The increase in platelet aggregability in whole blood in hypocapnia under the influence of ADP, a powerful natural inducer of platelet aggregation, may thus become a risk factor for intravascular thrombosis. It must also be taken into account that during the investigation of any factors influencing the vessel — blood system, in which homeostasis is maintained by a combination of the effect of many factors of varied importance, belonging to different morphological and functional systems, the study of this process is more appropriate and informative when carried out in whole blood.

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PARKINSON'S SYNDROME SIMULATED BY INJECTION OF KAINIC ACID INTO THE CAUDATE NUCLEI

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The creation of generators of pathologically enhanced excitation (GPEE) in certain parts of the CNS, which convert these formations into pathological determinants, inducing the formation of pathological systems, provides a method of modeling various neuropathological syndromes. It has been shown that the creation of a GPEE in the rostral portions of the caudate nuclei (CN) by microinjection of tetanus toxin (TT) induces the development of Parkinson's syndrome [3, 4]. Under these conditions GPEE formation was connected with blockade of dopamine secretion by TT, as a result of which the cholinergic neurons of CN, which form the GPEE, were disinhibited. The question arose, can Parkinson's syndrome be induced by forming a GPEE in CN through activation of the main cholinergic neurons without primary blockade of dopamine secretion.

The aim of this investigation was to study this problem. To create a GPEE we used kainic acid (KA), an analog of glutamic acid, the natural mediator of certain afferent connections of CN [9]. The mechanism of the epileptogenic action of KA is connected with its direct excitatory action and blockade of GABA-ergic neurons, with the result that cholinergic neurons are disinhibited [8].

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino rats of both sexes weighing 250-350 g. The animals were anesthetized with hexobarbital (100 mg/kg) after which metal cannulas (exter-

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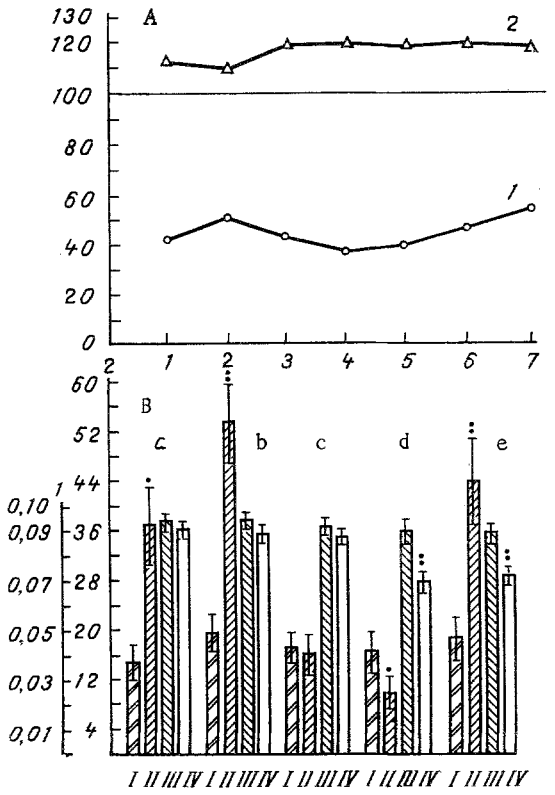

Fig. 1. Effect of anti-parkinsonian drugs on behavioral changes induced in rats by injection of KA into CN. A) Motor activity and PR in rats after injection of KA (0.1 μg) into CN. Abscissa, days after injection of KA; ordinate, number of squares crossed (NSC) and PR (in % of control, indicated by horizontal line). 1) NSC, 2) PR; B) effect of injection of benzhexol and L-dopa on motor activity and PR in rats receiving KA. Ordinate: 1) PR, 2) NSC; a, b) effect of benzhexol in doses of 5 and 10 mg/kg, respectively; C, D) effect of L-dopa in doses of 50 and 200 mg/kg, respectively; E) effect of combined injection of L-dopa (50 mg/kg) and benzhexol (2 mg/kg), I) NSC after injection of KA, II) NSC after injection of drugs, III) PR after injection of KA, IV) PR after injection of drugs. *$p < 0.05$, **$p < 0.01$.

