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Immunotropic Properties of GABA-ergic Agents in Suppression

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1. Introduction

Vigorous development of immunology and immunopharmacology is closely interrelated with formation of new scientific “branches” of neuroimmunology and neuroimmunopathology which predetermines the hypotheses of mutual regulation-interaction of the nervous and immune systems, of the significance of neuroimmune mechanisms in the regulation of most physiological functions and development of pathophysiological processes (Alford L., 2007; Irwin M.R., 2008; Freund G.G., 2009). The formulated hypotheses that immunologic mechanisms are involved in the pathogenesis of the CNS diseases (stroke, multiple sclerosis, chronic fatigue syndrome, epilepsy, etc.), their systematization within the scope of special neuroimmunopathology have laid the groundwork for a vigorous pharmacological search of agents for eliminating neuroimmune disturbances (Kryzhanovsky G.N. et al., 2003; Fleshner M., Laudenslager M.L., 2004; Alexandrovsky Y.A., Chekhonin V.P., 2005; Samotrueva M.A. et al., 2009).

In light of this problem and considering the abundant factual material testifying to the involvement of GABAergic system in immunomodulation, substances which are GABA analogues become of particular interest (Devoyno L.V., Iliuchenok R.U., 1993; Korneva E.A., 2003). Thus, in this experimental work we studied the immunotropic properties of the known representatives of group of GABAergic agents such as phenotropil (N-carbamoyl-methyl-4-phenyl-2-pyrrolidone), phenibut (hydrochloride of γ -amino and β -phenylbutyric acids) and baclofen (γ -amino-para-chloro- β -phenylbutyric acid). Having a wide range of psychotropic effects these drugs improve cognitive activity, decrease emotional tension and anxiety, normalize sleep; diminish asthenic manifestations, vasovegetative symptoms, etc. (Arushanian E. B., 2004). In this work aiming to widen the activity range of the above-mentioned drugs as well as to search for drugs capable of eliminating immune imbalance which is often in causal relationship with the CNS pathologies we studied the immunomodulating activity of gamma-aminobutyric acid (GABA) derivatives using an experimental immunosuppression model and determined the most effective doses and regimens.

2. Materials and methods

The study was performed on 544 CBA-line mice both male and female aged 3-4 months weighing 20-25g. The animals were kept in standard vivarium conditions – in plastic cages

on a sawdust bedding at a room temperature of +18-22°. They were fed twice a day with natural foods in the amount corresponding to daily doses (P 50258-92 State Standard) had a free access to water. The lighting in the daytime (12 hours) was combined (natural and luminescent). The animals were kept according to the guidelines on laboratory practice for preclinical trials in the RF (3 51000.3-96 and 51000.4-96 State Standards) and the Order of the Health Care Ministry of the RF № 267 of 19.06.2003 'On the Approval of the Guidelines on Laboratory Practice' (GLP) as well as in conformity with the International recommendations of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes, 1997.

By the moment when the experiments were held the animals had adapted to the human factor, acclimatized and were in good health (no changes in behaviour, appetite, circadian cycle, condition of fur and visible mucosas were detected). All the experiments were carried out in the spring-autumn period (without abrupt changes of weather conditions) within one and the same time interval (from 11.00 till 19.00) to avoid the influence daily biorhythms on the investigation results. The rodents were euthanized by means of fast cervical dislocation.

The pathology of the immune system was simulated by a single intraperitoneal introduction of cyclophosphamide (CPA) in a dose of 150 mg/kg 1 hour after the immunization with sheep erythrocytes (SE) (Arkadyev V.G. et al., 2003). The investigated substances were introduced 1 hour after the immunodepressant ("Biochimik", Russia).

The model of cyclophosphamide (CPA) immunosuppression was used to estimate the immunocorrective properties of phenotropil, phenibut and baclofen in a "dose-effect" respect, when administered intraperitoneally as a single dose and introduced during a peroral course of treatment; to study the immunopreventive and/or immunotherapeutic aspects of the action of the drugs when administered at different times in relation to CPA immunosuppression introduction ("time-effect").

