DETECTION OF POSTURAL ASYMMETRY FACTORS IN PITUITARY GLAND TISSUE CULTURE IN THE PRESENCE OF CEREBROSPINAL FLUID FROM CATS WITH UNILATERAL CORTICAL LESIONS

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The formation of a stable pathological state (SPS) in the CNS as a result of an organic brain lesion is a multifactorial multistaged process [6]. The presence of two stages in the formation of the new homeostasis in the CNS has now been established after local traumatic lesions of the motor system of the brain. Each of these stages is associated with the appearance of certain types of substances in the cerebrospinal fluid (CSF) and brain tissue: postural asymmetry factors (PAF) and compensatory factors blocking the action of PAF [4, 8]. However, existing experimental data on the time course of postural asymmetry (PA) formation in the presence of unilateral lesions of the motor cortex [4] suggest the existence of at least one other (earlier) stage of reorganization associated with activity of compounds of a certain type, functioning within the framework of the innervating structure — innervated target structure system. In the case of a disturbance of innervation of the latter, the hypothetical chemical factors, which we conventionally call PAF inducers (PAFI), ought to appear initially in the CSF and induce secretion of PAF from the hypothalamo-hypophyseal system of the brain [7]. Direct proof of secretion of PAF by the pituitary under the influence of PAFI can be obtained by long-term organotypic pituitary tissue culture [12]. If these hypothetical PAFI really exist, addition of CSF, taken from an animal in the early post-traumatic period before the appearance of PAF in its CSF, to the nutrient medium for pituitary gland culture ought to lead to the appearance of PAF in the conditioned culture medium, which would be detectable by the usual biological test for PAF activity [4].

The aim of this investigation was to test the hypothesis of the existence of PAFI as a PAF releasing factor, acting on pituitary cells.

EXPERIMENTAL METHOD

Five cats in which a circumscribed zone of the motor cortex of one cerebral hemisphere, stimulation of which by needle electrodes evoked contraction of the contralateral hind limb, was removed by suction under pentobarbital anesthesia (40 mg/kg), were used as donors of "pathological" CSF. The CSF was taken from the cisterna magna of the animal 2-3 h after the operation. Noninbred male rats were used as recipients for biological testing. To obtain conditioned medium (CM) 46 pituitary gland explants taken from the brain of 46 noninbred newborn rats were used. The tissue was cultured in revolving tubes in a modification using "flying coverslips" (Nunc, Denmark) on collagen supports in an atmosphere containing 5% CO_2, 25% O_2, and 70% N_2, at 37°C, in nutrient medium of the following composition: MEM medium (Gibco, USA) 60%, embryonic calf serum (Gibco) 15%, Hanks' salt solution 25%, glucose 600 mg %, and insulin 0.4 U/ml, in the presence of gentamicin 100 μg/ml. The cultures were grown in vitro in Petri dishes for 3 days under the conditions specified above, then transferred to revolving tubes [11]. The nutrient media were changed 3 times a week. "Pathological" CSF was obtained from the cats undergoing operations 6 days after the beginning of culture. It amounted to 20% of the volume of the nutrient medium. The pituitary glands were cultured in medium containing CSF for 2, 4, 8, 16, and 24 h. The CS was then withdrawn to determine PAF activity [3]. For this purpose, under superficial ether anesthesia, 25 μl of CM was injected into the cisterna magna, after with the spinal cord was divided at the

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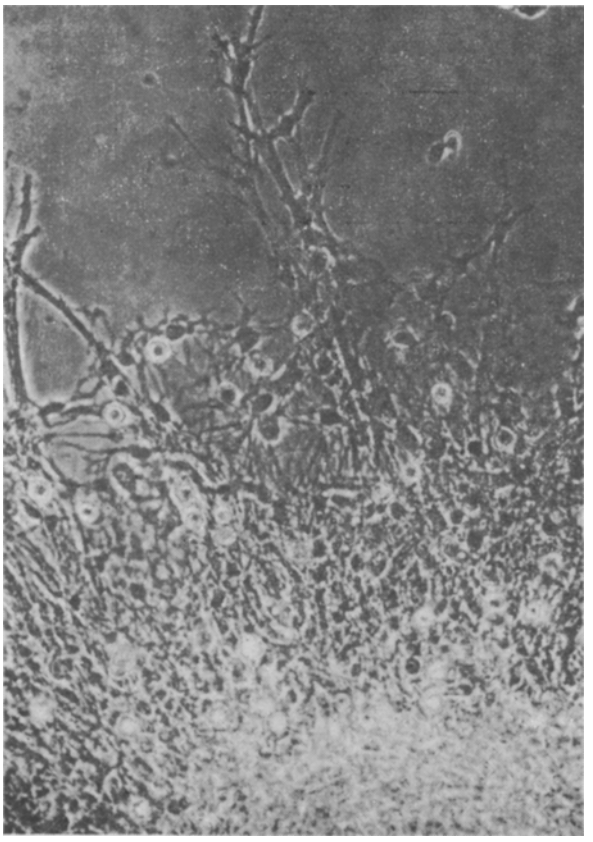

Fig. 1. Zone of neonatal rat pituitary in culture (6 days of culture). Phase contrast. 100 ×.

