ELECTRICAL STIMULATION OF THE FELINE CEREBELLAR VERMIAN CORTEX AS A MODEL OF TORSION DYSTONIA

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KEY WORDS: torsion dystonia; electrical stimulation; feline cerebellum; generator of pathologically enhanced excitation

The successive formation of a generator of pathologically enhanced excitation GPEE — a pathological determinant — in the CNS has been shown to be the basic pathogenetic mechanism of the onset and development of neuropathological syndromes [3]. These data, which determine the principles of modeling of neuropathological syndromes, provided the basis for the present study, with the aim of determining whether the appearance of a GPEE in the cortex of the cerebellar vermis can induce the torsion dystonia syndrome. The choice of cerebellum as the key structure for creation of a model of torsion dystonia is explained by its role in the regulation of muscle tone of the synergisms responsible for the maintenance of posture [1], a disturbance of which is observed in this disease [2].

EXPERIMENTAL METHOD

Experiments were carried out on male and female cats weighing 2.5-3.5 kg. Under pentobarbital anesthesia (40 mg/kg) bipolar electrodes were implanted into the cortex of the cerebellar vermis of the animals (interelectrode distance 3-5 mm, lobules V-VI according to the classification in [6]), recording electrodes were implanted into the region of the anterior sigmoid gyrus, and electrodes for recording the myogram into the biceps and triceps brachii muscles. The reference electrode was fixed in the nasal bones of the skull. The animals were used in the experiments 10-14 days after the operation. Electrical stimulation (ES) was applied by means of an ÉSU-1 electrostimulator as series of square pulses with a frequency of 100 Hz, pulse duration 0.25 msec, and amplitude from 1.0 to 20.0 V. The duration of ES was 20-30 sec. The electroencephalogram (EEG) and electromyogram (EMG) were recorded on a 4-ÉÉG-3 electroencephalograph. Frequency discrimination was used for quantitative analysis of the EMG changes: discharges during a 5-sec interval were counted on an F5264 counter, to the input of which electrical signals were sent by means of a UBP-2-03 amplifier. Behavioral responses were recorded visually in a chamber measuring 80 × 60 × 30 cm, and were also recorded on an "Élektronika VL-1103" videotape recorder. Diazepam (Gedeon Richter, Hungary) was injected intraperitoneally (0.1-1.0 mg/kg) 20 min before testing ES. After the end of the experiment the zone of stimulation was coagulated electrically (5.0 mA for 1 min) and the location of the electrodes verified.

EXPERIMENTAL RESULTS

Cessation of movement of the animal was observed 1-2 sec after the beginning of ES (5-10 V) — under these circumstances the cat pressed its limbs beneath itself, pressed back its ears, and closed its eyes. The animal lay on its side 3-5 sec after the beginning of ES, turned its head to the side opposite to that on which it lay, or held it against the chest, the limb muscles were tense (Fig. 1a, b). Slow lifting of one of the forelimbs was then observed, and it performed circular movements, during

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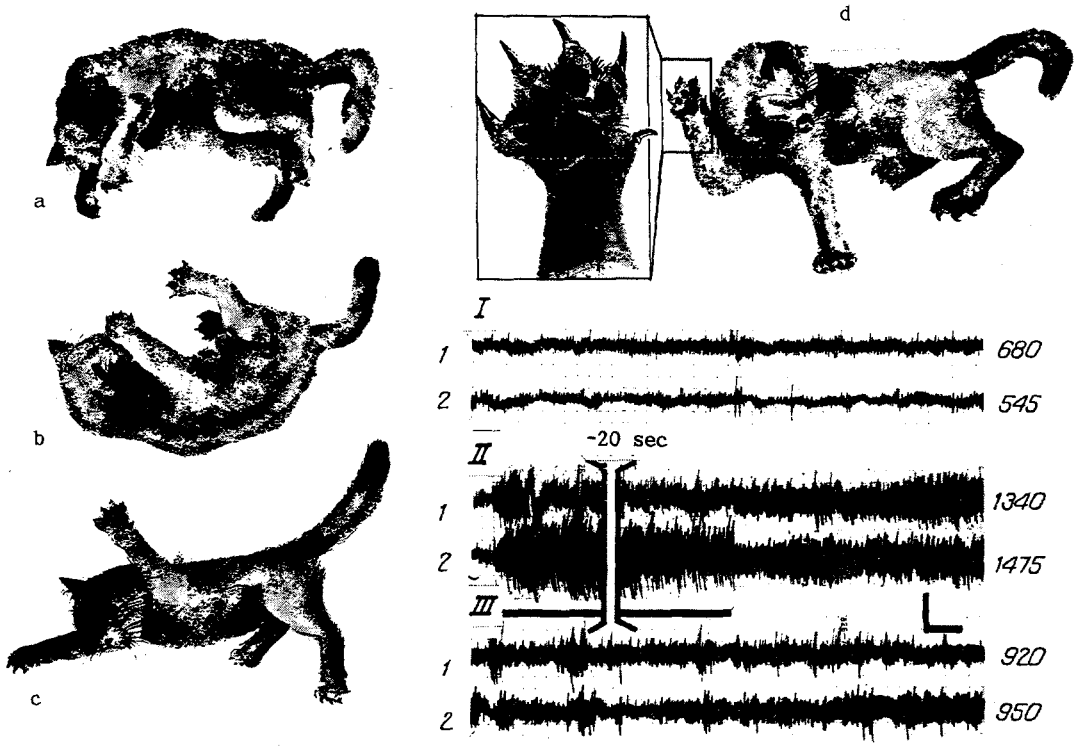