2.1 Immunopharmacological tests

The immune status of the animals was evaluated on the basis of standard immunopharmacological tests: delayed hypersensitivity reaction (DHR) involving the reaction index definition, passive hemagglutination reaction (PHAR) involving the antibody titre definition, latex test to study the phagocytic activity of peripheral blood neutrophils as well as a leukogram, to define the weight and cellularity of immunocompetent organs (thymus, spleen) (Khaitov R.M. et al., 2005).

The DHR was used to evaluate a cellular link of the primary immune response to sheep erythrocytes (SE). The animals of all groups were immunized by receiving a single hypodermic SE injection (a sensitizing injection) (2×10^8) in the interscapular area. The antigen booster dose was introduced on the 5th day (1×10^8 SE) in 0,02 ml of physiological saline into a hind leg paw – "experimental" leg (the booster injection). The equivalent amount of physiological saline was introduced into the contralateral leg – "control" leg. The reaction was estimated 24 hours later by euthanizing the animals by fast cervical dislocation, after which both legs were cut off at the level of an ankle-joint and weighed using analytical balances. The reaction index (DHRI) was calculated using the formula: $RI = (M_{ex} - M_c) / M_c \times 100\%$, where RI is a reaction index, M_{ex} is an "experimental" leg weight; M_c is "control" leg weight.

PHAR was used to estimate the humoral link of the primary immune response to SE. The animals were immunized once intraperitoneally in a dose of 5×10^8 in the amount of 500 mcl 1-2 hours after the introduction of the investigated substance. 7 days later the animals were withdrawn from the experiment by administering serum. To inactivate the complement the

serum was heated for 30 min at a temperature of 56°C. The hemagglutination reaction was carried out in 96-well plates in the amount of 500 µl of a diluent (0.5% solution of bovine serum albumin (BSA), solved in physiological saline) in which the investigated serums were successively diluted twice. After the dilution of the serums 25 µl of 1% SE suspension was introduced into each well. The preliminary analysis of the PHAR results was done after one hour of incubation at a temperature of 37°C, the plates were placed into a refrigerator and kept at a temperature of +4°C. The reaction was finally evaluated 18 hours later. The antibody titre (the maximal serum dilution in which SE agglutination was registered) was indicated using average compound indices.

To estimate the phagocytic activity of neutrophils the animals' heparinized blood was used. A suspension of latex particles of 1.3-1.5 µm size ("MinMedBioprom", Russia) was prepared in advance. The original latex suspension was three times washed with a 0.9% NaCl solution at 3000 rpm for 10 minutes. The sediment was resuspended in medium 199, the number of particles was calculated in a Goryaev's chamber and brought to the ultimate concentration of 150000 in 1ml. 24-48 hours after the introduction of the investigated substances (in the control group it was physiological saline) the animals were withdrawn from the experiment and their blood samples were taken. 50 µl of the latex work solution was mixed with 50 µl of heparinized blood and placed into a temperature-regulated chamber at a temperature of 37°C. The test-tubes were shaken by hand every ten minutes and then centrifuged. Smears were taken from the sediment, they were dried and fixed in Nikiforov's mixture consisting of equal parts of absolute ethyl alcohol and ether (10 min). The next day they were stained by the Romanovsky-Giemsa method (20-30 min). After this the smears were washed with water and dried in the air. The stained smears were examined under a microscope in an immerse system. The number of leucocytes and neutrophils with latex and without it was calculated in a smear. At the same time the number of latex particles in neutrophils was counted. The phagocytic activity of neutrophils was evaluated on the basis of the following indices: phagocytic index (% of phagocytosis) – the number of neutrophils with latex in 100; phagocytic count = the number of latex particles/100.

The total leukocyte count was calculated in a Goryaev's chamber. 0.4 ml of a diluent (3-5% of acetic acid dyed with methylene blue (acetic acid lyses erythrocytes, methylene blue stains leukocyte nuclei)) and 0.02 ml of blood was placed in a test-tube. Leukocytes were counted in 100 large squares. The total leukocyte count was calculated using the formula: $X = A \times 50$, where X is the number of leukocytes in 1ml of blood, A is the number of leukocytes calculated in a Goryaev's chamber.

A blood leukogram was calculated in blood smears stained by the Romanovsky-Giemsa method. The count of neutrophils (stab and segmented neutrophils), eosinophils, monocytes, and lymphocytes was calculated in a smear.

