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NEUROSENSITIZATION IN RESUSCITATED ANIMALS AND ITS CORRECTION BY PYRACETAM

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The most general principles governing injury to and restoration of the functions of organs and systems during dying and resuscitation have now been established [4]. Post-resuscitation brain pathology, known in the literature as postanoxic encephalopathy, resuscitation-induced encephalopathy, the respiratory brain, postresuscitation encephalopathy, and so on, is particularly interesting. In most publications on this matter the role of hemodynamic disorders, metabolic changes, and neurophysiological mechanisms in the genesis of the cerebral changes has been examined [3, 4, 11]. Meanwhile the possible role of immune mechanisms in postresuscitation brain pathology has been studied quite inadequately [8]. Further investigations also are required into the problem of correction of these disturbances by drugs.

The aim of the present investigation was to study neurosensitization in animals recovering from a terminal state and the effect of pyracetam, a drug which has begun to be used in resuscitation practice [10], on this process.

EXPERIMENTAL METHOD

Experiments were carried out on Wistar rats in which clinical death was induced under superficial ether anesthesia by the method of external cardiac tamponade [2]. The resuscitation measures included closed cardiac massage and artificial ventilation of the lungs. There were two series of experiments, differing in the duration of the terminal state: 4-5 min in series I, 7-8 min in series II. Under the experimental conditions used, the number of animals successfully resuscitated in these series of experiments was 53.8 and 33.6% of the total number of resuscitated rats respectively. The state of neurosensitization was evaluated by the following methods: the complement fixation test in the cold [1], tests of activation of spontaneous rosette formation [9], and tests of inhibition of migration of cells from splenic fragments [5]. Antigens for the immunologic studies were saline extracts from the cerebral cortex and skeletal muscle tissues of rats. Lymphocytes were isolated from the blood in a Ficoll—Verografin density gradient and their viability was determined by the trypan blue test. These experiments were carried out on intact animals and also on animals resuscitated on the 7th, 14th, 21st, and 30th days after the terminal state. In some experiments, starting with the 1st day after resuscitation, the animals were treated with pyracetam (100 mg/kg, subcutaneously) for 10 days.

EXPERIMENTAL RESULTS

The experiments showed that clinical death induced a state of neurosensitization in the animal. The development of this phenomenon proceeded in two ways: humoral and cellular. Immunologic reactions of humoral type were reflected in the discovery of antibrain antibodies in the blood of the resuscitated rats. It follows from the data given in Table 1

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TABLE 1. Effect of Pyracetam on Dynamics of Immunologic Parameters in Resuscitated Rats (n = 10)

