NATURE OF FACTORS INACTIVATING POSTURAL ASYMMETRY FACTOR ON INITIAL STAGES OF COMPENSATORY ADJUSTMENTS IN ANIMALS WITH UNILATERAL DESTRUCTION OF THE MOTOR NEOCORTEX

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Unilateral injury to structures of the motor cortex [1-3] leads to disturbances of the function of the segmental apparatus of the spinal cord, manifested as fixation of postural asymmetry (PA) by the lumbar segments. The active principle for PA fixation consists of peptide postural asymmetry factors (PAF), found in the CSF and brain extracts of animals in the first 2 weeks after CNS injuries of this type. After 3-4 weeks the CSF and brain extracts of the animals lose their ability to induce reliable PA of the hind limbs [4-6]; disappearance of PAF activity in the CSF and brain extracts of these animals, moreover, is due not to diminution of the PAF pool, but to the appearance of a thermolabile factor [5, 6] inactivating PAF, namely inactivation factor (IF).

The aim of the investigation described below was to study the chemical and biological nature of IF.

EXPERIMENTAL METHOD

Experiments were carried out on three adult male cats weighing 3.5-4.0 kg (donors) and on noninbred male albino rats weighing 160-180 g (recipients). The cortical representation of the right hind limb of the cats was removed by the method described previously [6] and, on the 2nd and 21st days after the operation, 0.5-1.0 ml of CSF was taken from the cisterna magna (under ether anesthesia). The CSF was freeze dried and kept at −20°C. The freeze-dried product from 3-week donors was fractionated by column gel-filtration on Sephadex G-25 superfine ("Pharmacia," Sweden) into high (mol. wt. > 5.0 kD) and low-molecular-weight (mol. wt. < 5.0 kD) fractions. The fractionated material (10 mg of freeze-dried CSF) was dissolved in 0.1 ml of 1 mM acetic acid and applied to a column (12 cm long, internal diameter 1.0 cm) and eluted with 1 M acetic acid, with a rate of flow of 3.5 ml/$cm^2 \cdot h$. The fractions were freeze-dried. For biological testing, the freeze-dried product of each fraction was dissolved in 1 ml Tris-HCl buffer, pH 7.4, containing 0.15 ml NaCl. Molecular-weight fractionation of the freeze-dried CSF also was carried out on Sephadex G-200 ("Sigma," USA; 10-40 μ fraction) on a column 16 cm long and with an internal diameter of 0.8 cm; Tris-HCl buffer, pH 7.4, containing 0.15 M NaCl, was used as the eluant. Between 1 and 2 mg of the freeze-dried CSF from 3-week donors, dissolved in 25 μM elution buffer, was applied to the column. The eluted material was collected in 0.2 ml fractions. Dextran blue, mol. wt. 2000 kD ("Serva," West Germany), human IgG, mol. wt. 150 kD, serum albumin ("Sigma"), mol. wt. 68 kD, and cytochrome C ("Reanal," Hungary), mol. wt. 13 kD, were used as the standards for calibration. Heat treatment of the CSF and its fractions was carried out in a sealed glass 1.0-ml ampul at 100°C for 15 min. For enzymic treatment of the high-molecular-weight fraction of CSF from 3-week donors, the freeze-dried product of this fraction was dissolved in 1 ml of Tris-HCl buffer, pH 8.1, containing 0.15 M NaCl, and applied to a 2-ml column, filled with sepharose-4B with immobilized trypsin ("Serva"); the quantity of immobilized trypsin was 20 mg. After incubation for 20 h at 37°C the hydrolysate was eluted and used for further investigation. The incubation time was chosen in accordance with the kinetics of enzymic hydrolysis of a solution of azaalbumin ("Calbiochem," USA).

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TABLE 1. PAF Activity in CSF of Donors after Unilateral Injury to Motor Neocortex

Substance tested	Time after operation	PAF activity in freeze-dried CSF			
		total number of recipients	number of recipients with right-sided PA	number of recipients with left-sided PA	PAF activity
Freeze-dried CSF	2	15	14	0	+
without heat treatment	21	14	3	4	−
after heat treatment	21	12	11	1	+

Legend. A solution of CSF with a concentration of 1 mg in 1 ml buffer was used.

TABLE 2. PAF Activity in Fractions of 21-Day CSF, Fractionated by Gel Filtration on Sephadex G-25

Substance tested	PAF activity in fractions			
	total number of recipients	number of recipients with right-sided PA	number of recipients with left-sided PA	PAF activity
Fraction V_e	15	13	1	+
Fraction V_0	10	4	3	−
Total of fractions V_e and V_0	11	4	0	−
Total of fractions of V_0 after treatment with trypsin and V_e	12	11	0	+

Legend. V_e) Inner volume fraction (mol. wt. under 5 kD); V_0) fraction of void volume (mol. wt. over 5 kD).