TABLE 1. PA Formation in Intact Recipients of Pituitary CM

Recipients	Containing 20% CSF from donor with motor cortex lesions		"CSF-free" medium	CSF from donors with lesion of motor cortex	
	right	left		right	left
With right-sided PA	2	10	0	2	1
With left-sided PA	16	1	1	2	2
Without PA	1	2	9	8	8

Legend. CSF was taken 2-3 h after unilateral injury to the cerebral cortex.

thoracic level [3]. The morphological and functional state of the pituitary culture was assessed by monitoring changes in the color of the nutrient medium during culture [9] and also by methods described previously [1]. Morphological analysis of the explant as a whole and of its individual cells comprising the zone of growth, was undertaken by E. I. Chumasov. The CSF was treated with hydrolytic enzymes: trypsin (Sigma, USA) and protease-free ribonuclease A (Sigma), in 0.15 M NaCl (pH 8.0-8.1). To treat 1 ml of CSF 0.1 mg of trypsin and 0.02 mg of ribonuclease A were used. Hydrolysis was carried out for 5 h at 37°C. The reaction was stopped by addition of 10% embryonic serum [13]. The results were subjected to statistical analysis by Bernoulli's method [2].

EXPERIMENTAL RESULTS

Coverslips with pituitary cultures were placed in the center of Petri dishes 35 mm in diameter, and surrounded by 0.5 ml of nutrient medium, without covering, but only slightly moistening, the explants. After 1 day the dishes were aerated with the gas mixture mentioned above and returned to the thermostat. Pituitary cultures preincubated for 3 days became

TABLE 2. Action of Trypsin and Ribonuclease on PAFI of CSF after Left-Sided Destruction of Donor's Motor Cortex

Enzyme	PA		Absence of PA	PAF activity
	right-sided	left-sided		
Trypsin	2	2	8	—
Ribonuclease	11	1	3	+

TABLE 3. Time of Appearance of PAF Activity in Pituitary CM

Parameter	Culture of pituitary gland with "pathological" CSF, h								
Presence of activity PAF	1	2	3	4		8	16	24	48
	0	0	±	+		+	+	+	+

Legend. 0) Absence of PAF activity, ±) activity not significant; +) PAF activity present in CM.

sufficiently well adapted and adherent to the collagen supports. The coverslips were then transferred to revolving tubes containing 1-1.5 ml (depending on the type of tubes) medium and, after aeration for 15 sec, were left for culture for 72 h. By that time, an active zone of growth 300-500 μ wide, containing fibroblast-like cells and actively proliferating glia, was observed around the spreading explants (Fig. 1). Next, medium containing "pathological" CSF was added to the pituitary culture. Addition of CSF to the nutrient medium stimulated metabolism, as shown by the more rapid yellowing of CM compared with the usual rate.

After removal the CM was tested for PAF activity by a double blind control method. "CSF-free" media, early "pathological" CSF, CM-containing CSF from an intact animal, and CSF hydrolysates were tested in the same way (Tables 1 and 2).

The data given in Table 1 indicates that addition of "pathological" CSF taken in the early stages of the post-traumatic period, before any PAF activity was present in the CSF, led to the appearance of PAF activity in the CM of the pituitary culture with an action similar in type to that subsequently observed in the donor. For instance, medium with the addition of CSF from a donor with a lesion on the right side induced PA of the corresponding sign (lef-sided) in more than 80% of recipients, whereas medium to which CSF from donors with lesions on the left side was added induced right-sided asymmetry in the overwhelming majority of recipients. Meanwhile, "CSF-free" medium and early "pathological" CSF had no FA activity and induced PA only in very few recipient rats.

Consequently, in the early stages of the post-traumatic period (2-3 h) the donor's CSF contained factors specific relative to the side of the cortical lesion, inducing the production of PAS of the corresponding sign by pituitary cells. In other words, these experiments indicate that special hypophysiotropic releasing factors, or PAFI, appear in the CSF during the first few hours after trauma.

It will be clear from Table 2 that PAFI from the CSF of "left-sided" donors is inactivated by trypsin but not destroyed by ribonuclease. PAFI from CSF of donors with right-sided cortical lesions likewise is destroyed by trypsin and resistant to the action of ribonuclease, i.e., these results are evidence of the protein nature of PAFI.

Determination of the time during which PAF accumulates in CF in an amount sufficient for its activity to be detected by biological testing is an interesting problem.

It will be clear from Table 3 that PAF begin to appear in CM after 3-4 h. However, we know that in vivo, PAF activity does not appear in the CSF of animals with trauma to the cerebral cortex until after 18-20 h [4], whereas in man it appears 2-3 h after cortical injury [10].

These results are the first direct experimental proof of the existence of factors in the CSF justifying the name of "PAF inducers," which are precursors of factors of the PAF type.

Production of certain PAF by the rat pituitary in culture under the influence of CSF from cats indicates that PAFI are species-nonspecific. This fact in principle provides an opportunity for the practical use of the experimental data for the early diagnosis of brain trauma.

We do not yet know the site of formation of PAFI. Whether it is formed in the innervating structure-target structure system or, by analogy with releasing hormones, the hypothalamus plays a part in its formation, are problems which require further experimental investigation. At the present stage of study it can only be concluded that the ability of the pituitary gland in tissue culture to produce certain specific factors under the influence of early "pathological" CSF opens the way for production and subsequent analysis of significant quantities of sufficiently pure humoral factors associated with various CNS lesions.

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