Parameters	Series of experiments	With (+) or without (–) pyracetam	Intact rats	Time after resuscitation (days)			
				7	14	21	30
Antibrain antibodies, log_2 of titer	I	–	0,44±0,36	1,56±0,38	3,05±0,43**	2,58±0,47*	2,1±0,56
		+		1,14±0,27	2,21±0,35*	1,85±0,41	1,26±0,35
	II	–		1,88±0,43	5,2±0,64***	3,5±0,45**	2,8±0,50*
		+		1,48±0,31	3,4±0,51**	2,43±0,42*	2,0±0,44
Antimuscle antibodies, log_2 of titer	I	–	0,23±0,20	0,35±0,27	0,56±0,15	0,48±0,16	0,33±0,18
		+		0,39±0,22	0,41±0,15	0,34±0,20	0,18±0,10
	II	–		0,40±0,18	0,65±0,30	0,72±0,28	0,51±0,23
		+		0,29±0,14	0,57±0,33	0,55±0,27	0,42±0,17
E-RFC with brain antigen, %	I	–	36,8±2,12	32,1±3,02	41,5±2,84	49,4±2,51*	44,3±2,72
		+		28,9±2,11	39,7±3,16	43,7±2,88	42,4±3,00
	II	–		38,3±4,03	48,2±3,1*	53,4±3,42**	55,2±3,83**
		+		31,6±3,22	41,5±2,35	47,7±2,85*	46,8±3,14
E-RFC with muscle antigen, %	I	–	31,3±3,42	29,7±2,56	28,4±3,27	30,0±2,42	29,3±3,04
		+		27,6±2,84	30,5±2,46	28,3±2,65	33,2±2,92
	II	–		28,8±2,60	33,4±3,15	27,8±2,90	31,7±3,22
		+		32,1±3,18	29,0±2,58	31,4±3,31	28,7±2,35
Inhibition of zone of splenocyte migration by brain autoantigen	I	–	1,07±0,07	0,95±0,06	0,86±0,04	0,80±0,05*	0,83±0,04*
		+		0,93±0,04	0,90±0,05	0,84±0,05	0,88±0,05
	II	–		0,98±0,07	0,83±0,05*	0,61±0,06**	0,77±0,07*
		+		1,01±0,06	0,89±0,04	0,84±0,05*	0,86±0,05
Inhibition of zone of splenocyte migration by muscle autoantigen	I	–	0,98±0,06	0,92±0,03	0,91±0,06	0,96±0,06	0,93±0,05
		+		0,95±0,05	0,97±0,08	0,90±0,03	0,95±0,06
	II	–		0,91±0,04	0,90±0,05	0,93±0,04	0,87±0,04
		+		0,97±0,06	0,86±0,04	0,95±0,06	0,95±0,05

Legend. Significance of differences calculated relative to corresponding values for intact animals; *P < 0.05, **P < 0.01, ***P < 0.001. Numerator – without pyracetam, denominator – with pyracetam.

that the maximal level of circulating antibrain antibodies was observed on the 14th day after resuscitation. Later their titer fell gradually.

When the data given above are analyzed, a number of factors must be picked out. First, the frequency of discovery and the level of complement-fixing antibrain antibodies in the blood serum clearly depended on the duration of the terminal state. In the experiments of series II the intensity of humoral neurosensitization was found to be higher. Second, such a sharp rise in the level of antibodies against muscle tissue antigens was not observed in the blood serum of the resuscitated animals.

Hypersensitivity of cellular types was recorded in the resuscitated rats on the basis of the results of both tests used. Preincubation of lymphocytes with an autohomogenate of the brain (but not of skeletal muscles) was accompanied by activation of the spontaneous rosette formation test with an increase in the number of detectable rosettes with sheep's erythrocytes (E-RFC). Addition of brain antigen to the incubation medium significantly inhibited migration of splenocytes of resuscitated animals. No such effect was observed when either fragments of the spleen from intact animals or saline extract of skeletal muscles was used in the test.

The immunologic processes tested were found to follow a definite time course, characterized by the somewhat later development of cellular neurosensitization compared with the appearance of antibrain antibodies in the blood serum of the resuscitated animals. For instance, the greatest change in the number of E-RFC and inhibition of cell migration from splenic fragments were observed on the 21st-30th days after the terminal state.

Administration of pyracetam had a marked effect on the development of neurosensitization in the postresuscitation period. A clear decrease was observed in the serum level of antibrain antibodies when the drug was used. This action was exhibited as early as on the 7th day after resuscitation, but it was most marked by the 14th day of the postresuscitation

period. The drug also affected the indices of cellular neurosensitization. This is shown by the statistically significant decrease in inhibition of splenocyte migration in tests with brain antigen. Simultaneous weakening of the inhibitory effect of brain antigen of resuscitated animals was observed on spontaneous rosette formation of autologous lymphocytes with sheep's erythrocytes.

Considering information in the literature on the beneficial effect of pyracetam on the microcirculation and metabolism in the CNS in hypoxic cerebral edema [6], the importance of this aspect of the pharmacodynamics of the drug in minimizing the neurosensitization-inducing action of a period in the terminal state can be postulated. This suggestion is also in agreement with the fact that pyracetam has marked antihypoxic properties [7]. At the same time the possibility of a direct action of the drug on immunogenesis cannot be ruled out.

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