Lymphoproliferative processes in the immunocompetent organs were determined on the basis of the weight and cellularity of the thymus and spleen. After the animals were euthanized, the organs were extracted and weighed; cell suspensions were prepared in medium 199 with 50 mg/ml concentration for the spleen and 10 mg/ml for the thymus. They were filtrated, washed twice with medium 199 to remove adipose tissue particles (for 10 min at a rate of 1500 revolutions). After that they were resuspended in medium 199 to the original concentration in medium 199. To make calculations the suspensions of lymphoid organs were previously mixed in the ratio 1:1 with 3% acetic acid, dyed with methylene blue; the number of nucleated cells (NC) was counted in a Goryaev's chamber. The number of NC was indicated in absolute and relative (in relation to the weight of the lymphoid organ) values.

2.2 Experimental series and groups

A few sets of experiments were carried out: the 1st aimed to study the immunomodulating activity of GABA derivatives in a “dose-effect” respect; the 2nd explored the immunopreventive and/or immunotherapeutic aspects of the effect of GABA derivatives when administered at different times in relation to CPA immunosuppression introduction (“time-effect”); the 3rd targeted to evaluate the activity of GABA derivatives when introduced during a peroral course of treatment; the 4th aimed to study the capability of the substances to eliminate leukogram disturbances and to restore lymphoproliferative and biochemical processes in the immunocompetent organs (thymus, spleen).

The animals in each set of experiments were divided into groups (n=8): control 1 was represented by mice receiving physiological saline as a placebo in the equivalent amount (similarly to the way and frequency of administration of the investigated substance in each set); control 2 included species with an immunopathology model, they also received physiological saline; experimental groups included animals with immunosuppression, which received GABA derivatives in accordance with the goal pursued in each set of experiments.

In the 1st and 3rd experimental sets CPA was introduced 1 hour after the immunization with SE to the animals of the control 2 group and experimental groups; in the 2nd set CPA was administered twice (simultaneously with the immunization and 24 hours after the immunization with SE).

