ROLE OF THE PITUITARY IN FIXATION OF POSTURAL ASYMMETRY AT THE SEGEMENTAL LEVEL AFTER HEMISECTION OF THE SPINAL CORD

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Unilateral injury to central systems of motor control, namely the cortex of the anterior lobe of the cerebellum [2, 10, 11], the motor cortex [1], and Deiters' nuclei [6], leads to the formation of postural asymmetry (PA) of the hind limbs, fixed at the level of the lumbar segments of the spinal cord, i.e., preserved even after spinalization of the animal. Fixation of PA is induced by low-molecular-weight species-nonspecific factors of peptide nature, specific for the side of injury [1, 4], which have been called postural asymmetry factors (PAF).

Considering the important role of the neuroendocrine system in the metabolism of most neuropeptides, an attempt was made to investigate the role of the pituiarty in the regulation of PAF activity in the CNS.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 160-180 g. Under ether anesthesia lateral hemisection of the spinal cord was performed on the right side at level T4-T5 by means of a sharp razor. The PA thus developing was manifested as flexion of the right hind limb (right-side PA). Fixation of PA at the lumbar level in the spinal cord was recorded visually 1 h after spinalization in the T1 region by the method described previously [3]. Transauricular hypophysectomy was performed by the method in [7]. Completeness of the hypophysectomy was verified by examination of the region of the sella turccia after sacrifice of the animals. Mock hypophysectomy consisted of transauricular application of a needle to the region of sella turcica without damage to the pituitary. Cerebrospinal fluid (CSF)was collected from the occipital cistern. Brain tissue was minced in liquid nitrogen and homogenized in a fivefold excess (by volume) of 0.2 M HCl. The homogenate was centrifuged at 100,000g for 90 min and the supernatant was neutralized with 0.2M KOH to pH 6.7, freed from residue, and freeze-dried. Activity of PAF in the CSF and freeze-dried product was expressed as the number of minimal active doses per microliter CSF and per milligram protein of the extract. The minimal active dose was taken to be the smallest quantity of CSF or extract to induce PA in recipients. Bioassay consisted of intracisternal injection of CSF or aqueous solution of the freeze-dried extract in a volume of 50 µl, followed by a sequence of tenfold dilution, into intact animals. The recipients were spinalized 15 min after injection and the presence or absence of PA was recorded. Each dose was tested on at least 10 recipients. Protein in the extract was determined by the method in [12]. The results were subjected to statistical analysis by the signs test [8].

EXPERIMENTAL RESULTS

To study the role of the pituitary in fixation of PA the first step was to determine the time required for fixation of PA after hemisection of the spinal cord. It will be clear from Table 1 that during the first 5 h after hemisection reorganizations leading to fixation of PA were taking place in the lumbar segments of the spinal cord during the first 5 h after hemisection. This is a longer time than in the case of injury to the cortex of the anterior

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TABLE 1. Fixation Time of PA After Hemisection of Spinal Cord

Time between hemisection of spinal cord and spinalization, h	Number of animals with PA/ total number of animals	Fixation of PA
3	0/12	—
4	5/10	—
5	14/16	+
24	17/18	+
72	12/12	+

Legend. Here and in Table 2, "+" denotes presence of fixation of PA at 95% level of significance.

TABLE 2. Effect of Hypophysectomy on Fixation of PA

Operation	Number of animals with PA/ total number of animals	Fixation of PA
Hemisection of spinal cord after hypophysectomy:		
1 Day	1/7	—
3 Days	0/12	—
5 Days	0/5	—
The same 1 day after mock hypophysectomy	8/9	+

lobe of the cerebellum (45 min) and shorter than after destruction of the motor cortex (20 h) [3, 10]. It was postulated previously that the fixation time of PA depends on the distance between the site of injury of the axon and its synaptic contact [3]. Since this distance (between the thoracic region, the site of injury, and the lumbar region of the spinal cord, where PA of the hind limbs is fixed) occupies an intermediate position between the length of terminals of short-axon Purkinje cells of the cerebellar cortex and the long-axon pyramidal cells of the cerebral cortex, the results confirm this hypothesis.

In the next series of experiments the effect of hypophysectomy on fixation of PA was studied after hemisection of the spinal cord. Considering the results of previous series of experiments (Table 1), the presence or absence of fixation of PA in hypophysectomized rats was recorded after spinalization, carried out 5 h after hemisection. As Table 2 shows, hypophysectomy prevents fixation of PA.

Since fixation of PA by the spinal centers is induced by PAF [1], it might be supposed that the absence of fixation of PA in animals after hypophysectomy was due to PAF deficiency. Accordingly, the effect of hypophysectomy on PAF activity in the CNS was investigated 1 day after hemisection of the spinal cord (Table 3).

Table 3 shows that hypophysectomy led to a decrease in PAF activity in the CSF by 100,000 times and in the brain by 2000 times. Specific activity of PAS expressed per microgram protein was 50,000 times higher in the pituitary than in brain tissue. Since no purified preparation of PAF was available, it is impossible to answer the question whether the site of PAF synthesis is the pituitary. Meanwhile the percentage distribution of total PAF activity, calculated relative to the total protein content in extracts of pituitary (100 µg) and brain (1550 µg) and per volume of CSF (100 µl) was as follows: pituitary 93.72%, CSF 6.25%, brain 0.03%. These data are evidence of the predominant localization of PAF activity in the pituitary. The discovery of comparatively low PAF activity in the CSF and brain tissue after hypophysectomy may be due also to localization of activiation or synthesis of PAF outside the pituitary, as has recently been established for ACTH [13] and α-MSH [14], which are synthesized mainly in the pituitary. The final answer to this question will be obtained after more prolonged observation on the level of PAF activity in the CNS of hypophysectomized animals.

The results of this investigation indicate that the neuroendocrine system participates in biochemical regulation of the functions of paired formation of the central motor system after unilateral injury to them.

TABLE 3. Effect of Hypophysectomy on Activity of PAF in CNS

Operation	Pituitary		Activity of PAF			
			CSF		brain	
	per microgram protein	total	per microliter	total	per microgram protein	total
Hemisection of spinal cord	$1,5 \cdot 10^6$	$1,5 \cdot 10^8$	10^5	10^7	$3,2 \cdot 10^1$	$5 \cdot 10^4$
The same 1 day after hypophysectomy	—	—	1	10^2	$1,6 \cdot 10^{-2}$	$2,5 \cdot 10^1$

It can be tentatively suggested that participation of the hypothalamo-hypophyseal system in the selective accumulation (and also, perhaps, in the biogenesis) of PAF is not confined simply to the post-traumatic state of the CNS. We know that stress induced by pain, immobilization, and cold also induces the formation of postural asymmetry [5]. Considering that opioid peptides, on the one hand, play an essential role in the reaction of the organism to stressors [15] and, on the other hand, that they can induce PA [9], it can be postulated that PAF formed in the CNS in the presence of organic lesions also belongs to the class of opioid peptides. The final answer to this question will be given by research into the secretion and chemical analysis of the structure of PAF.

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