number of animals with generalized convulsions
Control—injection of 1.0 μl of 0.9% sodium chloride solution into SC (n = 35)	21,2±1,3	3,8±0,1	26
Injection of sodium salt of benzylpenicillin into SC, 5,0 (*n*=14)	25,8±2,2	0,8±0,08**	0*
10,0 (*n*=14)	29,2±2,2**	0,83±0,08**	0*
15,0 (*n*=7)	32,2±2,3**	1,0±0,09**	0*
Injection of bicuculline hydrochloride into SC, 50,0 (*n*=13)	45,0**	0*	0*
	36,6±2,2**	1,1±0,08**	0*

Legend. Significance of differences between experimental and control groups of animals: *p < 0.025; **p < 0.001.

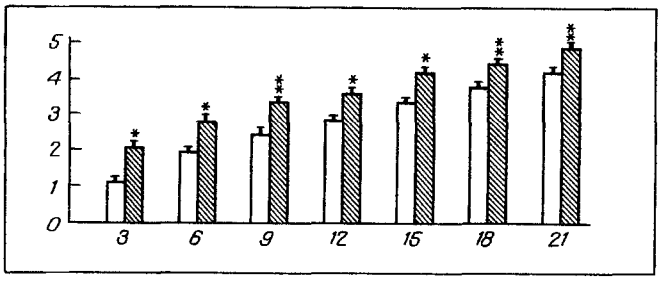

Fig. 2. Development of picrotoxin kindling in rats after destruction of SC. Abscissa, period of observation (in days); ordinate, severity of seizures (in points). Unshaded columns — severity of convulsions in animals of control group (1.1 mg/kg picrotoxin), shaded columns denote rats of experimental group (receiving 0.8 mg/kg picrotoxin). Significance of difference between experimental and control group by Wilcoxon—Mann—Whitney test: *p < 0.001, **p < 0.025.

The use of picrotoxin in addition to bilateral injection of bicuculline into SC (50 ng) caused single paroxysmal spasms of the muscles of the snout and trunk in 7 of the 13 animals. The latent period of the seizures was significantly longer than in animals of the control group, but the paroxysmal reactions were significantly less severe than in the control (Table 1).

Effect of Destruction of SC on Picrotoxin Kindling. In the next series of experiments the effect of destruction of SC was studied on the seizure threshold relative to picrotoxin and on the development of seizure activity during picrotoxin kindling (Fig. 2). After destruction of SC, ED_{50} for picrotoxin was 0.88 mg/kg (0.77-0.99 mg/kg), significantly lower than in animals with an intact SC, namely 1.42 mg/kg (1.30-1.54 mg/kg, $p < 0.05$). After the first injection of picrotoxin, single paroxysmal spasms were observed in 16 of the 21 animals of the experimental group and in 15 of the 21 animals of the control group. In all animals of the experimental group, after the second injection of picrotoxin, marked clonic spasms of the muscles of the whole trunk developed, and after the 5th injection of the convulsant, four of the 21 animals developed generalized convulsions. In the control group, after the first eight injections of the convulsant the number of animals with seizure manifestations and with the appearance of clonic contractions of the limb and trunk muscles increased. Starting with the 9th injection, in three of the 21 rats generalized clonicotonic convulsions were noted. Generalized convulsions were observed in all animals of the experiment and control groups after 15 and 21 days respectively.

The investigations thus showed that microinjections of penicillin and also of bicuculline into SC lead to the formation of a GPEE. Activity of the GPEE created in SC is responsible for suppressing the generalized picrotoxin seizures. This effect of the GPEE was manifested as lengthening of the latent period of onset of the first seizures and a decrease in their severity by complete prevention of generalized clonicotonic convulsions. The nonspecific character of the antiepileptic effect (injection of substances with different mechanisms of neurotropic action) is evidence of the importance

of the generator mechanisms themselves in maintaining the antiepileptic function of SC Further confirmation of the antiepileptic role of SC is given by data showing lowering of the seizure thresholds in animals after bilateral destruction of SC. The results are confirmed by others [11] showing abolition of the anticonvulsant effects of intranigral injection of GABA agonists on a model of electric shock convulsions after mechanical destruction of SC. It has also been shown that injection of bicuculline (50 mmoles) into SC causes the appearance of tonic electric shock convulsions in rats [12].

It is an interesting fact that in order to create a GPEE in SC using penicillin, a relatively low dose of the preparation (10 MU) is required, whereas to create of GPEE in the hippocampus, injection of 250 MU penicillin has been used [14], and in the cerebellar cortex penicillin did not cause the appearance of a GPEE whatsoever [15]. This fact may be evidence that under conditions of systemic injection of convulsants, the formations of SC are the first to respond to the epileptogen, carrying out some form of "detection" of the epileptogenic stimulus and activating the antiepileptic system of the brain.

The investigations described above thus showed that SC is an important functional stage in the antiepileptic system of the brain. The role of this structure may perhaps be to integrate the separate links of the system and to maintain the development of an adequate health-promoting effect.

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