APPEARANCE OF POSTURAL ASYMMETRY OF HIND LIMBS IN RATS FOLLOWING UNILATERAL COLCHICINE BLOCK OF AXONAL TRANSPORT IN CORTICOLUMBAR PROJECTIONS

E. I. Varlinskaya, G. P. Obukhova, and G. A. Vartanyan

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The writers showed previously [2, 3] that after unilateral injury to the motor cortex functional asymmetry arises in the segmental apparatus, and is manifested as postural asymmetry (PA) of the hind limbs, and participation of specific endogenous neurohumoral factors in its formation was demonstrated experimentally. The time required for development and fixation of functional asymmetry of the spinal cord was found to depend on the distance between the site of injury to the neuron body or axon and the synaptic terminal [1]. On this basis it was postulated that the signal about injury to nerve cell bodies or conducting tracts is cessation of the normal secretory function of the terminal as a result of a disturbance of axon transport [2].

To test this hypothesis the possibility of formation of functional asymmetry of the segmental apparatus, due to the appearance of PA factor in the cerebrospinal fluid (CSF) was studied after blocking of axonal transport in corticolumbar projections in the absence of organic damage to brain and spinal cord tissue. Axonal transport was blocked by colchicine. To identify disturbances of axonal flow the histochemical retrograde transport of horseradish peroxidase (HRP) method was used.

EXPERIMENTAL METHOD

Noninbred male rats weighing 180-220 g were used. Under ether anesthesia a microinjection of colchicine (from Merck, West Germany) in a dose of 1 μg, in 1 μl of physiological saline, was given into the motor cortex on the left side at a depth of 1 mm into the underlying white matter. The animals were then divided into groups and spinalized at level T_1 at different times after injection of colchicine. All the animals were tested for the presence of PA of the hind limbs visually and electromyographically. EMGs of two groups of corresponding thigh muscles (biceps and quadriceps) of both hind limbs were analyzed. Steel bipolar needle electrodes (diameter 0.5 mm, interelectrode distance 3 mm) were used. The results were processed directly in the course of the experiment on a nonlinear MN-10M simulation apparatus by calculation of the coefficient of asymmetry, the ratio of the difference between bioelectrical activity of the homonymous muscles, integrated by frequency and amplitude, and their total. The results were subjected to statistical analysis by Student's test at a $P < 0.05$ level of significance. Blocking of axonal transport in corticolumbar projections was identified by microinjection of 0.1-0.2 μl of 30-40% solution of HRP (Sigma-VI) into the gray matter of the spinal cord. The animals were killed 48 h after injection by perfusion of the brain with physiological saline, followed by perfusion with a fixing solution consisting of 0.4% paraformaldehyde and 25% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. The brain was removed and kept for 24 h at 4°C in the same fixing solution, after which it was transferred into 30% sucrose solution in 0.1 M phosphate buffer, in which it remained for 24-48 h at 4°C. Serial sections 40 μ thick were then cut on a freezing microtome. The sections were stained by the histochemical method in [6]. Biological testing was used to reveal PA-inducing factors (PAF) in the CSF of the donor animals with blocked axonal transport in corticolumbar projections. For this purpose donors' CSF in a volume of 50 μl was injected into the cerebromedullary cisterna of intact recipient rats. The recipients were spinalized 10-15 min later and tested for the presence or absence of PA.

I. P. Pavlov Physiological Department, Research Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR N. P. Bekhtereva.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 97, No. 3, pp. 259-261, March, 1984. Original article submitted April 22, 1983.

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TABLE 1. Time of Onset and Maintenance of PA of Hind Limbs at the Spinal Level in Rats after Injection of Colchicine into Left Hemisphere

Time of spinalization after injection of colchicine, days	Total number of animals	Presence of PA			
		right-sided flexion		left-sided flexion	
		no. of animals	%	no. of animals	%
1	10	0	0	0	0
2	10	10	100	0	0
3	10	9	90	0	0
5	10	6	60	3	30
7	10	2	20	8	80
10	10	0	0	3	30
14	9	0	0	0	0

TABLE 2. Induction of PA of Hind Limbs in Intact Animals by CSF from Donors with Unilateral Blocking of Axonal Transport in Corticolumbar Projections by Injection of Colchicine into Left Cerebral Hemisphere

Time of collecting CSF after injection of colchicine, days	Total number of recipients	Number of recipients with PA	Activity of PAF
1	10	0	—
2	10	9	+
3	10	10	+
7	11	9	+
14	10	1	—

Legend. +) Presence of active PAF with 95% confidence level; —) absence of PAF.

