investigation of the shortening function [3] and with changes in configuration [1] of the wall of LV and the apex during the work cycle of the heart. Consequently the wall of LV and apex of the heart make different contributions to the formation of parameters of the central hemodynamics, namely the pressure inside LV and the stroke volume of the heart, under the influence of inotropic agents.

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INACTIVATION OF POSTURAL ASYMMETRY FACTOR AT THE STAGE OF COMPENSATION OF A POSTURAL DISTURBANCE DUE TO UNILATERAL ABLATION OF THE MOTOR CORTEX

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Unilateral injury to the cortex of the anterior lobe of the cerebellum [1], the vestibular system [2], and the motor area of the cerebral cortex [3] lead to functional modifications of the segmental apparatus manifested as fixation of postural asymmetry (PA) of the hind limbs by the lumbar segments of the spinal cord. Fixation of PA has been shown to be induced by PA factors (PAF) of peptide nature, formed in the injured CNS, as has been shown for cerebellar and vestibular factors [1, 2]. The chemical nature of PAF produced after removal of the motor cortex (cortical PAF) has not been investigated.

Comparison of the dynamics of fixation of PA with changes in PAF activity showed that PA of the hind limbs disappears (is compensated for) 3 weeks after unilateral removal of the motor cortex, against the background of a decrease in PAF activity in the CSF and brain tissue to zero [4]. The cause of the decrease in cortical PAF activity has not been explained.

The aim of this investigation was to study this problem and also to determine the chemical nature of cortical PAF.

EXPERIMENTAL METHOD

Experiments were carried out on 120 noninbred male albino rats weighing 160-180 g. Under ether anesthesia the left motor area of the necortex – the zone of cortical representation of the right hind limb, was removed. On the 3rd and 21st days after the operation the animals were decapitated, the brain removed, frozen in liquid nitrogen, and kept at −20°C; 5 g of tissue was homogenized in 0.2 M HCl as described previously [5]. The supernatant obtained after centrifugation (100,000 g, 60 min) was collected and the pH adjusted to 6.7 by the addition of 0.2 M KOH, the residue was removed by centrifugation, and the supernatant was freeze-dried. Next, 100 mg of the freeze-dried products containing 10 mg protein [10] was dissolved in 1 M acetic acid and applied to a K 26/40 column (Pharmacia, Sweden), filled with Sephadex G-25 ("fine"). Gel-filtration was carried out in 1 M acetic acid with a flow rate of 3.5 ml/h·cm^2. The fractions collected were neutralized with 1 M

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TABLE 1. Sensitivity of Cortical PAF to Boiling and to Enzymes

Test material	No. of recipients with PA/ total number of recipients	Activity of PAF
NaCl (0,9%)	2/12	—
Extract	10/11	+($P<0,05$)
Extract after boiling	15/15	+($P<0,01$)
Extract + trypsin	0/15	—
Extract + ribonuclease	8/8	+($P<0,01$)

Legend. Here and in Tables 2 and 3, when number of animals with PA was determined only those recipients with flexion of the right hind limb were counted.

ammonia, then tested for induction of PA. Under the same conditions 5 g of brain tissue from intact rats was extracted and subjected to gel-filtration. The column was calibrated by peptides with mol. wt. of 1450, 710, and 528 daltons (from Sigma, USA) and also with blue dextran (from Serva, West Germany). Sensitivity of PAF to enzymes was determined in 1.0 ml of incubation mixture to which a sample of freeze-dried extract containing 500 μg protein was added. Incubation with 20 μg of trypsin (from Sigma) was carried out in 0.01 M NH_4HCO_3, pH 8.0, at 37°C for 5 h. The incubation time was chosen in accordance with the kinetics of trypsin hydrolysis of aza-albumin (from Calbiochem, USA) under the same conditions. The extract was treated with 5 μg of ribonuclease A, free from proteases (from Sigma) in 0.1 M Tris-HCl, pH 7.5, at 37°C for 4 h. The duration of incubation was determined by the kinetics of enzymic hydrolysis of mRNA from E. coli (from Sigma) at 260 nm. Enzymes were inactivated by boiling in a water bath for 10 min followed by sedimentation (10,000 g, 10 min), after which the supernatant was treated. In control experiments the extract was incubated under conditions similar to those described above, but without addition of the enzymes. The protein content in aliquots of the incubation mixtures injected into the recipients was 1 μg. Activity of PAF was determined by bioassay, based on induction of flexion of the right hind limb in intact recipients after slow injection of 50 μl of an aqueous solution of the test substance into the cisterna magna, followed (5 min after injection) by cordotomy at the level T_1-T_2. PA was recorded visually (1) 1 h after division of the spinal cord. PAF activity was expressed as the number of minimal active doses, determined by serial tenfold dilutions with distillate of the test material. The criterion of signs [6] was used for statistical analysis of the data.