Fig. 1. Behavioral and EMG changes during ES of cerebellar vermian cortex. a, b, c, d) 8, 15, 20, and 25 sec, respectively, after ES; I) EMG before beginning of ES; II) 5 sec after I (region of continuous recording of EMG for 20 sec cut out); III) 15 sec after II. 1) Right biceps and 2) triceps brachii muscles; numbers on right indicate frequency discrimination of EMG — number of discharges in a 5-sec interval. Horizontal line, marker of stimulation, parameters of ES: 100 Hz, 0.25 msec, 10 V. Calibration: 100 μV; time marker, 2 sec.

which the limb muscles remained tense, the limbs were spread out sideways, and the cat showed its claws (Fig. 1c, d). During an attempt to change the position of the limb passively or to withdraw the head, definite resistance was noted. In this period an increase in the frequency and amplitude of the discharges in the antagonist muscles of the arm was recorded (Fig. 1, II). In this case the number of discharges counted in a period of 5 sec was 2 to 3 times greater than initially. After the end of ES the animal still remained lying on its side, slow circular movements of the forelimbs were observed, the head continued to rotate and resistance to passive withdrawal of the limbs and of the head was still noted. Frequent and high-amplitude activity was recorded in the EMG at this time and the number of discharges was 1.4-2.0 times greater than initially (Fig. 1, III). These behavioral and EMG changes were recorded for 30-40 sec after termination of ES, and after a further 10-20 sec the animal assumed a vertical posture, sniffed the floor, and made licking movements. The limb movements of the animal at this period were characterized by slowness, the length of the step was reduced, and 1.5-3.5 min after the end of ES normal motor activity was restored.

In the course of the postural-tonic and motor disturbances described above, the animals' pupils were dilated, and horizontal and vertical nystagmus was present, accompanied by salivation, urination, and defecation.

The disturbances noted above were not reproduced during analogous ES of adjacent areas of the cerebellum after electrical coagulation (5 mA for 60 sec) of the vermis, or likewise after division of the cerebellar cortex between the stimulating electrodes. Application of ES with an intensity of 1-5 V caused rotation of the head in 5 of the 10 cats, and this continued for 5-10 to 20-30 sec after the end of stimulation. ES with a strength of 10-20 V was accompanied by falling of the animal on to its side, sudden contraction of the limbs, and rotation of the trunk around its long axis, at the rate of 0.5-1.0 rotation/sec. After the end of ES the trend of the rotations became opposite in direction and their intensity fell to one rotation in 3-5 sec, and they were recorded for 10-20 sec, after which the cat continued to lie on its side for a further 15-45 sec. During this period slow movements of the forelimbs of a rotary character were observed, with contraction of the limb muscles and rotation of the head to the side opposite to that on which the cat lay, or with the head pressed against the chest. The animal stood up and began to move 30-80 sec after the end of ES, but rigidity of movements persisted for a further 2.0-4.5 min, after which normal motor activity was restored.

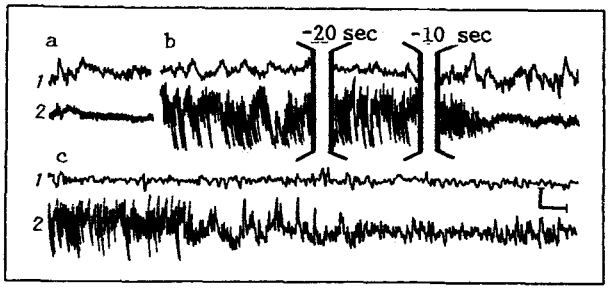

Fig. 2. Activity of GPEE in cerebellar vermian cortex, created by ES, and changes it produced by diazepam: a) initial electrical activity; b) 10 sec after ending of ES (2 segments of a continuous trace of the EEG 20 and 10 sec, respectively, in duration have been cut out); c) 30 min after intraperitoneal injection of diazepam (1.0 mg/kg) and 10 sec after ending testing ES. 1) anterior sigmoid gyrus, 2) cerebellar vermian cortex. Parameters of ES: 100 Hz, 0.25 msec, 10 V. Calibration: 100 μV; time marker, 1 sec.

Before the beginning of ES low-amplitude (20-40 μV) activity was recorded in the cerebellar vermian cortex with a discharge frequency of 30-80 Hz (Fig. 2a). Immediately after the end of ES synchronized activity developed with a frequency of 10-20 to 40-50 Hz, and with an amplitude of separate potentials of 100-200 μV (Fig. 2b). Similar activity was recorded for 20-40 sec after the end of ES, and this was followed by restoration of low-amplitude (20-40 μV) discharges with a frequency of 30-80 Hz (see Fig. 2b, the last fragment).