EXPERIMENTAL RESULTS

As the control for left-sided blocking of axonal transport by colchicine in corticolumbar projections, bilateral injection of HRP into the region of the spinal cord 3 days after injection of colchicine was used. The animals were killed 48 h after injection of HRP (5 days after injection of colchicine) and the spinal cord was processed for detection of HRP-containing cells.

Morphological study of the motor cortex revealed the following pattern of distribution of HRP-containing neurons. Enzyme-containing neurons were absent in the cerebral cortex on the left side, into which colchicine was injected, in all fields of vision, except for a few single palely stained pyramidal cells (Fig. 1a, b). The opposite side differed sharply from the region of injection of colchicine. Here pyramidal cells of various sizes, in groups of five to ten cells, with intensive staining of the neuron body and processes given off by it could be observed (Fig. 1b). The action of colchicine was evidently manifested as blocking of retrograde HRP transport in corticolumbar projections. We know from the literature that this substance has an even more marked action on the rapid component of anterograde axon transport [4]. This suggests that after injection of colchicine, transport along the axon is disturbed not only in the retrograde, but also in the anterograde direction.

The morphological data formed the basis for investigations aimed at detecting fixation of PA in animals with unilateral blocking of axonal transport in corticolumbar projections (Table 1).

The results showed that blocking of axonal transport in cortico-lumbar projections, like trauma to the motor cortex [2, 3], leads to the formation of functional asymmetry of the segmental apparatus, manifested as PA of the hind limbs and characteristic structural changes in the EMGs (Fig. 2).

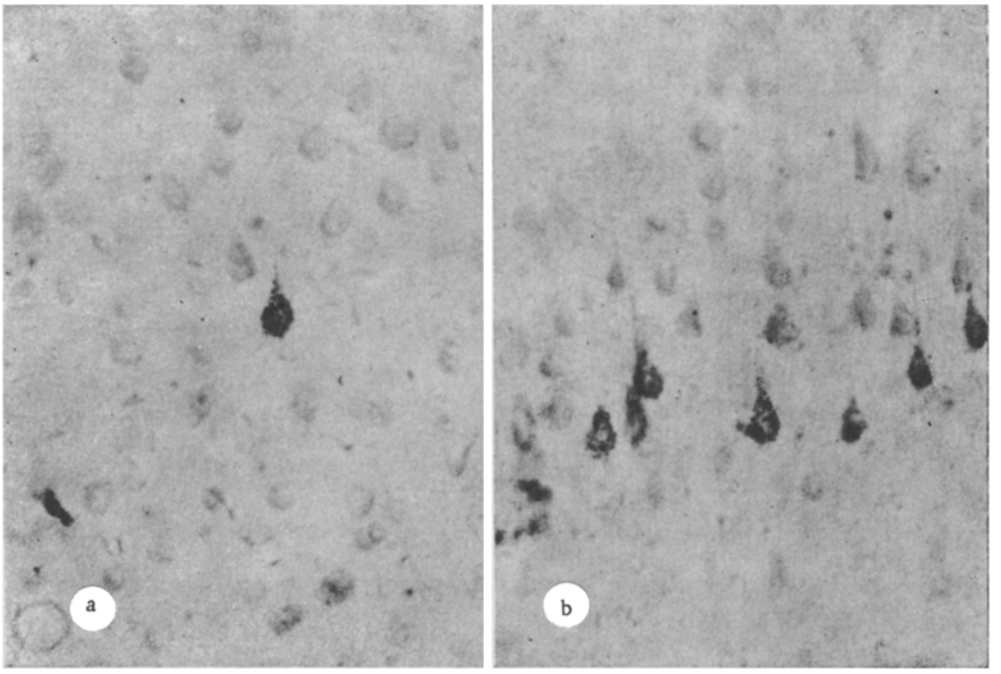

Fig. 1. Distribution of HRP-containing neurons in layer V of motor cortex after unilateral blocking of axonal transport in corticospinal projections: a) left hemisphere (region of injection of colchicine); b) right hemisphere (intact). 320×.

