The facts described above suggest the existence of two systems of regulation of immunogenesis: an older system, represented by amino acids, and a system of peptide regulation, reflecting the later stages of evolution.

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IMMUNOMODULATING PROPERTIES OF NONSTEROID ANTI-INFLAMMATORY AGENTS

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The writers previously showed that certain iodine derivatives of pyrazolone stimulate the humeral immune response to erythrocytic and viral antigens [1]. The aim of this investigation was to study the immunostimulating and immunosuppressive properties of some representative nonsteroid antiinflammatory agents (NSAIA).

EXPERIMENTAL METHOD

The test compounds butadione, antipyrin, acetylsalicylic acid, and sodium salicylate were obtained from the Novosibirsk Pharmaceutical Chemical Factory, and 4-iodoantipyrin, 4-bromoantipyrin, and stampyrine were synthesized by E. V. Shmidt et al. (Tomsk Polytechnical Institute). In some experiments tslorone, obtained from the Research Institute of Therapeutic Substances (Stariya Kupavna, Moscow Region) was used as the control.

Experiments were carried out on 350 noninbred male mice weighing 18-20 g, obtained from the animal house of the "Vector" Research-Production Combine (Koltsevo village, Novosibirsk Region) and 80 male (CBA × C57BL)F_1 mice obtained from the animal house of the Institute of Clinical Immunology, Siberian Branch, Academy of Medical Sciences of the USSR (Novosibirsk).

To evaluate the immune response we used a heterologous antigen (sheep's red blood cells — SRBC) and also Coxsackie A13 virus (Flores strain), adapted to reproduction in J-41 cell culture in the presence of Eagle's MEM medium, liter of virus 5.0 log TCD_{50}/0.1 ml, The number of antibody-forming cells (AFC) in the spleen of mice immunized with the heterologous antigen was determined [7] against a background of intraperitoneal injection of the NSAIA, once a day for 4 days, in half the maximal tolerated dose (for 4-iodoantipyrin the dose was established experimentally previously [1]). Immunization of the mice with Coxsackie A13 virus and injection of the NSAIA were carried out by the program developed by the writers previously [1]. T-suppressor cell function was evaluated by the method in [3]. The significance of the results was assessed by Student's test.

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TABLE 1. Effect of NSAIA on Immune Response to Heterologous Antigen

Serial No.	Name of NSAIA	Dose, mg/kg	Relative number of AFC/10^6 splenocytes	
			expt.	control
1.	Antipyrin	500	1732.7±345.9	1784.4±155.6
2.	Stampyrine	500	1154.6±213.4	107.3±141.7
3.	Butadione	62.5	271.3±70.5*	846.4±147.1*
4.	Acetylsalicylic acid	125	517.0±127.9	876.4±147.1
5.	Sodium salicylate	250	658.4±161.4*	1154.0±213.4*
6.	4-iodoantipyrin	5.0	254.7±26.7*	144.3±22.2*
7.	4-bromoantipyrin	250	1079.1±143.4	880.9±189.7

Legend. Asterisk indicates significant differences compared with the corresponding control group.

EXPERIMENTAL RESULTS

The effect of NSAIA on the immune response to heterologous antigen showed in different series of experiments (Table 1) that three of the compounds tested, namely 4-iodoantipyrin, 4-bromoantipyrin, and stampyrine stimulated the immune response. This effect was most marked in the case of 4-iodoantipyrin — the number of AFC in the mouse spleen was significantly increased compared with the control group of animals ($254.7 \pm 26.7 \cdot 10^6$ and $144.3 \pm 22.2 \cdot 10^6$ respectively).

Meanwhile, the salicylate and butadione had a suppressive action; in the case of butadione and sodium salicylate, moreover, the decrease in the number of AFC compared with the control group was statistically significant.

