EFFECT OF PYRACETAM DERIVATIVES ON ANTIBODY FORMATION

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The widespread use of substances with nootropic activity in medical practice has brought with it the need for a more intensive study of the pharmacodynamics of this comparatively new group of drugs. In particular, their effect on immunologic responses requires investigation, for this has not been discussed in the literature. This aspect of the study of compounds with nootropic activity is based on existing information indicating close interaction between the nervous and immune systems at ontogenetic and neurotransmitter levels [2, 10, 11].

An effective drug with nootropic activity is pyracetam, which has been shown to inhibit serum brain antibody tissues in animals resuscitated from the terminal state [7]. It is not clear, however, whether this effect of the drug is connected with its direct action an antibody formation or whether it is due entirely to the known antihypoxic properties of pyracetam and its beneficial effect on the intracerebral hemodynamics and microcirculation [1, 8, 9].

The aim of this investigation is to study the immunotropic activity of pyracetam and some of its derivatives synthesized in the Institute of Pharmacology, Academy of Medical Sciences of the USSR, in order to reveal any connection between the trend of their neurotropic and immunomodulating action.

EXPERIMENTAL METHOD

Experiments were carried out on (CBA × C57BL)F_1 hybrid mice weighing 18-21 g. There were four series of investigations. In series I (control) the primary immune response of the animals to intraperitoneal injection of thymus-dependent antigen (sheep's red blood cells) in a dose of 10^8 cells/10 g body weight was studied. The intensity of the immune response was tested by determining the number of antibody-forming cells (AFC) in the spleen of the immunized animals, by the Cunningham plaque technique [12] on the 5th day after injection of the antigen. Viability of the spleen cells used in the reaction was estimated from the results of the test with 0.1% trypan blue solution. In the experiments of series II, simultaneously with immunization and during the next 2 days the mice were given intraperitoneal injections of pyracetam or one of its derivatives, whose neurotropic activity was described previously [5]. The test substances were given in doses of 50, 100, and 300 mg/kg and their effect on the primary immune response was analyzed, depending on the dose of antigen injected (series III). Since pyracetam is given under clinical conditions in long courses, it was administered perorally in a dose of 200 mg/kg daily for 15 days (series IV), after which, besides the number of AFC in the animals, the weight of the spleen, number of nucleated cells, and ratio between the number of viable and nonviable splenocytes (cell viability index, CVI) in the spleen were determined. During all these investigations the mice were immunized by the method described above on the 1st day after the end of pyracetam administration.

EXPERIMENTAL RESULTS

Pyracetam in a dose of 200 mg/kg had a definite inhibitory effect on the primary immune response and caused a statistically significant decrease in the number of antibody-producing

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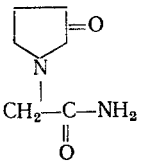
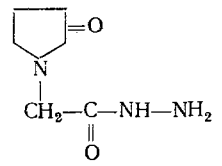
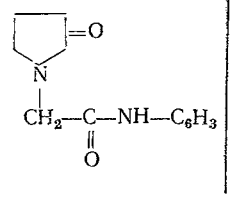
TABLE 1. Changes in Number of AFC in Mouse Spleen Depending on Dose of Drugs and Dose of Antigen Injected (M ± m)

Name of compound	Chemical structure	Dose of antigen injected (number of SRBC/10 g body weight)	Dose of compound, mg/kg	Number of AFC	
				per 10^6 splenocytes	in per cent of control
Control	—	10^8	—	435,0±38,0	100,0
		10^7	—	348,0±52,5	100,0
Pyracetam		10^8	100	379,0±25,8	87,0
		10^8	200	315,0±21,0	72,4*
		10^7	50	254,0±19,5	73,2*
		10^7	100	210,0±20,1	60,5*
		10^7	200	181,0±15,3	52,0*
Hydrazide analog of pyracetam		10^8	100	780,0±51,2	179,0*
		10^8	200	825,0±65,8	190,5*
		10^7	50	418,0±35,4	120,3
		10^7	100	532,0±29,0	153,0*
		10^7	200	617,0±73,2	177,4*
N-phenylpyracetam		10^8	100	242,0±15,3	55,7*
		10^8	200	80,0±6,4	18,5*
		10^7	100	244,0±28,3	73,0*
		10^7	200	138,0±9,5	39,8*