After administration of diazepam (0.5-1.0 kg 30 min before the beginning of ES to the cerebellar vermis) the changes described above in posture did not arise, there was no head turning, and tonic contraction of the limbs was observed only during ES and for 5-10 min after its termination. Synchronized activity was recorded in the cortex of the cerebellar vermis for 10-15 sec after the end of ES, high-frequency (30-80 Hz) activity was restored with an amplitude of individual discharges of between 20 and 80 μV (Fig. 2c).

The results of these experiments show that during ES of the cerebellar vermian cortex behavioral changes, consisting of postural-tonic and motor disturbances develop: loss of balance, rotation of the trunk around its long axis, slow movements of the limbs accompanied by simultaneous contraction of antagonist muscles (athetoid movements). These disturbances are observed in the clinical picture of torsion dystonia [2]. One of the characteristic manifestations of the combination of disturbances described above is rotation of the head, which can be regarded as the equivalent of torticollis.

Torsion dystonia is known to have antagonist relations with epilepsy [2]. It was shown previously [3, 5], that ES of the cerebellar vermian cortex leads to suppression of activity of epileptic foci in the cerebral cortex. CSF obtained from animals after ES of the cerebellar vermian cortex has an antiepileptic effect [4]. The present model also corresponds to torsion dystonia.

Investigations of changes in electrical activity demonstrate that GPEE arises in the cortex of the cerebellar vermis under the influence of ES. Its activity persists for several tens of seconds, and during this period the features of the syndrome described above are exhibited. Thus the formation and activity of the GPEE in the cortex of the cerebellar vermis constitute the pathogenetic mechanism of the syndrome. This conclusion is supported by the results of investigations of effects of diazepam: under its influence activity of the GPEE in the cerebellar cortex is inhibited and manifestations of the syndrome are reduced. Probably in genetically determined torsion dystonia in mice the structural changes described [7] in the cerebellar cortex determine the state of permanent high excitability of the cerebellar cortical neurons.

It can be concluded from the data described above that the pathological determinant of this model of torsion dystonia is formed in the cerebellar vermian cortex. This conclusion of confirmed by the data showing that the syndrome cannot be produced after electrical coagulation of the cerebellar cortex or after an incision made in it between the stimulating electrodes.

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EFFECT OF THE ANTIHYPERTENSIVE ACTION OF CAPTOPRIL ON LEVEL OF HYPERTROPHY AND SOME METABOLIC PARAMETERS OF HEART MUSCLE

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Captopril, an inhibitor of angiotensin converting enzyme, is known to limit renal hypertension to a significant degree or to abolish it [3, 4, 7]. However, the question of to what extent this antihypertensive effect influences the hypertrophy of the myocardium and its energy metabolism has been the subject only of sporadic research [6], and many aspects of it remain unexplained.

The aim of this investigation was to discover how the antihypertensive effect of captopril in experimental renal hypertension affects the mass of the heart muscle, activity of creatine kinase and glycolytic enzymes, the tricarboxylic acid cycle, and the pentose phosphate shunt.

EXPERIMENTAL METHOD

Experiments were carried out on 28 male Wistar rats, divided into three series: I) control animals, II) animals with experimental hypertension, and III) animals with experimental hypertension treated with captopril. There were 10 rats in each series. Renal hypertension was induced by the method in [8], i.e., by constricting the abdominal aorta between the two renal arteries. The operation was performed retroperitoneally in the modification in [1]. The rats received a standard diet and water ad libitum. The animals were used in the experiments 30 days after the operation for electromanometric recording of the pressure in the carotid artery. Pentobarbital anesthesia was used for the experiments. Animals of series III were given captopril in a dose of 30 mg/kg twice a day per os. The drug was obtained from "Farmakhim" (Bulgaria). The myocardium of the left ventricle was homogenized in a "Pottez" homogenizer with teflon pestle. The homogenate was diluted with bidistilled water in the ratio of 1:10 and centrifuged in the cold for 30 min at 10,000 rpm. Activity of the enzymes was determined in samples taken from the supernatant with the aid of test kits from "Böhringer." Activity of the following enzymes was determined and expressed in international units (IU) per gram protein: a) glycolytic – lactate dehydrogenase (LDH, EC 1.1.1.27, cat. no. 124893), α-hydroxybutyzate dehydrogenase (α-HBDH) or lactate dehydrogenase (LDH-1-isozyme, EC 1.1.1.27, cat. no. 124818), and pyzuvate kinase (PK, EC 2.7.1.40, cat. no. 126047); b) of the pentose cycle – glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49, cat. no. 124672); c) of their tricarboxylic acid cycle – isocitrate dehydrogenase (ICDH, EC 1.1.1.42, cat. no. 125989), and malate dehydrogenase (MDH, EC 1.1.1.37, cat. no. 124940); d) creatine phosphokinase (CPK, EC 2.7.3.2, cat. no. 126322), and creatine phosphokinase MB isozyme (CPK-MB, EC 2.7.3.2, cat. no. 189219). The weight of the whole heart of animals of all three series was expressed per 100 g body weight. The results were subjected to statistical analysis.

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