MOTOR AND EMOTIONAL DISTURBANCES DEVELOPING IN RATS RECEIVING DAILY SUBTHRESHOLD INJECTIONS OF PICROTOXIN

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Chronic administration of metrazol in a subconvulsive dose causes the development of predisposition to seizures, manifested as an increase in the intensity of responses to metrazol, ranging from individual twitches to generalized clonicotonic convulsions [3, 8, 10]. The phenomenon also is characterized by lowering of the seizure threshold relative to metrazol for a long period of time after its administration has ceased, i.e., it has the basic features of kindling and is defined as pharmacologic kindling [3, 8]. Besides the seizure changes, during kindling due to repeated injections of lidocaine in animals, stable behavioral changes arise [12]. Hence the importance of studying whether pharmacologic kindling can develop as a result of administration of other epileptogens, differing in the mechanism of their convulsant action from metrazol and lidocaine, and also of studying changes arising under these circumstances in behavior. The work was devoted to a study of the development of epileptic activity (EpA) in rats during chronic administration of subconvulsive doses of picrotoxin, whose epileptogenic effect is linked with its action on the GABA-ionophore complex [13], and also to a study of behavioral disorders arising under these conditions.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 170-250 g. Each group consisted of no fewer than 10 animals. Picrotoxin, in doses of 5, 2.5, 1.5, or 1.25 mg/kg was injected intraperitoneally in a volume of 0.5 ml of 0.9% NaCl solution daily under identical conditions (the same time of day, in a room with the same intensity of illumination and with the same intensity of illumination and with the same noise effect). Animals of the control group received injections of physiological saline under identical conditions. The animals were observed for 90 min after receiving the injection of picrotoxin. The intensity of the seizures was expressed in points [3]. The latent period of the first seizure responses and of generalized convulsions also was determined. Defensive responses were evaluated in accordance with the character of the animals' behavior when attempts were made to handle them, and these also were expressed in points [12]. To investigate the electrical activity of the brain, rats were anesthetized with hexobarbital (100 mg/kg) and monopolar constantan electrodes were inserted into the hippocampus, caudate nucleus, sensomotor cortex, and cortex of the cerebellar vermis, stereotaxically taking coordinates from the atlas [11]; the reference electrode was fixed in the nasal bones. The results were subjected to statistical analysis [7].

EXPERIMENTAL RESULTS

Injection of picrotoxin (5 mg/kg) caused generalized convulsions or spasms of the forelimbs (the intensity of the convulsions was 3.6 ± 0.2 points). After injection of picrotoxin in a dose of 2.5 mg/kg clonic convulsions were observed in eight of the 12 animals (1.8 ± 0.7 points), whereas in a dose of 1.5 mg/kg spasms were observed in four of the 12 rats (0.3 ± 0.2 points). After injection of picrotoxin in a dose of 1.25 mg/kg no seizure responses were observed. This dose was chosen for chronic administration.

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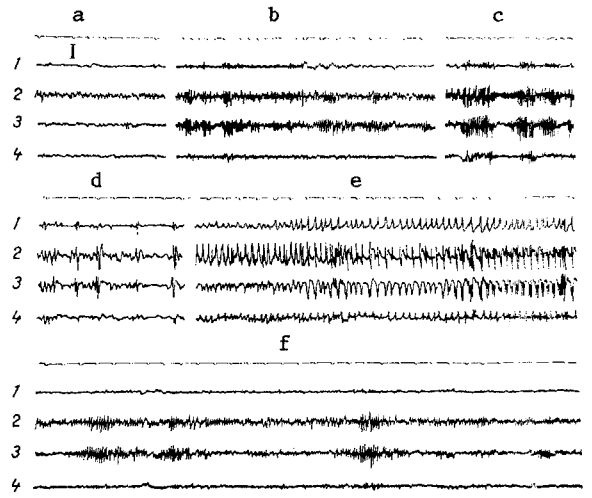

Fig. 1. Changes in brain electrical activity during kindling induced by picrotoxin. a) Initial activity, b, c, d) after 2, 5, and 9 injections of subthreshold dose of picrotoxin respectively, e) 6.5 min after 9th injection, f) 24 h after e, before routine injection of picrotoxin. 1) Sensomotor cortex, 2) dorsal hippocampus, 3) caudate nucleus, 4) cerebellar cortex. Calibration: 200 μV, time marker 1 sec.

