EFFECT OF GABA-ERGIC SUBSTANCES ON THE HUMORAL IMMUNE RESPONSE

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An oriented search for drugs capable of acting on immunological reactivity is an urgent task in modern immunopharmacology. The role of cholinergic and adrenergic systems in mechanisms of regulation of immune responses has recently been established [4]. There is information in the literature on the effect of serotoninergic and dopaminergic processes on immunogenesis [1, 3]. The presence of adrenoreceptors and acetylcholine receptors in lymphoid tissue has also been demonstrated [2]. These data, as well as the fact that GABA is one of several neuromediators [11], provided a basis for the suggestion that a connection may exist between the functional state of the GABA-ergic system and immune responses.

This paper gives the results of a comparative study of the effect of several GABA-ergic substances on formation of the humoral immune response.

EXPERIMENTAL METHOD

Experiments were carried out on 144 CBA albino mice weighing 18-20 g. One of the following preparations was injected intraperitoneally into the animals: GABA (from Reanal, Hungary) in a dose of 100 mg/kg, the cetyl ester of GABA (CE GABA) in a dose of 20 mg/kg, sodium hydroxybutyrate (HOBA) – 300 mg/kg, and sodium valproate – 250 mg/kg. To block GABA receptors, bicuculline (from Sigma, USA) was injected subcutaneously in a dose of 2 mg/kg. In the control series, instead of the test substance, physiological saline was injected into the mice. The animals received one of the above GABA-ergic substances simultaneously with injection of the test antigen and during 2 days after immunization. A suspension of sheep's red blood cells in a dose of 1.5×10^8/10 g body weight served as the test antigen. The number of antibody-forming cells (AFC) in the spleen was determined on the 5th, 7th, and 9th days after antigenic loading by the method in [10] (Table 1). To determine the direct effect of the preparations on lymphoid cells a suspension of mouse splenocytes was incubated for 1 h at 37°C in Hanks' medium, after which the number of viable cells was determined by the trypan blue test.

EXPERIMENTAL RESULTS

Injection of substances potentiating GABA-ergic inhibition was accompanied by marked depression of the AFC population in the spleen. There were only slight differences in the degree of immunodepression induced by the various substances. For instance, after injection of GABA the most marked decrease in the number of immunoglobulin-producing class M cells was observed in the spleen, which at the height of the immune response amounted to only 43.6% of the control level. CE GABA inhibited the primary immune response less strongly, as shown by a mean reduction of not more than 40.1% in the number of AFC.

An inhibitory effect on immunogenesis also was observed in the experiments with HOBA, in which the AFC population on the 5th day after injection of the test antigen into the animals was reduced by 67.3% compared with the control.

Reduction of the immune response also occurred in the experiments with sodium valproate, which, in the dose used, can increase the functionally significant GABA mediator reserves [13]. Injection of this substance was followed by a reduction in the number of AFC in the spleen by 58.3% of the control value.

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TABLE 1. Number of AFC of Mice Receiving GABA-ergic Substances (M ± m)

Preparation	No. of animals	Time after immunization, days		
		5	7	9
Control	32	288,9±31,3	93,2±7,4	25,9±6,4
GABA	24	126,1±18,3†	39,0±5,15†	18,0±4,5
CE GABA	20	115,8±27,25*	31,1±4,7†	21,1±5,1
HOBA	26	83,6±17,7†	26,6±5,4†	16,1±3,4
Sodium valproate	20	123,5±12,7†	34,5±3,5†	18,2±4,3
Bicuculline + GABA	22	108,6±11,2†	46,6±8,3*	21,7±2,7

*P < 0.01.
†P < 0.001.

Allowing for data in the literature on the temporal characteristics of the immune response to administration of drugs [5], it was considered important to study the effect of the various test substances on the kinetics of the primary immune response. These experiments showed that the maximal immunodepressant effect of the drugs was recorded on the 5th day, i.e., at the peak of the immune response, and it was much weaker on the 7th day of immunogenesis.