3. Results and discussion

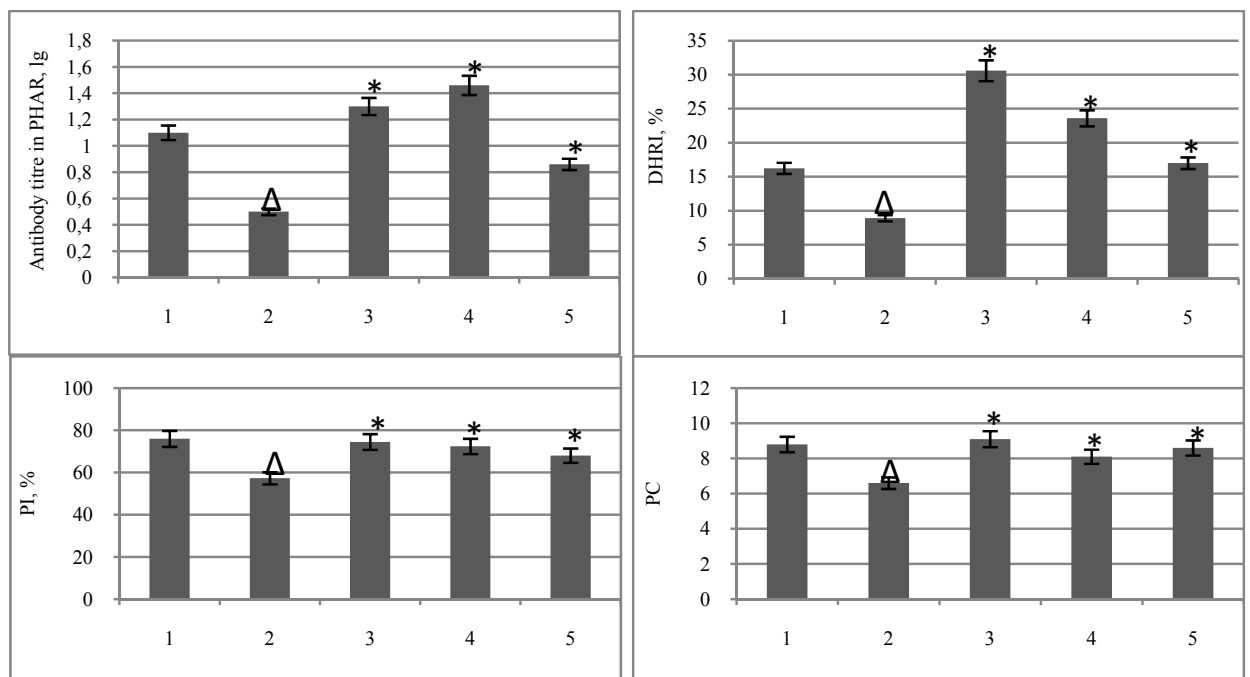
3.1 Immunosuppression

All the conducted experimental sets proved the development of immunological insufficiency in the control 2 group mice: a reliable decrease by more than 50% in the DHR index, by more than 35% in the hemagglutinin titre, by more than 40% in the neutrophil phagocytic activity as compared to the similar indicators in the control 1 group was observed. Moreover, depressed leucopoiesis manifesting itself both as a statistically relevant decrease of the total leukocyte count (by more than 20% with $p_1 < 0.05$) and a pronounced change in the cell ratio in the leukogram was registered in the animals exposed to CPA cytostatic drug. Particularly, a reliable decrease by more than 30 % in the count of leukocytes and segmented neutrophil leukocytes as compared to the background values in control 1 ($p_1 < 0.05$) and a total absence of eosinophils were observed in CPA immunosuppression. It should be noted that the number of stab neutrophils was more than 50% higher ($p_1 < 0.05$) in this group of mice. The study of lymphoproliferative and biochemical processes in the immunocompetent organs revealed involution of the thymus and spleen and a decreased cell count in them ($p_1 < 0.05$), as well as a reliable increase in the lipid peroxidation intensity associated with decreased catalase activity in the investigated organs ($p_1 < 0.05$).

3.2 The immunomodulating activity of GABA derivatives in a “dose-effect” respect

In the 1st experimental set the model CPA immunosuppression was used to study the activity of phenotropil, phenibut, and baclofen in the following doses: phenotropil – 25 mg/kg; 50 mg/kg; 100 mg/kg; phenibut – 12.5 mg/kg; 25 mg/kg; 50 mg/kg; 100 mg/kg and baclofen – 2 mg/kg; 5 mg/kg; 10 mg/kg; 20 mg/kg (Samotrueva M.A. et al., 2010; Tyurenkov I.N. et al., 2008, 2009, 2010).

The conducted investigation demonstrated that a single intraperitoneal introduction of phenotropil in all studied doses promoted an over 50% restoration of the indices of cellular DHR and the level of antired-cell antibodies in PHAR as compared to the corresponding values in the animals with simulated immunopathology ($p_2<0.05$) (here and below p_1 and p_2 – are reliability degrees in the relatively intact animals and animals with immunosuppression correspondingly). Moreover, phenotropil introduced in doses of 25mg/kg and 50 mg/kg showed pronounced immunostimulating properties regarding DHR: the quantitative parameter of the local reaction was statistically reliably higher than that of control 1 ($p_1<0.05$). Phenotropil in a dose of 100 mg/kg had a less pronounced immunocorrective action in relation to the humoral and cellular immunity links: the indices of DHRI and the antibody titre were 30-40% higher than those in the immunosuppressed species ($p_2<0.05$). However, they did not exceed the values in the intact animals (fig. 1).



Experimental groups: 1 - control 1 (physiological saline); 2 - control 2 (CPA); 3 – phenotropil (25mg/kg) + CPA; 4 – phenotropil (50mg/kg) + CPA; 5 – phenotropil (100 mg/kg) + CPA

Notes: Δ and * – $p<0.05$ – reliability of differences as compared to controls 1 and 2 correspondingly (Student’s t-criterion with Bonferroni and Newman-Keuls’ adjustment for multiple comparisons, single-factor analysis of variance involving the definition of Tukey-Kramer criterion and Scheffe’s criterion)

In what follows: DHRI – delayed hypersensitivity reaction index, PHAR – passive hemagglutination reaction, PI – phagocytic index, PC – phagocytic count.