All the animals exhibited a marked reduction of motor activity combined with muscle relaxation 3-5 min after the first 3 or 4 injections of picrotoxin in the above doses, which lasted for 5-7 min and was followed by resumption of motor activity. Periods of locomotor activity were interrupted by brief episodes of complete immobility. The frequency of these episodes was 2-5/min and their duration 2-6 sec. During this time bursts of slow waves and spike-wave complexes with a frequency of 8-9/sec were recorded on the EEG and were most marked in the caudate nucleus (Fig. 1b). Behavioral and EEG changes were noted for 15-60 min. Starting with the 5th injection of picrotoxin, no periods of reduction of motor activity with muscle relaxation were observed in the animals. During this period, 10-18 min after injection of picrotoxin, immobility and convulsive spasms of the head and neck, accompanied by tremor of the head, were observed. An increase in frequency of the bursts of slow waves to 10-15/min and in their duration to 5-8 sec, an increase in amplitude of the potentials to 250 μV, and the appearance of spike discharges with an amplitude of 200-400 μV, more marked in the caudate nucleus and hippocampus (Fig. 1c), were recorded on the EEG. The duration of the above changes was 30-60 min. Injection of picrotoxin on the subsequent days was accompanied by the appearance of convulsive spasms of the muscles of the snout and trunk with brief (up to 2 min) periods of clonic convulsions of the forelimbs. These responses were accompanied on the EEG by spikes and spike-wave complexes with an amplitude of 200-500 μV, which were recorded in all structures tested (Fig. 1d). Clonic convulsions were converted into generalized convulsions with the animal falling on its side and with the development of postictal depression. During these convulsions, frequent (2-3/sec) regular potentials synchronized in all structures and with a duration of 20-50 sec were observed on the EEG (Fig. 1e). The amplitude of EpA at the beginning of the seizures was maximal (up to 500 μV) in the hippocampus (Fig. 1e, zone 2). The changes observed on the EEG began 7-10 sec later in the caudate nucleus than in the hippocampus and the amplitude of their EpA reached 300 μV (Fig. 1e, zone 3).

After 10 injections of picrotoxin generalized convulsions were observed in four of the 19 animals, and after 20 injections — in 12 of 17 animals (Fig. 2). At this stage generalized convulsions occurred after every injection of picrotoxin. The total number of injections of the drug required for the development of generalized convulsions was 6-17. Between 2 and 24 h after injection of picrotoxin, during the absence of convulsions, bursts of slow waves and spike-wave complexes were recorded on the EEG and were most marked in the caudate nucleus (Fig. 1f), which corresponded to episodes of immobility and of tremor of the head. Bursts of slow waves and of spike-wave complexes were weaker in the hippocampus and they were not recorded at all in the other brain structures tested. In five of 17 rats no convulsions were observed after 20 injections of picrotoxin, but only weak spasms were noted. After injection of picrotoxin in a dose of 1.25 mg/kg, generalized convulsions were observed 20 days after the

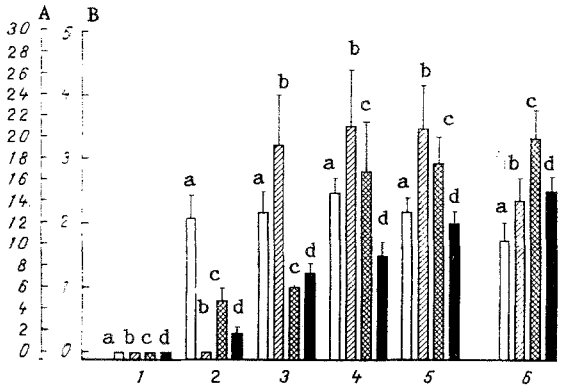

Fig. 2. Intensity of seizure activity and defensive responses during daily injection of picrotoxin in a dose of 1.25 mg/kg. Abscissa, duration of injection of picrotoxin, days: 1-5) 3rd, 5th, 10th, 15th, and 20th injections of picrotoxin respectively. 6) 20 days after last (21st) injection of picrotoxin. Ordinate: A) time of observation after injection of picrotoxin, min; B) intensity of seizures and of defensive responses, points. a) Latent period of 1st seizure responses, b) latent period of convulsions, c) intensity of convulsions, d) intensity of defensive responses.

