Antiserotonin Antibodies as Stimulators of Mouse Peritoneal Macrophages

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 124, No. 12, pp. 663-665, December, 1997
Original article submitted June 3, 1996

Stimulating effect of antiserotonin antibodies on phagocyte activity of peritoneal macrophages from C57Bl/6 mice is demonstrated both after systemic administration and in a cell culture. *In vitro* experiments show that serotonin and antiserotonin antibodies exert similar effects. A possible mechanism of neurotropic effect of antiserotonin antibodies involving immunocompetent cells is discussed.

Key Words: *neurotransmitters; antibodies; macrophages*

Ample experimental material is now accumulated on a strict relationship between the nerve and immune system [7,13]. Cells of these systems produce similar regulatory factors (interleukins, interferons, endorphins, etc.) and bear the corresponding receptors [1,7]. L. V. Devoino *et al.* [4] have found that neurotransmitters exert both suppressive (serotonin, 5-HT) and stimulating (dopamine) effects on the immune system. Enhanced generation of autoantibodies against various neurotransmitters in different neuropathological states has been demonstrated clinically and experimentally [3,12,15]. We previously studied neuromodulating effects of antitransmitter antibodies (Ab) in some neuropathological states: alcoholism, drug addiction, and parkinsonism [5,8,9].

On the other hand, the effect of Ab to neurotransmitters on the central nervous system (CNS) and their role in the interplay between the nervous and immune systems remain poorly understood. Long-lasting neurotropic effect of Ab to neurotransmitters may attest to the involvement of immunocytes producing bidirectional cytokines (interleukins, interferon, and tumor necrosis factor) into this process [6]. The aim of the present study was to verify the hypothesis that immunocytes are activated by Ab to neurotransmitters.

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MATERIALS AND METHODS

Experiments were carried out on male C57Bl/6 mice weighing 18-20 g (n=50). The effect of anti-5-HT antibodies on phagocytic activity (PA) of peritoneal macrophages was studied after systemic administration, in cell culture, and in comparison with the effect of 5-HT.

Anti-5-HT Ab were isolated from rabbits immunized according to standard protocol [2] with 5-HT—bovine serum albumin conjugate synthesized as described previously [14]. The titer of anti-5-HT Ab from the data of enzyme-linked immunosorbent assay [3] was 1:12,000. The specificity of isolated Ab was tested by competitive inhibition with free 5-HT (enzyme-linked immunosorbent assay). γ-Globulin fraction from the serum of immunized and intact rabbits was isolated by ammonium sulfate precipitation [11], dialysed, lyophilized, and stored at 4°C.

Phagocytic activity of peritoneal macrophages toward *Staphylococcus aureus* was assessed by phagocytic number (percentage of phagocytizing macrophages) and phagocytic index (number of phagocytized bacteria per macrophage) [10].

In series I, the animals (n=16) were intraperitoneally injected with anti-5-HT Ab in 0.2 ml physiological solution in doses of 25 and 75 mg protein/kg body weight one day before evaluation of PA in cell culture. The same doses of γ-globulins from intact

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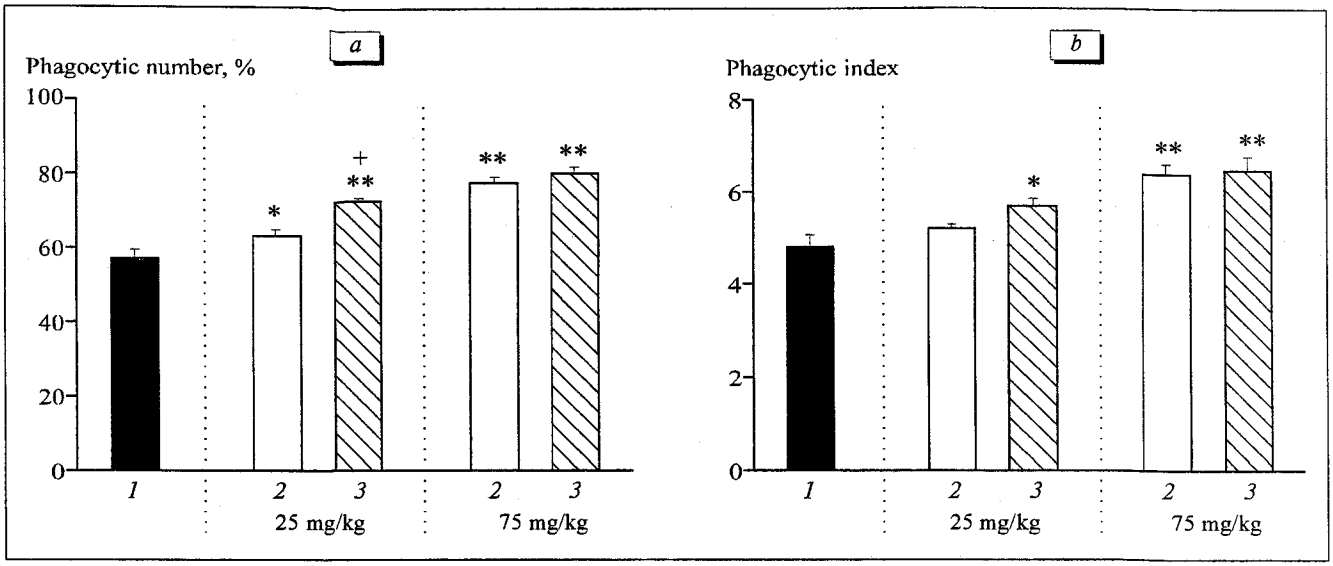

Fig. 1. Functional activity of peritoneal macrophages from mice treated with various doses of antiserotonin antibodies. Here and on Fig. 2: *1*) control (injection of physiological solution); *2*) injection of normal γ-globulin; *3*) injection of antiserotonin antibodies. *$p<0.05$, **$p<0.01$ compared with the control; +$p<0.01$ compared with γ-globulin.

rabbit and an equal volume of normal saline were used as control.