The effect of NSAIA on the immune response to viral antigen in the noninbred mice was assessed by determining the time course of synthesis of antibodies neutralizing Coxsackie A13 virus, using as the comparison preparation tilorone, which has immunostimulating properties [2]. The experiments showed (Fig. 1) that four compounds (antipyrin, 4-iodoantipyrin, stampyrine, and sodium salicylate) stimulated the humeral immune response more actively than tilorone; this property was most characteristic, moreover, of the pyrazolone derivatives. Butadione and acetylsalicylic acid, under the experimental conditions used, had an immunosuppressive action. It must be emphasized that Coxsackie A13 virus is not pathogenic for adult mice and, since it is weakly immunogenic, it can exert an immunosuppressive action in mice [6].

Suppression of antibody formation with the aid of specific suppressor T-cells is one method of natural regulation of immunopoiesis. It has been shown, in particular, that after immunization of mice with SRBC, the number of suppressor T cells in the body of the animals increases, and injection of spleen cells of syngeneic donors, immunized with a large dose of SRBC, into intact mice led to marked and specific suppression of the recipients' immune response [3, 11].

This model was used by us to study the effect of 4-iodoantipyrin and 4-bromoantipyrin on induction of specific suppressor T cells. Spleen cells of immunized (CBA × C57BL)F_1 donor mice were incubated in vitro with 4-iodoantipyrin and 4-bromoantipyrin, 100 μg/ml, for 3.5 h, after which they were injected into syngeneic recipients. The results (Table 2) showed that treatment of the cells with 4-iodoantipyrin led to a 2.5-fold increase in the number of AFC compared with the control (immune spleen cells); treatment of the spleen cells with 4-bromoantipyrin caused a sevenfold increase in the number of AFC compared with the control.

Thus inhibition of antibody formation by specific suppressor T cells was corrected by NSAIA; 4-bromoantipyrin, moreover, completely abolished the suppressor effect of the spleen cells.

In view of the diversity of substances combined into the group of "nonsteroid anti-inflammatory agents," the mechanism of their antiphlogistic activity has been linked with inhibition of prostaglandin biosynthesis [10]. Sufficient factual evidence of the role of prostaglandins in the regulation of various types of immune reactions has now accumulated. Prostaglandin E2 (PGE_2), which has been studied the most in this respect, reduces migration of B lymphocytes, precursors of AFC, from the bone marrow into the spleen, and potentiates the induction of specific suppressor T cells. Being an immunodepressant, PGE_2 reduces the number of AFC in animals immunized with SRBC and inhibits the B-cell response stimulated by staphylococci [4, 9]. Physiological concentrations of PGE_2 can depress several functions of the T-system of immunity: antigenic stimulation, E-rosette formation, lymphokine production, etc. [8].

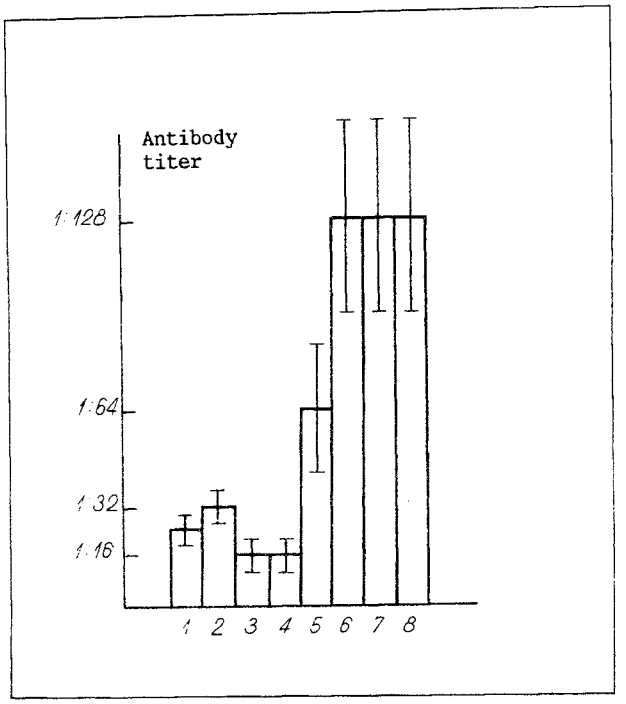

Fig. 1. Effect of NSAIA on immune response to Coxsackie A13 virus. Legend: 1) immunization control, 2) tilorone, 3) butadione, 4) acetylsalicylic acid, 5) sodium salicylate, 6) 4-iodoantipyrin, 7) antipyrin, 8) stampyrine.