Fig. 1. Effect of phenotropil in different doses on PHAR, DHR development and the phagocytic activity of neutrophils in the immunosuppression conditions

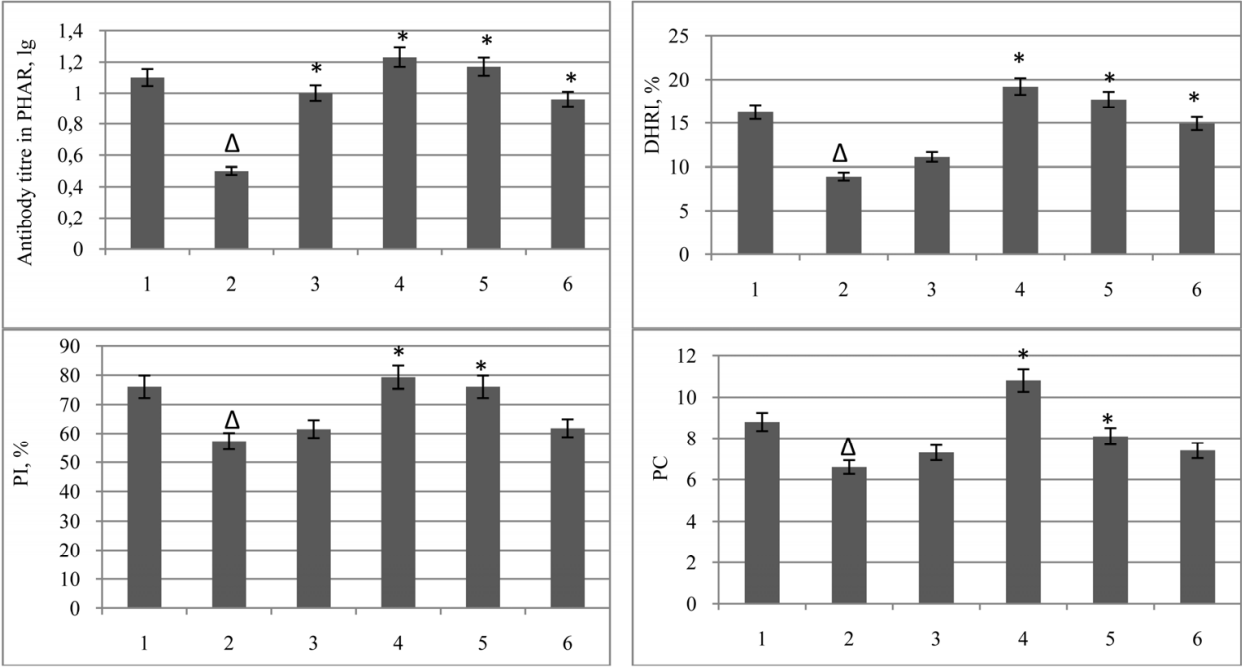
The evaluation of phenotropil effect on the indices of the non-specific link of immunogenesis in the conditions of CPA-induced immunosuppression revealed that the drug eliminated the inhibiting action of the immunodepressant. A statistically reliable increase of PI (by more than 20%) when phenotropil was introduced in doses of 25 mg/kg ($p_2<0.05$) and 50 mg/kg ($p_2<0.001$) and PC (by more than 20%) in doses of 25 mg/kg ($p_2<0.05$) and 100 mg/kg ($p_2<0.05$) as compared to the immunosuppressed animals was

registered. The administration of phenotropil in a dose of 25 mg/kg was associated with a restoration of the indices demonstrating the phagocytic activity of neutrophils almost to the “norm” (fig. 1).

Proceeding from the obtained results we selected the doses of 25 mg/kg and 50 mg/kg for a further study of phenotropil as an immunocorrective drug.

The administration of phenibut in doses of 25 mg/kg and 50 mg/kg was associated with a rise by more than 80% in the indices of both specific (the antibody titre in PHAR and the DHR index) and non-specific (PI and PC) immunoreactivity as compared to the similar values in the group of animals with simulated immunopathology (control 2) ($p_2<0.05$) and a rise by more than 20% as compared to control 1 ($p_1<0.05$) which proves the immunostimulating properties of phenibut in the specified doses (fig. 2).

A single intraperitoneal introduction of phenibut in a dose of 100 mg/kg promoted the elimination of CPA suppressor effect on the development of a primary immune response to SE: antibody titre in PHAR and DHRI reached the background values in the placebo control ($p_2<0.05$). No reliably significant change in phagocytosis indices induced by phenibut administration in a dose of 100 mg/kg was registered which proves that in this dose the drug has no corrective effect in respect to nonspecific resistance. A dose of 12.5mg/kg of the investigated substance appeared to be effective only with respect to the humoral link of pathogenesis: antibody titre in PHAR almost reached “the norm” values in control 1 ($p_2<0.05$) (fig. 2).