21st injection in those animals in which they had appeared after the culmination of kindling (21st day). In the remaining animals, injection of picrotoxin after an interval of 3 weeks did not induce a seizure response.

Besides behavioral disturbances and a seizure syndrome, the appearance of defensive responses and an increase in their intensity were observed in response to repeated injections of picrotoxin into the animals. Starting with the 5th injection of the drug, 5-7 min after this injection passive defensive responses were observed in six of 19 rats: running away or jumping to avoid being handled, increased resistance to capture.

After 10 injections of picrotoxin passive defensive responses were observed in all the animals (Fig. 2). Active defensive responses (assumption of a defensive posture, striking back with the forelimbs) were observed in only 2-4 animals starting with the 11th injection of picrotoxin. Although the mean intensity of the seizures and defensive behavior increased parallel to each other (Fig. 2), there was no obvious dependence of the intensity of one on the other in individual animals. Thus after the 15th injection of picrotoxin, the index of defensive behavior in animals exhibiting convulsions was 1.6 ± 0.2 point, compared with 1.4 ± 0.4 point in rats without convulsions ($p > 0.05$), and after the 20th injection the corresponding values were 1.9 ± 0.5 and 2.2 ± 0.4 points ($p > 0.05$). The coefficient of correlation between these parameters varied on different days from +0.1 to −0.16 ($p > 0.05$). Injection of physiological saline into animals of the control group, and also into animals subjected to kindling (instead of the routine injection of picrotoxin) did not lead to the appearance of defensive responses.

The investigations thus showed that repeated injection of picrotoxin in a subconvulsive dose leads to the appearance not only of a seizure syndrome, to which all workers who have studied kindling have drawn attention [3, 8-10], but also to the onset of three syndromes: 1) seizure, which begins with very small myoclonic twitches and ends in generalized convulsions; 2) manifestations of marked akinesia in the form of paroxysms of immobility followed by weak tremor, and 3) emotional–behavioral disorders, expressed as pathologically enhanced defensive responses. The maximal intensity of these disturbances differed in the course of kindling.

On the basis of the general theory of generator, determinant, and systemic mechanisms of neuropathological syndromes [2] it can be postulated that the pathogenesis of each of the above syndromes is based on its own pathological system and the corresponding pathological determinant. Development of the seizure syndrome is associated with the formation of an epileptic system, the primary determinant of which is the hyperactive hippocampus [3]; manifestations of akinesia and tremor constitute part of a Parkinson's syndrome, in which the pathological determinant is located in the caudate nuclei [2, 5, 6]. The emotional–behavioral disorders are, as we know, associated with hyperactivation of structures of the limbic system (amydala, hippocampus), which are the pathological determinant of this syndrome. Emotional–behavioral

disorders can be induced by the creation of a generator of pathologically enhanced excitation in structures of the limbic system [4]. Emotional–behavioral changes of a similar kind also were well marked during kindling induced by metrazol [3]. In their investigations on kindling induced by lidocaine, Post and co-workers [12] also observed increased aggressive–defensive responses. However, unlike the observations of the authors cited, we ourselves did not observe that the intensity of the defensive responses depended on the intensity of the seizures, although active defensive responses were weaker. The reason for these differences may have been a difference in the action of the substances used by ourselves and by Post and co-workers. During kindling induced by picrotoxin mainly passive defensive responses arose in the rats, which are regarded as characteristic of animals of this species [1]. The appearance of the first defensive responses simultaneously with initial seizure manifestations and the subsequent absence of dependence of the intensity of these responses on the severity of the seizures in the stage of culmination of EpA formation is evidence that the mechanisms of initiation of seizures and of defensive behavior in picrotoxin kindling may be linked, whereas the mechanisms maintaining them may be independent of one another.

Thus during chronic administration of picrotoxin a complex set of neuropathological syndromes develops and is responsible for the succession of stages of the process. The pathogenetic structure of this set of syndromes differs, and involves different pathological systems [2].

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