Inhibition of spike activity by dermorphin took place through activation of opiate receptors, for the effects of dermorphin were blocked by naloxone (10^{-5} M).

To discover any possible influence of dermorphin on the conduction of excitation in "en passant" fibers the electroneurogram of the intracardiac nerve was recorded during stimulation of two or three sympathetic ganglia; under these curcumstances postganglionic sympathetic axons running in the intracardiac nerve without relaying were activated. Dermorphin in a concentration of 10^{-6} M did not affect his "en passant" impulsation.

Our results thus show that the endogenous amphibian opioid peptide, dermorphin, can specifically inhibit intracardiac ganglionic transmission by acting through opioid receptors. The study of where these receptors are located – whether at the presynaptic or the postsynaptic level – will be a task for future experiments.

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ACTION OF BENZODIAZEPINES ON THE IMMUNE RESPONSE

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KEY WORDS: GABA-ergic system; benzodiazepine receptors; diazepam; oxazepam; immune response.

Isolated studies of the effect of GABA and GABA-positive substances on immunologic processes have been published in recent years. GABA, its cetyl ester, and sodium hydroxybutyrate and valproate have been shown to affect antibody formation in the spleen [4]. Injection of GABA and γ-hydroxybutyric acid stimulates immunogenesis, as shown by morphological and functional changes in the organs of immunity [1]. There are also data on the effect of GABA and γ-hydroxybutyric acid on the nonspecific factors of immunity: lysozyme activity and phagocytosis [6]. Changes in the GABA concentration in the tissues of the posterior lobe of the hypothalamus have been demonstrated under the influence of factors inhibiting function of the immune system [5]. The immunostimulating effect of muscimol, a direct agonist of the GABA receptor, and the suppressive effect of bicuculline, a competitive inhibitor of the GABA receptor, and also the depressive action of the chloride channel blocker picrotoxin on the immune response [2] have been established.

The benzodiazepines, widely used cytotropic agents, are known to modulate the GABA receptor, i.e., they increase pre- and postsynaptic GABA-mediated inhibition [10]. According to data in the literature [8], diazepam in doses of 1 and 2 mg/kg does not affect the delayed type hypersensitivity reaction, whereas in a dose of 8 mg/kg it inhibits this reaction strongly [7].

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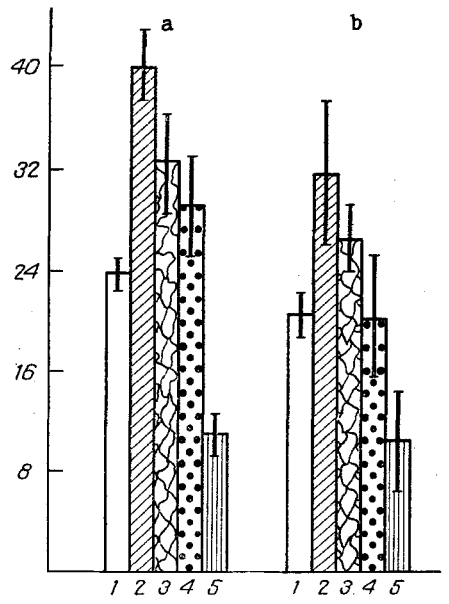

Fig. 1. Effect of diazepam (a) and oxazepam (b) on immune response of CBA mice to immunization with SRBC ($5 \cdot 10^8$ cells). Ordinate, number of RFC per 10^3 cells. 1) Control; 2) 0.5 mg/kg; 3) 1 mg/kg; 4) 2 mg/kg; 5) 8 mg/kg. The drugs were given by a single intraperitoneal injection 30 min before immunization.

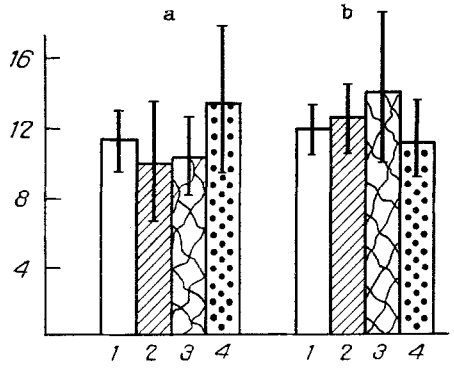

Fig. 2. Number of RFC in CBA mice immunized with SRVC ($5 \cdot 10^6$ cells) under the influence of diazepam (a) and oxazepam (b). Legend as to Fig. 1.

For the reasons given above it was interesting to examine the effect of benzodiazepines of the immune response, and the investigation described below was carried out for this purpose.

EXPERIMENTAL METHODS

Experiments were carried out on 300 CBA mice aged 3-4 months and weighing 18-24 g. The animals were immunized with sheeps' red blood cells (SRBC) in doses of $5 \cdot 10^6$ and $5 \cdot 10^8$ intravenously, by a single injection. The magnitude of the immune response was determined as the number of rosette-forming cells (RFC) per 1000 nucleated cells in the spleen of the mice on the 5th day after immunization.

The benzodiazepines, namely diazepam (Seduxen, from Gedeon Richter, Hungary) and oxazepam (Polfa, Poland), were injected in doses of 0.5, 1, 2, and 8 mg/kg. Control animals were injected with physiological saline in the same volume as the test drugs (0.2 ml). The preparations were injected intraperitoneally in a single dose (oxazepam in suspension form) 30 min before immunization.