The freeze-dried CSF from 3-week donors was incubated with protein A (from the Pasteur Institute) in 1.0 ml of 0.05 M Tris-buffer, pH 7.4, containing 0.15 M NaCl. The composition of the incubation mixture was: 1 mg freeze-dried CSF and 0.1 mg protein A. The incubation time was 60 min. After incubation the mixture was fractionated on a column with Sephadex G-200. Activity of PAF in the freeze-dried CSF and its fractions was detected on the basis of their ability to induce flexion of the right hind limb in recipient rats after slow injection of 50 µl of a solution of the test material into the cisterna magna, followed by division of the spinal cord 5 min later at the level T1-T2. PA was recorded by the method [1, 2, 4] 1 h after spinalization of the recipients. The results were subjected to statistical analysis by the signs test [7].

EXPERIMENTAL RESULTS

The CSF of the cats 2 days after the operation of removal of the cortical representation of the right hind limb induced significant right-sided PA of the hind limbs in the recipient rats, but CSF from 3-week-old donors did not possess this property. Meanwhile, heat treatment of the CSF of the 3-week donors revealed PAF activity in it (Table 1). This suggests that the CSF of compensated donors contains thermolabile factors inactivating PAF. The action of IF on PAF activity can also be abolished by gel-filtration on Sephadex G-25 in 1 M acetic acid. In this case, PAF activity was found in the inner volume fraction, corresponding to biopolymers with mol. wt. of below 5.0 kD. The free volume fraction, corresponding to biopolymers with mol. wt. of over 5.0 kD did not possess this property. Pooling aliquots of the free and inner volume fractions led to inactivation of PAF (Table 1). Pooling aliquots of the free volume fraction, after treatment with trypsin, and the inner

TABLE 3. Determination of Molecular Weights of PAF–IF Complexes by Gel-Filtration on Sephadex G-200

Range of molecular weights, kD	Biological testing of fractions											
	freeze dried CSF							freeze-dried CSF and protein A				
	before heat treatment			PAF activity	after heat treatment			PAF activity	after heat treatment			PAF activity
	T	R	L		T	R	L		T	R	L	
Over 200	8	2	2	—	17	5	7	—	12	12	0	+
200—160	9	4	0	—	11	2	4	—	9	4	1	—
160—130	8	1	3	—	17	15	2	+	13	5	3	—
130—90	11	2	1	—	13	2	5	—	9	3	4	—
90—70	9	4	2	—	18	17	1	+	12	11	0	+
Under 70	7	2	1	—	11	4	5	—	10	4	2	—

Legend. T) Total number of recipients, R) number of recipients with right-sided PA, L) number of recipients with left-sided PA.

volume fraction did not lead to inactivation of PAF (Table 2), evidence of the protein nature of IF.

It was suggested previously that inactivation of PAF at the compensation stage takes place due to the formation of a soluble PAF–IF complex, unable to induce PA [5]. Biological testing of the fractions obtained after molecular-weight fractionation of the freeze-dried CSF on Sephadex G-200 did not detect any significant PAF activity in them. However, heat treatment revealed PAF activity in fractions with an approximate mol. wt. of 160-130 and 90-70 kD (Table 3). Considering the low molecular weight of PAF (under 5 kD) it can be tentatively suggested that the molecular weight of IF does not differ significantly from that of the IF–PAF complexes. On the basis of previous data on the character of the time course of IF activity in the CSF of donors [6] and values obtained in the present investigation for mol. wt. of IF it can be postulated that the IF fraction with mol. wt. of 160-130 kD consists of antigen (IgG). To test this hypothesis, the unique property of protein A of forming complexes with Ig of the G class, without thereby disturbing interaction of antibody with antigen [8], was used. Fractionation of the incubation mixture of freeze-dried CSF with protein A on Sephadex G-200 followed by heat treatment of the fractions obtained revealed PAF activity as before in fractions with mol. wt. of 90-70 kD and the void volume fraction. In the fraction with mol. wt. of 160-130 kD, however, no PAF activity could be found. This can be explained by the formation of a bond between protein A and the PAF–IF complex, leading to an increase in molecular weight and to a significant change in the elution volume of the complex containing PAF.

To sum up the results it can be concluded that in the initial stages of compensation after unilateral injury to the motor neocortex, inactivation of PAF takes place due to the appearance of high-molecular-weight protein factors in the CNS, consisting partly of antibodies, and inactivating PAF by forming a soluble complex with it.

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