TABLE 2. Effect of 4-Iodoantipyrin and 4-Bromoantipyrin on Realization of the Effector Function of Specific Suppressor Cells in (CBA × C57BL)F_1 Mice

Serial No.	Groups of recipient animals	Treat. of spleen cells in vitro for 3. 5 h with	Relative number of AFC/10^6 splenocytes
1.	Intact spleen cells (SC)	–	252,0±53,6
2.	Immune SC		55,25±28,2
3.	Immune SC	4-iodoantipyrin 100 μg/ml	139,0±45,5*
4.	Immune SC	4-bromoantipyrin 100 μg/ml	396,0±86,0*

Legend. Asterisk indicates significant difference compared with Group 2.

The immunomodulating effect of NSAIA which we found in relation to erythrocytic and viral antigens and the role of prostaglandins in the regulation of immune reactions are evidently interconnected. This hypothesis is confirmed by the inhibition of the suppressor T-cell effect by halogen derivatives of pyrazolone, which we discovered. It was shown previously that PGE_2 is an essential component of the generation of suppressor T-cells, on whose membranes the corresponding prostaglandin receptors are located. The nonsteroid antiphlogistic indomethacin, which blocks PGE_2 synthesis, inhibited induction of suppressor T cells, depressed their suppressor function, and ultimately stimulated immunogenesis [5]. This picture is similar in many ways with that which we obtained. It is not contradicted by suppression of the humeral immune response by several of the NSAIA studied, since the immunomodulating effect of the latter probably varies depending on dose, schedule of administration, and so on.

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FUNCTIONAL BLOCKING OF THE HIPPOCAMPUS BY TARGETED NEUROIMMUNIZATION

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To create models of disturbances in the activity of different parts of the brain, including the hippocampus, as a rule the methods used are quite crude and are associated with brain tissue destruction. In this respect the method of neuroimmunization (NI), which in some cases allows targeted functional inactivation of different parts of the brain, possessing antigenic specificity, to be achieved [1]. This method is widely used in the creation of models of autoimmune diseases of the nervous system [9, 10] and also in experiments involving procedures directed toward brain-specific proteins [2]. The method is based on induction of an immune response of the body to endogenous "barrier" antigens (AG), by immunizing animals with homologous AG-material. This paper gives physiological evidence of the applicability of NI for functional blocking of the hippocampus.

EXPERIMENTAL METHOD

Experiments were carried out on Wistar rats of different ages. Adult animals (males weighing 200-250 g) were immunized with tissue cytosol of the hippocampus (HP) or the convex surface of the neocortex (NC), twice at weekly intervals with 2 mg of total protein (in 0.5 ml) mixed with 0.05 ml of Freund's complete adjuvant. Young rats aged between 1 and 7 days received injections of 0.13 mg of the same AG-material without the adjuvant, and maintenance immunizations were carried out on the 35th and 48th days (1 mg each time without adjuvant) and the 86th and 93rd days (2 mg with 0.05 ml of adjuvant each time) after birth. The physiological effects of NI in the adult animals were assessed 7 days after the second immunization, in the "open field" (OF) test, twice for 5 min each time, separated by an interval of 1 h, and during the formation of a conditioned active avoidance reflex (CAAR) on six consecutive days, with 30 combinations of acoustic and electrical stimuli each time. The same OF test was carried out on the young rats (a circular arena 95 cm in diameter) at the age of 23 days, and CAAR formation was carried out one week after the last reimmunization.

*Deceased.

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