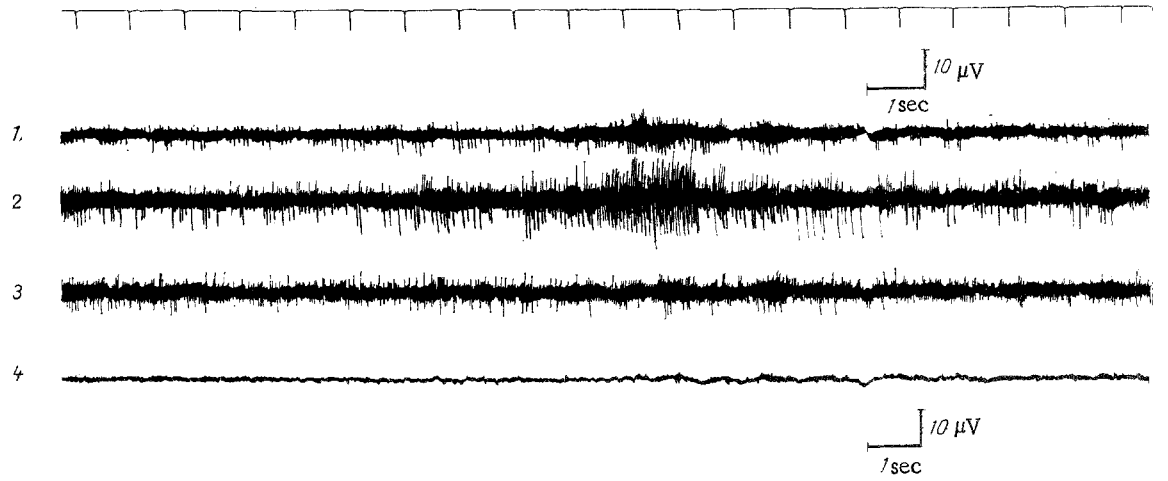

Fig. 2. EMG of thigh muscles of animal with unilateral blocking of axonal transport in corticospinal projections: 1) EMG of right quadriceps femoris muscle; 2) EMG of left quadriceps femoris muscle; 3) EMG of right biceps femoris muscle; 4) EMG of left biceps femoris muscle.

PA was expressed as stronger flexion of the hind limb contralateral to the side of colchicine injection; the EMG of the biceps femoris muscle of this same limb increased in frequency and amplitude under these circumstances.

Blocking axonal transport was accompanied by the same sequence of functional modifications to the segmental apparatus as injury to the motor cortex, namely the appearance of functional asymmetry, reversal of this state, and a return to the symmetrical level of function. The process of successive modifications during blocking of axonal transport takes place within a period of 2 weeks (Table 1), whereas after trauma it takes 3 weeks [5]. In the dose used, colchicine evidently has a gentler action, involving fewer conducting tracts than removal of the motor cortex.

PAF activity in the CSF of animals with blocked axonal transport in corticolumbar projections was determined by biological testing (Table 2).

PAF activity in intact recipients was detected between 2 and 7 days after injection of colchicine.

Unilateral blocking of axonal transport in corticolumbar projections thus leads to the same effects as destruction of brain tissue: formation of PAF, leading to the appearance of an asymmetrical functional state of the segmental apparatus. The results support the previous conclusion that the signal about injury to elements of CNS is disturbance of normal axonal transport of materials.

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MECHANISM OF THE HYPOTENSIVE EFFECT OF PARATHORMONE

G. K. Zoloev, B. D. Slepushkin, and Yu. B. Lishmanov

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The parathyroid hormone is the most important regulator of calcium metabolism in the body [3]. However, it has recently been shown that it also has many other biological effects. One of these is its ability to induce vasodilatation and to lower the arterial blood pressure (BP) [6, 7-8, 10], which has been found to be true both of synthetic parathyroidin and parathyroid gland extract [13]. Although the hypotensive action of parathormone (PH) is very short in duration, the authors cited above consider that it may have definite biological importance and that it is actually older phylogenetically than its hypercalcemic effect [11].

The mechanism of action of parathyroid hormone on the vascular wall is not yet fully understood. To shed some light on this mechanism the investigation described below was undertaken.

EXPERIMENTAL METHOD

Experiments were carried out on 56 male albino rats weighing 180-200 g under thiopental anesthesia. BP was recorded by the direct method by means of an electromagnetic manometer-polygraph (Thomson, France) in the common carotid artery. Drugs for study were injected into the femoral vein. To block α-adrenoreceptors, droperidol was used [2], and verapamil was used as the calcium antagonist [12]. The action of PH was also investigated after administration of a stable Leu-enkephalin analog (D-Ala^2-Leu^5-Arg^6-enkephalin), capable of inhibiting the adenylate cyclase – cAMP system [9]. The cAMP concentration was determined in weighed samples of the diaphragmatic portion of the aorta by radioimmunoassay using kits from Amersham Corporation (England) and radioactivity was counted on a Mark III scintillation counter (USA). The numerical results were subjected to statistical analysis by Student's t test.

Laboratory of Pathophysiology, Siberian Branch, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, Tomsk.

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