nal diameter 0.22 mm) were implanted bilaterally in the rostral zones of CN, corresponding to coordinates (AP = −1, L = 2.5, H = 3.5) taken from the atlas in [7]. Monopolar recording electrodes were implanted at the same time in CN and the sensomotor cortex. By means of a microinjector, 5 μl of KA solution was injected 5-7 days after the operation (0.1-0.15 μg into each nucleus). The animals of the control group received injections of the same volume of phosphate buffer solution. The animals' motor activity was studied in the open field test [1], counting the number of squares crossed during 5 min of observation while the test animal moved about in the center of the field. As the parameter of rigidity (PR) we used the reciprocal of the measured distance from the base of the head to the base of the tail during the period of cessation of the animal's movements, since this characteristic symptom of parkinsonism is manifested in rats as lordosis [3, 4], and is directly dependent on PR. Electrical activity (EA) of the sensomotor cortex and CN was recorded by a monopolar method; the reference electrode was fixed in the nasal bones. Potentials were recorded on a 4 EEG-3 ink-writing electroencephalograph. Dopamine (100-200 μg) was injected into CN by means of a microinjector in a dose of 5 μl. The results were subjected to statistical analysis by variance and nonparametric methods.

EXPERIMENTAL RESULTS

From 3 to 15 min after injection of KA into CN, 14 of the 22 animals developed transient akinesias, from 10-30 sec to 1-1.5 min in duration, interspersed with short periods of activ-

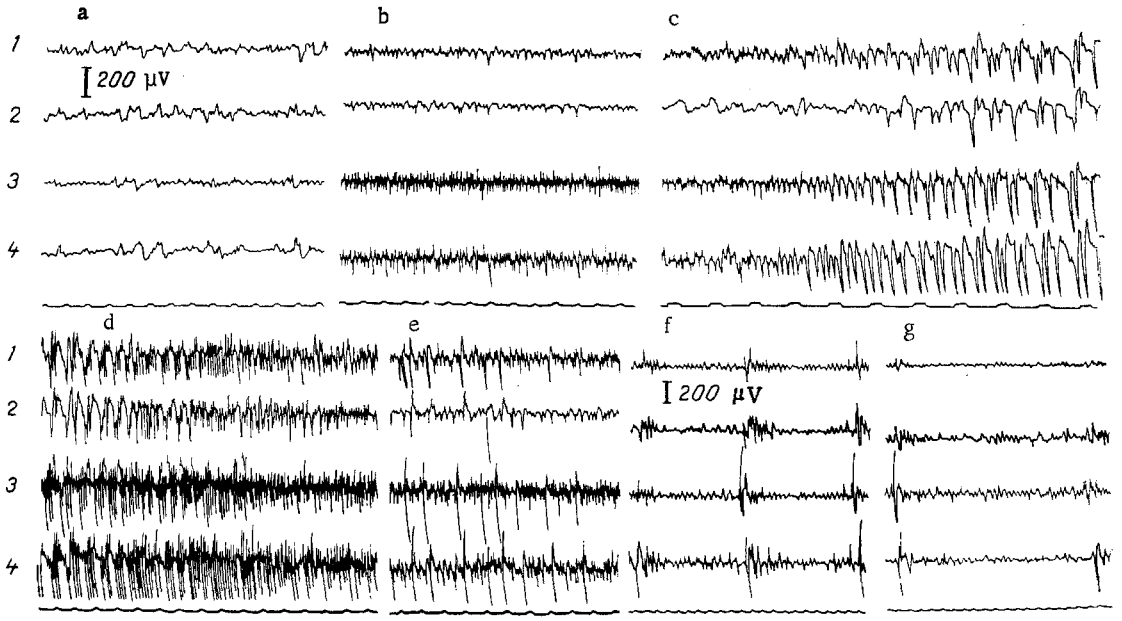

Fig. 2. Changes in EA of brain structures after injection of KA into CN. a) Initial background; b) 3 min after microinjection of KA (0.12 μg); c) 11 min after b; d) 1.2 min after c; e) 1.5 h after d; f) 25 h after e; g) 7th day after injection of KA into CN. 1, 2) left and right sensomotor cortex, respectively; 3, 4) left and right CN, respectively. Calibration 200 μV, time marker 1 sec.

ity. In the period between akinesias the animals sniffed the floor and walls of the chamber, and peered into the holes in the floor. After a further 5-20 min, changes of the animals' posture were observed during akinesias; lordosis developed, the rats splayed their hind limbs and moved them forward. The head was bent down and pressed against the forelimbs. In three of 17 cases an irregular tremor of the head was observed. The duration of the periods of akinesia 1.5-2 h after injection of KA was between 30-40 sec and 2.5-3 min. The animals became increasingly inert. During the short periods of locomotion the movements were constrained, the length of the step was reduced and the tone of the tail increased. The number of squares crossed under these circumstances was 17 ± 4, which is significantly fewer than in the control (42 ± 5; Fig. 1A). During this period PR^{-1} was 10.9 ± 0.2 cm, which also was significantly less than in the control (12.1 ± 0.3; Fig. 1A).