EXPERIMENTAL RESULTS

To determine the chemical nature of PAF brain extract from animals killed 3 days after ablation of the left motor cortex was used. Table 1 shows that boiling does not abolish the ability of the extract to induce right-sided flexion on bioassay, evidence of the thermostability of PAF. This property of the factor enabled boiling the incubation mixture to be used to stop the enzyme reaction. Since PAF loses its activity in the presence of trypsin, and is not inactivated by incubation with ribonuclease, it can be postulated that cortical PAF is peptide in nature. It was shown previously that proteases inactivate PAF formed in the CNS after unilateral injury to the cortex of the anterior lobe of the cerebellum [1] and of the vestibular nuclei of Deiters [2], and also factors from the right and left hemispheres of the intact brain, inducing PA [5, 7]. Leu- and Met-enkephalins [11] and some of their synthetic analogs [8] also have the ability to induce PA. On the basis of these results and those of the present investigation it can be concluded that functions of paired structures of the CNS controlling motor coordination under normal conditions and after unilateral brain injuries are controlled by neuropeptides. Participation of the opioid peptides – opiate receptors system in these mechanisms is highly probable.

As was shown previously [4], cortical PAS can be identified by bioassay at the stage of formation of early signs of disturbance of corticospinal connections, manifested as fixation of PA at the segmental level during the first 2 weeks after cortical injury. The next stage of post-traumatic reorganization of the CNS, characterized by restoration of the original symmetrical function of spinal centers, is accompanied by a fall of PAF activity to zero in total brain extract [4]. These of the donor's brain at the stage of 3 days after ablation of the left motor cortex, on the contralateral side to flexion in the biological test, was 0.001 mg (0.1 μg of protein). Injection of brain extract of animals with compensated PA (21 days) into intact recipients did not cause cause the development of postural asymmetry, even in a dose of 1 mg (100 μg protein). The specific activity

TABLE 2. PAF Activity in Brain Tissue 3 and 21 Days after Ablation of Left Motor Cortex

Time after ablation of cortex, days	PAF activity in 100 mg freeze-dried extract			PAF activity in fractions after gel-filtration of 100 mg of freeze-dried extract			
	Number of recipients with PA/total number of recipients	Total PAF activity in extract	Specific PAF activity per milligram protein of extract	Fraction No.	Weight in kilodaltons	Total PAF activity in fraction, *	Specific PAF activity per milligram protein of fraction
3	10/11	10^5	10^4	1	5	0/15	
				2	5—2	0/10	10^5
				3	2—1	18—20	
				4	1	0/10	
21	0/11			1	5	0/17	
				2	5—2	0/17	10^5
				3	2—1	25/27	
				4	1	0/15	

TABLE 3. Inactivation of Cortical PAF of Void Volume Fraction (V_0) Obtained after Gel-Filtration of Brain Extract from Animals with Compensated Postural Asymmetry

Protein content in aliquots of fraction, μg	Testing of solution containing cortical PAF and aliquot of V_0 fraction			
	V_0 fraction obtained from brain extract of intact animals		V_0 fraction obtained from brain of animals with compensated PA	
	No. of recipients with PA/total number of recipients	Activity of PAF	No. of recipients with PA/total number of recipients	Activity of PAF
300 μg	10/12	+($P<0,05$)	0/9	—
30 μg	9/9	+($P<0,01$)	3/9	—
3 μg	9/10	+($P<0,05$)	4/9	—
0,3 μg	11/11	+($P<0,01$)	9/10	+($P<0,05$)

of PAF in total brain extract thus decreased toward the end of the 3rd week after trauma by not less than 4 orders of magnitude. After gel-filtration virtually all PAF activity of the brain extract from animals recently undergoing the operation could be found in fraction No. 3, containing peptides (total quantity 330 μg) with mol.wt. of between 1 and 2 kilodaltons (Table 2). The minimal active dose of this fraction was $3.3 \cdot 10^{-6}$ mg as protein. Comparable total and specific PAF activity was contained in a fraction with the same elution volume, isolated from brain extract of animals with compensated PA (protein content 500 μg; minimal active dose $5 \cdot 10^{-6}$ mg as protein). Consequently, disappearance of PAF activity in the unfractionated brain extract at the stage of compensation of postural asymmetry was due, not to degradation of the factor, but to its inactivation. Reduction of PAF activity in the total brain extract during 3 weeks after trauma took place against a background of virtually unchanged activity of the factor in fractions No. 3, possible indirect evidence of an increase in the degree of inactivation of PAF during compensation of postural asymmetry.