The numerical results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

The experiments showed that during immunization with SRBC ($5 \cdot 10^8$ cells) diazepam and oxazepam in a dose of 0.5 mg/kg stimulated the immune response considerably and increased the number of RFC by comparison with the control (Fig. 1). The use of both substances in a dose of 1 mg/kg led to weaker stimulation. When the dose of the drugs was increased to 2 mg/kg, the number of RFC approached the control level. When a larger dose of diazepam and oxazepam (8 mg/kg) was used significant inhibition of the immune response was found: the number of RFC was reduced by half compared with the control. Consequently, the benzodiazepines had a dose-dependent effect, consisting of a change in the immune response from stimulation to inhibition with an increase in the dose of the drugs.

The immunostimulating action of diazepam and oxazepam in small doses is connected with activation of the GABA-ergic system. The modulating influence of these drugs on the GABA receptor is confirmed by synergism between the action of muscimol and the benzodiazepines. The results of the present experiments with diazepam and oxazepam in doses of 0.5 and 1 mg/kg are close to those obtained by the writers previously when using the GABA receptor activator muscimol.

The synaptic mechanism of the dose-dependent effect of agonists of the benzodiazepine receptor, which have an allosteric effect on the system controlling affinity of the GABA receptor for neurotransmitter GABA is not yet clearly understood. There are at present two points of view regarding benzodiazepine receptors. According to one of them there are two types of receptors (with high and low affinity); according to the other, there are several subtypes of GABA-benzodiazepine receptor, each of which has four different binding sites for benzodiazepines, which may exist in at least three different conformations [12]. The possibility cannot be ruled out that during the action of different doses of benzodiazepines on the immune response, different benzodiazepine receptors are involved in the process, but the inhibitory effect of the high dose of diazepam and oxazepam on the immune response may perhaps be due to the nonspecific action of these substances.

It can be tentatively suggested that participation of the GABA-benzodiazepine receptor—ionophore complex in the mechanism of immunomodulation may be determined by interaction of the GABA system with the monoamine systems of the brain, for we know that the GABA-ergic system, consisting mainly of interneurons, has a modulating action on other neurotransmitter systems (specifically the serotoninergic and dopaminergic systems [11, 14]), whose role in the regulation of immunogenesis has been proved [3]. There are definite grounds for this suggestion. It has been shown [9], for instance, that the GABA-mimetic muscimol inhibits serotonin metabolism in the nuclei raphe. Conversely, the GABA-receptor antagonist bicuculline stimulates serotonin turnover. Muscimol has also been found to stimulate activity of the dopaminergic neurons of the compact part of the substantia nigra [13].

After immunization with a low dose of SRBC ($5 \cdot 10^6$ cells) administration of benzodiazepine receptor agonists in doses of 0.5, 1, and 2 mg/kg caused no change in the immune response (Fig. 2). The results of these investigations showed that benzodiazepines exert their action only in response to a high dose of immunization, when more intensive proliferation and differentiation of immunocompetent cells takes place. It can be tentatively suggested that for the immunomodulating action of the benzodiazepines and, perhaps, of the whole GABA-ergic system to be manifested, a definite background of the immune response must be present, and only if the intensity of the immune response is sufficiently high are the effects of these psychotropic drugs exhibited.

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EFFECT OF DALARGIN, A SYNTHETIC ENDOGENOUS OPIOID ANALOG, ON NATURAL CYTOTOXICITY OF HUMAN LYMPHOCYTES

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KEY WORDS: dalargin; human lymphocytes; natural cytotoxicity.

Interest in the study of the role of opioid peptides in regulation of the immune system has increased considerably in recent years [2, 15]. Data have been obtained on the influence of opioid peptides on the proliferative response of mitogen-stimulated lymphocytes [5], on the suppressor activity of lymphocytes [11], antibody synthesis [6], the phagocytic function of neutrophils and macrophages [12, 14], and cellular cytotoxic reactions [7, 9, 10]. The writers showed previously that dalargin, a synthetic analog of Leucine-enkephalin with the structural formula Tyr-D-Ala-Gly-Phe-Ley-Arg possesses marked immunomodulating activity in vitro, and among other properties it stimulates the ability of T lymphocytes to form rosettes with sheep's red blood cells, it influences the proliferative response of lymphocytes stimulated by concanavalin A and by pokeweed mitogen, and it stimulates the phagocytic activity of leukocytes [1, 2].

The aim of this investigation was to study the action of dalargin on natural cytotoxicity (NCT) of human peripheral blood lymphocytes.

EXPERIMENTAL METHODS

Mononuclear cells were isolated by the method in [3]. Adherent cells were removed by incubation on plastic Petri dishes for 1 h at 37°C [8]. Nonadherent cells were resuspended in complete medium RPMI 1640 (flow Laboratories, England) with the addition of 10% embryonic calf serum (ECS; Flow Laboratories), 2 mM L-glutamine (Merck, West Germany), 10 mM HEPES (Flow Laboratories), and 50 μg/ml of gentamicin, the cell concentration being adjusted to $2 \cdot 10^6$/ml. The effector cells were preincubated with dalargin in a final concentration of 10^{-6}-10^{-14} M or with the control culture medium for 1 h at 37°C, washed twice, and studied in the cytotoxic test.

Human erythroleukemia cells line K-562, cultured in complete RPMI 1640 medium with 10% ECS were used as the targets.

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