In the period from the 2nd through the 7th day of observation the number of squares crossed and the value of PR remained the same as on the 1st day of the experiment (Fig. 1A). Seven of the 16 rats developed ptosis, and presentation of food did not evoke sniffing movements of the vibrissae. In addition, some animals exhibited a phenomenon akin to akathisia.

Disappearance of the lordosis was observed in 12 of the 20 rats 8-14 days after injection of KA, and PR^{-1} was 12.1 ± 0.2 cm ($p > 0.05$). The number of squares crossed was 40 ± 5, which likewise did not differ from the control. In five rats at this time increased motor activity developed (the number of squares crossed was 92 ± 15), accompanied by myoclonic contractions of the neck muscles and by rushing about. The remaining three animals continued to exhibit hypokinetic manifestations and lordosis.

From 1.5 to 3.5 min after injection of KA into CN all the animals exhibited relatively high-amplitude (100-250 μV) synchronized activity with a frequency of 10-25 oscillations/sec (Fig. 2b, zones 3 and 4). During the next 3-10 min the amplitude of the discharges in CN increased from 400-800 μV to 2 mV (Fig. 2c, zones 3 and 4). At the same time spike potentials began to appear in the cerebral cortex (Fig. 2c, zones 1 and 2). High-amplitude synchronized potentials were recorded in all derivations in the period from 30 sec to 2-3 min, after which the amplitude of the discharges in the neocortex was reduced to 80-200 μV, and in CN to 200--800 μV (Fig. 2d). Similar periods of grouped high-amplitude EA were observed during the period from 2-3 h to 18-34 h of observation, and their frequency of appearance in these cases varied from 15-20 to 1-2 per hour. In the course of the periods of increased brain EA most animals (15 of 17 rats) showed considerable hypo- and akinesia (the kinesias mentioned above), and also the characteristic lordosis. In the period between bursts of EA spike potentials

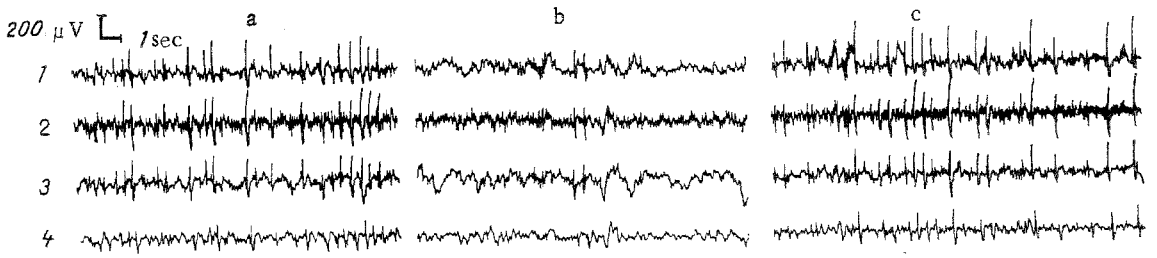

Fig. 3. Effect of dopamine on activity of GPEE induced by injection of KA into CN. a) 45 min after injection of KA (0.1 g); b) 1.5 min after bilateral microinjection of dopamine (200 μg) into CN; c) 10.5 min after b. 1, 2) Right and left CN, respectively; 3, 4) right and left sensomotor cortex, respectively. Calibration 200 μV, time marker 1 sec.

were recorded in CN with an amplitude of 500-900 μV to 1-1.5 mV and with a frequency of between 10 and 50/min (Fig. 2e, zones 3 and 4). At this time spike potentials with an amplitude of 150-200 to 400-800 μV were observed in the cerebral cortex (Fig. 2e, zones 1 and 2). On the 2nd-3rd day after injection of KA, spike discharges with an amplitude of 400-900 μV and with a frequency of between 1-4 and 15-20 per minute were recorded in CN of all animals (Fig. 2f, zones 3 and 4). Spike potentials lasted 7 days after the time of injection of KA (Fig. 2g, zones 3 and 4), and then disappeared.