Comparison of these results with data in the literature on the neurotropic activity of the drugs in the doses now used indicates the important role of peripheral mechanisms in the development of the changes taking place. First, this is confirmed by the absence of significant differences in the intensity of the immunosuppressive effect of drugs which penetrate poorly (GABA) or relatively better (HOBA, CE GABA) through the blood–brain barrier. Second, the hypothesis of the important role of peripheral mechanisms of inhibition of the immune response by the test substances is confirmed by comparison of their immunotropic and neurotropic activity. For instance, CE of GABA and HOBA, unlike GABA (100 mg/kg), in the doses tested caused a marked central depressant effect [6]. Meanwhile GABA, according to the data cited above, is indistinguishable in the degree of its immunodepressant activity from CE GABA and HOBA.

The concrete mechanisms of the inhibitory action of GABA-ergic drugs on antibody formation are not yet completely clear. It has been shown that a fall in the number of AFC in the spleen is not due to the direct cytotoxic action of the drugs on lymphoid cells. Incubation of a suspension of splenocytes with the addition of even high concentrations of the test drugs (1×10^{-2} to 3×10^{-2} M) to the medium did not change values obtained in the test with trypan blue.

In recent years much attention has been paid to interaction between drugs and the surface structures of immunocompetent cells [7]. A special series of experiments was carried out to analyze the immunotropic effect of GABA after blockade of the GABA receptors by bicuculline. The disturbances of immunogenesis were found to be in the same direction as those found after administration of GABA alone. The only difference was the rather weaker inhibition of the immune response when the substances were given simultaneously. The possibility cannot be ruled out that the absence of any more marked differences in the experiments with GABA alone and with a combination of GABA and bicuculline is due to the transient nature of the effect of the latter agent. In this connection attention in drawn to research which showed that bicuculline, because of the short duration of its action, does not completely abolish the central depressant effect if administered simultaneously with certain GABA-ergic substances [8]. An alternative explanation may be the view that GABA exerts its immunodepressive effect indirectly through receptors insensitive to bicuculline. Data in the literature on the heterogeneity of GABA receptors [12] allow a similar interpretation of the results of the series of experiments in which GABA was used in combination with bicuculline.

The emphasis on the important role of peripheral mechanisms in the manifestation of the immunotropic action of GABA-ergic substances in no way rules out the possibility of mediation of the effect of these drugs through central neurohumoral stages in the regulation of immune homeostasis [9].

The results described above are evidence that immunogenesis is definitely dependent on the state of the GABA-ergic system and the immunodepressive effect of substances potentiating GABA-ergic inhibition.

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NATURE OF POTENTIATION OF GABA EFFECTS BY BENZODIAZEPINE TRANQUILIZERS

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KEY WORDS: spinal cord; chlordiazepoxide; GABA; K^+ ions; Cl^- ions

The most important pharmacological effects of benzodiazepine tranquilizers are due to their influence on GABA-ergic synapses of the brain [6, 14, 15]. Diazepam has been shown to potentiate presynaptic inhibition of primary afferents in the spinal cord [7] and in the cuneate nucleus of 12 cats, whereas the depolarizing [4, 10] and hyperpolarizing [10, 14] effects due to the direct action of GABA are potentiated by benzodiazepine tranquilizers.

A model according to which benzodiazepines increase the affinity of receptors for GABA allosterically [9], probably by inhibition of a protein modulator which inhibits binding of GABA with receptors [8] or by displacing it from the receptor complex and facilitating coupling of GABA receptors with chloride ionophores [2], is regarded as the molecular mechanism lying at the basis of the potentiating influence of benzodiazepine on the effects of GABA. However, these views are contradicted by data showing that benzodiazepines have no effect on the cooperativeness of interaction between GABA and the GABA receptors of nerve cell membranes in tissue culture [5].

The results described in this paper are evidence that benzodiazepines can directly influence the function of chloride ionophores in nerve cell membranes.

EXPERIMENTAL METHOD

Experiments were carried out on the isolated perfused spinal cord of rats aged 9-15 days. Details of the method were described previously [1].

The characteristic action of chlordiazepoxide (10^{-5}-10^{-14} M) and its effect (exposure 15 min) on primary afferent depolarization or hyperpolarization of motoneurons into GABA (10^{-5}-10^{-3} M) were investigated. Synaptic transmission in the spinal cord was blocked by perfusion with a solution deficient (0.2 mM) in Ca^{++} ions and containing an excess (10 mM) of Mg^{++} ions. The effect of chlordiazepoxide on the evoked monosynaptic ventral root potential and the dorsal root potential evoked by stimulation of the dorsal root of the neighboring segment

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