In series II, anti-5-HT Ab (10^{-5}, 10^{-7}, and 10^{-9} M) were added to cultured mouse peritoneal macrophages (n=17). The effect of anti-5-HT Ab was compared with that of γ-globulin from intact rabbits and PA of normal macrophages.

In series III, the effects of anti-5-HT Ab were compared with those of 5-HT alone (10^{-3}, 10^{-5}, and 10^{-7} M) and in combination with anti-5-HT Ab (10^{-5} M) on cultured macrophages (n=17). Macrophages obtained from intact animals served as the control.

The data were processed statistically using Student's tests.

RESULTS

It was found that anti-5-HT Ab systemic administration of stimulates PA of mouse peritoneal macrophages. As seen from Fig. 1, injection of 25 mg/kg Ab significantly increased phagocytic number in comparison with injection of physiological solution ($p<0.01$) and normal γ-globulin ($p<0.01$). γ-Globulins in this dose had no effect on phagocytic index and slightly increased phagocytic number in comparison with administration of physiological solution ($p<0.05$). Higher doses of Ab (75 mg/kg) similarly to γ-globulin nonspecifically stimulated PA of peritoneal macrophages.

Addition of anti-5-HT Ab to cultured macrophages induced a more pronounced and dose-dependent effect on PA, maximum stimulating activity being observed with the dose of 10^{-7} M (Fig. 2). The stimulating effect of Ab in the studied concentration range significantly surpassed the nonspecific effect of γ-globulin ($p<0.01$), which in lower doses had no effect of PA.

Comparative analysis of the effects of anti-5-HT Ab and 5-HT showed that Ab and the neurotransmitter induced similar changes on PA: the effect of 5-HT also depended on the neurotransmitter dose. Phagocytic number increased 1.3- ($p<0.01$), 1.5-, and 1.6-fold, respectively, in comparison with the control

TABLE 1. Effect of Different Concentrations of 5-HT on PA of Mouse Peritoneal Macrophages ($M\pm m$)

Test substance	Dose, M	Phagocytic number, %	Phagocytic index
5-HT	10^{-3} (n=7)	61.4±1.4*	4.7±0.16*
	10^{-5} (n=7)	75.0±1.5**	5.3±0.07**
	10^{-7} (n=7)	76.4±0.9**	5.3±0.04**
5-HT+anti-5-HT Ab	10^{-5} (n=7)	50.7±1.3	4.4±0.14
Physiological solution	(n=6)	45.8±2.4	4.1±0.1

Note. *$p<0.01$, **$p<0.001$ compared with physiological solution, n is the number of samples.

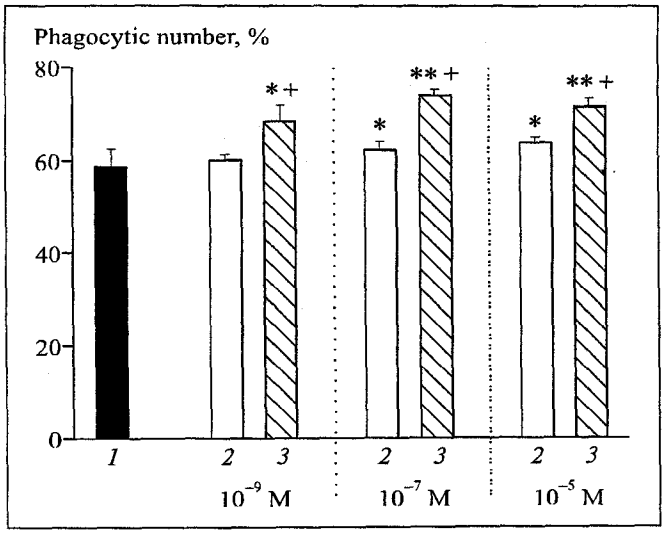

Fig. 2. Effect of antiserotonin antibodies on functional activity of cultured mouse peritoneal macrophages.

($p<0.001$, Table 1). A combination of anti-5-HT Ab and 5-HT (10^{-5} M) had no effect of PA of macrophages (Table 1).

Thus, our experiments showed that anti-5-HT Ab stimulate PA of mouse peritoneal macrophages after intraperitoneal administration and under conditions of cell culture. The effect observed *in vitro* was dose-dependent, more pronounced, and differed significantly from that of γ-globulin from an intact rabbit.

Serotonin also stimulated cultured mouse macrophages similar to that of anti-5-HT Ab, which was absent in their combined application. Presumably, anti-5-HT Ab pool contains anti-idiotypic antibodies interacting with serotonin receptors on macrophages.

Thus, our data confirm that anti-5-HT Ab affect the CNS through immune cells, specifically through macrophages. Activated macrophages produce the cytokines (interleukin-1 and tumor necrosis factor)[6] that penetrate the blood-brain barrier and modulate functional activity of the CNS [1].

The study was partially supported by the Russian Foundation for Basic Researches (grant No. 96-04-48576).

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