Injection of cyclodol (benzhexol) in a dose of 5 mg/kg into animals in states of hypo- and akinesia caused an increase in the number of squares crossed to 37 ± 5, which was much greater than in the control (15 ± 4; Fig. 1B, a). From 5 to 10 min after injection of benzhexol in a dose of 10 mg/kg, the rats began locomotor activity. During movements the animals sniffed the floor and walls of the chamber and peered into holes in the floor. During the next 10-25 min the duration of ambulation increased from 20-40 sec to 1-2 min. The number of squares crossed was 53 ± 5, significantly more than before injection of the drug (19 ± 4; Fig. 1B, b). Benzhexol did not affect PR.

Injection of L-dopa in a dose of 50 mg/kg caused no change in the number of squares crossed or in PR (Fig. 1B, c). A decrease in the flexor attitude of the trunk, and disappearance of lordosis were observed 10-15 min after injection of L-dopa (200 mg/kg), reflected in an increase in PR^{-1} from 10.7 ± 0.3 to 13.5 ± 0.3 cm ($p < 0.01$; Fig. 2B, d). The number of squares crossed under these circumstances was 10 ± 3, far fewer than before injection of the drug (19 ± 4; Fig. 1B, d). During ambulations the animals showed a marked reduction or complete absence of constraint of movements.

Combined administration of L-dopa and cyclodol in doses of 50 and 2 mg/kg, respectively, which if used separately were ineffective, caused an increase in motor activity; the animals under these circumstances showed no constraint of their movements and the rigidity disappeared. The animals sniffed the floor and walls of the chamber. The number of squares crossed and the value of PR were significantly changed (Fig. 1B, e). From 20 to 30 sec after injection of dopamine (100 μg) into both CN, inhibition of the discharges was observed (Fig. 3b), ambulation began, and there was a marked decrease of rigidity. These changes in behavior of EA were restored after 2-3 min (Fig. 3c) Dopamine, in a dose of 200 μg suppressed GPEE and restored the behavioral changes for up to 5-7 min.

The results thus demonstrate that the formation of a GPEE in the rostral zones of CN after injection of KA into them induces the appearance of symptoms of Parkinson's syndrome. These are manifested as oligo- and akinesia, rigidity, arching of the spine (lordosis), and pressing the head against the trunk and forelimbs. Less constantly the experimental equivalent of oligomimia appeared — absence of normal movements of the vibrissae and sniffing movements, and also the equivalent of akathisia — marking time. The data given above show that microinjection of KA into the rostral zones of CN leads to the development of the characteristic symptom-complex stimulating the akinetico-rigid form of parkinsonism.

Attention is drawn to the low reproducibility of a symptom so characteristic of parkinsonism as tremor. Tremor was observed in only a few animals in the late stages of development of the syndrome. During simulation of parkinsonism by the creation of a GPEE in CN with the aid of TT [3, 4] marked tremor was found very constantly. These differences can evidently

be explained by differences in the action of TT and KA and the different mechanisms lying at the basis of GPEE formation in response to injection of these substances. Depending on the state of the synchronizing and desynchronizing systems of the brain, the tremorous and akinetico-rigid forms of parkinsonism are regarded as two opposite functional states [2].

The development of manifestations of Parkinson's syndrome in the animals began immediately after injection of KA into CN and the formation of GPEE in these zones. The increase of EA in the nuclei correlated with the appearance of akinesia and rigidity in the rats. Confirmation of the generator nature of the Parkinson's syndrome and also of the pathogenetic agreement between this model and clinical forms of the disease was obtained in experiments in which anti-parkinsonian drugs were used. It was shown that microinjection of dopamine into CN led to inhibition of activity of the GPEE and weakening or even suppression of the symptoms of parkinsonism, whereas increased activity of the GPEE was accompanied by restoration of the characteristic behavioral disturbances. When benzhexol and L-dopa were used, benzhexol depressed the akinetic manifestations by a greater and the symptoms of rigidity by a lesser degree. It is an interesting fact that during combined use of benzhexol and L-dopa, potentiation of the anti-parkinsonian effect of the drugs was observed.

Injection of KA into CN is currently used as a model of chorea [9]. The development of choreiform disturbances is known to be based on depression of the functional activity of CN neurons [8], observed in the late stages (on the 10th day or later) of injection of KA [6]. Our investigations showed that on the first days of action of KA EA in CN is increased and a GPEE is formed, and this is accompanied by the development of Parkinson's syndrome.

The data given above thus confirm the generator nature of Parkinson's syndrome and they are evidence of the possible role of glutamatergic mechanisms in its pathogenesis.

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