Legend. *P < 0.05, here and in Table 2. SRBC) Sheep's red blood cells.

cells in the spleen (Table 1). The intensity of this effect depended on the dose of antigen injected. For instance, after injection of the test antigen in a dose of 10^8 cells/10 g body weight, pyracetam in a dose of 100 mg/kg did not reduce the immune response. However, the use of this dose of the drug after weaker antigenic stimulation was accompanied by inhibition of antibody formation.

Addition of the phenyl radical to the nitrogen in the side chain of the pyracetam molecule (N-phenylpyracetam) enhanced the immunosuppressive properties of the compound, as was shown by the greater decrease in the number of AFC in the spleen of mice receiving this compound compared with the action of pyracetam itself. When the amide group in the pyracetam molecule was replaced by a hydrazide group (the hydrazide analog of pyracetam) a change was observed in the trend of immunotropic activity, expressed as marked stimulation of antibody formation. This fact is in agreement with data in the literature indicating that substances containing a hydrazide group, such as hydrazine sulfate, hydrallazine, and the hydrazine derivative 1,1'-dimethylhydrazine, possess immunostimulating properties [13, 15].

The immunosuppressive action of pyracetam was still present in the case of prolonged peroral administration of the drug (Table 2). The reduction in the number of AFC in the spleen observed under these circumstances was not accompanied by a decrease in weight of the organ. Evidence that the immunosuppressive effect of pyracetam was not due to any toxic action on splenocytes was given by calculation of the CVI, reflecting the ratio between living and nonviable nucleated cells in the spleen (Table 2). The value of CVI for mice receiving pyracetam was about the same as in the control. This is evidence of a qualitative difference between pyracetam and the cytostatic immunodepressants, during administration of which the number of nonviable cells in lymphoid organs increases sharply.

The mechanism of the immunotropic action of pyracetam has not yet been finally established. There is evidence that the structure of this compound is similar to that of the cyclic form of GABA [3], and electrophysiological evidence of the presence of a GABA-ergic component in the action of pyracetam [4]. On the basis of these facts, and also considering recent data on the effect of GABA on lymphocyte activity [6, 14], it can be postulated that the reduction in the number of AFC observed in the spleen is the result of the inhibitory action of pyracetam on differentiation of B lymphocytes into antibody-producers.

It follows from the data given above that modification of the pyracetam molecule is accompanied by a change in the character of its immunotropic activity, which correlates with

TABLE 2. Effect of Pyracetam on Some Parameters of Splenic Cell Composition (M ± m, n = 15)

Parameter	Control	Pyracetam, 200 mg/kg, for 15 days
Weight of spleen, mg	184,0±9,7	173,0±10,4
Number of nucleated cells (·10^6)		
total	143,0±25,0	138,0±22,0
living	135,0±20,0	127,0±21,0
nonviable	8,0±1,5	10,6±1,8
CV1	16,8±3,2	11,9±2,5
Number of AFC (per 10^6 splenocytes)	435,0±38,0	236,0±15,0

the trend of the neurotropic action, discovered previously, of drugs of this class. For instance, enhancement of the immunosuppressive effect of N-phenylpyrrolidone corresponds to the marked central depressant activity of this pyracetam derivative. Conversely, the discovery that the hydrazide analog of pyracetam stimulates antibody production correlates with the stimulating action of this compound on the CNS [5].

The results of the present investigation are further evidence of the receptor analog of the nervous and immune systems. The results are evidence that pyracetam affects antibody formation, and this must be taken into account when this drug is used clinically. The opposite nature of the immunotropic activity of pyracetam derivatives also indicates that a search among α-pyrrolidone derivatives for substances for use in immunomodulating therapy may be useful.

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