The next series of experiments showed that PAF inactivation factor is contained in the void volume fraction (V_0) obtained by gel-filtration of brain extract of animals with compensated PA on Sephadex G-25, on the 21st day after operation. Addition of aliquots of the V_0 fraction to 1 mg of extract (100 μg protein, 1000 minimal active doses of PAF) from the brain of animals with PA (3 days after cortical ablation) led to inactivation of PAF. As Table 3 shows, the inactivation effect was characterized by dose-dependence. The V_0 fraction obtained similarly from brain extract of an intact animal did not possess the property of inactivating PAF in the same doses.

It can be concluded on the basis of the results that the stage of compensatory transformations of the CNS after unilateral ablation of the motor cortex is characterized by the appearance of a factor inactivating PAF in the brain tissue. The molecular weight of the inactivating factor exceeds 5 kilodaltons. Inactivation of PAF may be due to competition between PAF and its inactivating factor for binding sites on target cells of the segmental apparatus, not leading to induction of PA (Table 2), or to the formation of an inactive PAF – PAF inactivating factor complex, dissociating in an acid medium, and separated by gel-filtration. Similar properties are known for neurophysin–oxytocin (vasopressin) complexes [12] and also for antigen–antibody complexes [9].

Elucidation of the true mechanisms of PAF inactivation is of great importance for our understanding of the biochemical basis of compensatory processes after injury to the CNS and is an interesting topic for future research.

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EFFECT OF ACTH ON CORTICOSTEROID AND PROGESTERONE LEVELS IN FEMALE BABOONS DEPENDING ON THE PHASE OF THE MENSTRUAL CYCLE

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Dependence of adrenal secretion of glucocorticoids on ACTH has now been well studied. However, there is no unambiguous answer to the question of how the sensitivity of the adrenals to ACTH changes in relation to changes in sex hormone concentration in the peripheral blood. We know that the ACTH level in women changes in the course of the menstrual cycle [6]. Removal of the ovaries in rats leads to a decrease in synthesis of ACTH and its release from the pituitary [4]. An increase in adrenocortical activity in rats in the period of proestrus [11] and a preovulatory rise of the cortisol concentration in the peripheral blood of women have been discovered [6]. There is also evidence of a decrease in the trophic effect of ACTH in women after ovariectomy and its restoration by preliminary estrogen therapy [12].

To study the effect of ACTH on the endocrine function of steroid-producing glands depending on the level of sex hormones in the body, a comparative study of the dynamics of steroid hormones in the follicular and luteal phases of the menstrual cycle in response to a standard dose of ACTH was undertaken in experiments on hamadryad baboons.

EXPERIMENTAL METHOD

Five mature female hamadryad baboons weighing 12-21 kg and aged 8-13 years were used. The experimental animals had a regular menstrual cycle lasting 28-38 days. The state of the sex cycle of the baboons was monitored by observing swelling of the genital skin. The first day of menstrual bleeding was taken as the beginning of the cycle. Synthetic ACTH (B 1-24, ACTH, Synacthen) was injected intravenously in a dose of 1 U/kg body weight in the follicular (6th-9th days) and luteal phases of the cycle (21st-23rd day), during the period of maximal activity of the corpus luteus. Blood was taken from the cubital vein (8-10 ml) before and 30 min and 1, 2, 4, 24, and 48 h after injection of the ACTH. The plasma was obtained by centrifugation at 3000 rpm for 20 min and was kept at −20°C until required for assay. Concentrations of corticosterone, 11-deoxycortisol, and progesterone were determined in duplicate samples of plasma by radioimmunoassay [7]. Antisera obtained by the Laboratory of Experimental Endocrinology, Research Institute of Experimental Pathology and Therapy, Academy of Medical Sciences of the USSR, Sukhumi (TU 42.14, 333-82, 334-82, and 331-82) were used for determination. Cortisol (S) was determined by the competitive binding method [10]. Calibration curves were plotted between logit-log coordinates, and the steroid levels in the plasma were determined and the results subjected to statistical analysis by Student's t test on the "Élektronika 15-VSM-5" computer.

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