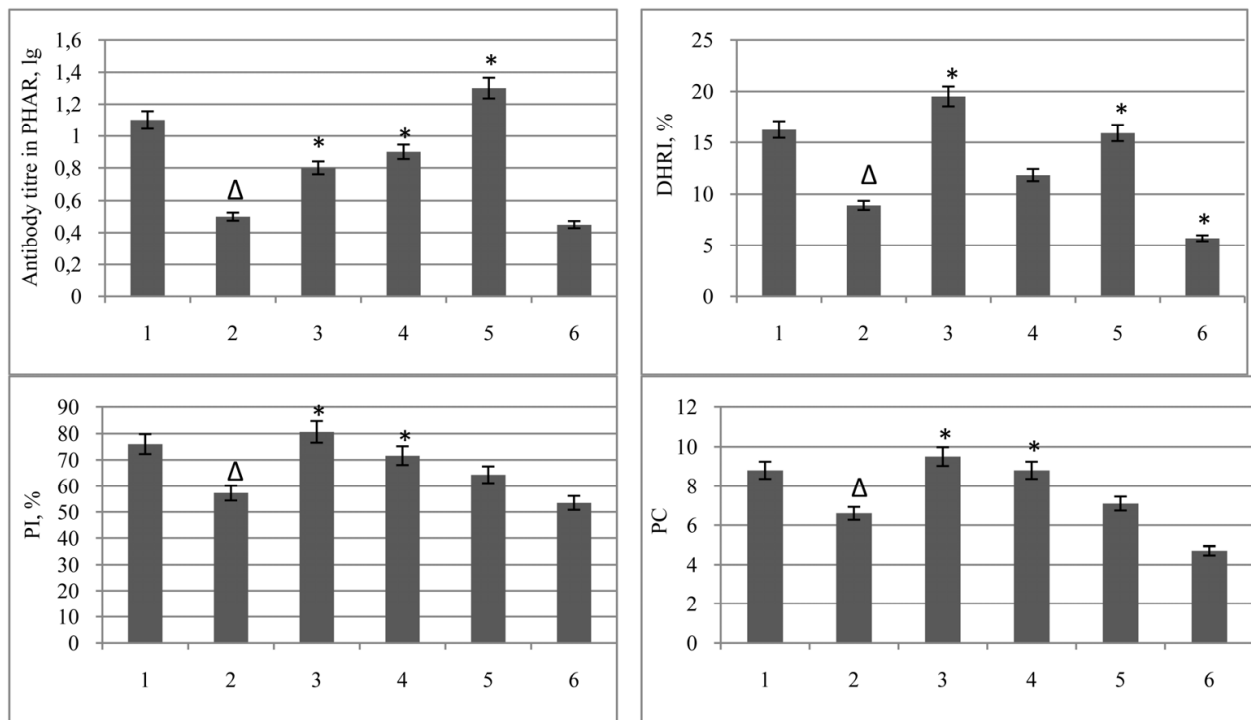
Experimental groups: 1 - control 1 (physiological saline); 2 - control 2 (CPA); 3 - phenibut (12.5 mg/kg) + CPA; 4 - phenibut (25 mg/kg) + CPA; 5 - phenibut (50 mg/kg) + CPA; 6 - phenibut (100 mg/kg) + CPA.

Notes: Δ and * - $p<0.05$ ← reliability of differences as compared to controls 1 and 2 correspondingly (Student’s t-criterion with Bonferroni and Newman-Keuls’ adjustment for multiple comparisons, single-factor analysis of variance involving the definition of Tukey-Kramer criterion and Scheffe’s criterion)

Fig. 2. Effect of phenibut in different doses on PHAR, DHR development and the phagocytic activity of neutrophils in the immunosuppression conditions

Therefore, the most significant immunoreactivity changes in the animals with experimental immunosuppression were observed when phenibut was administered in doses of 25 mg/kg and 50 mg/kg which were recommended for a further study of the immunomodulating activity of phenibut.

The evaluation of the immunocorrective properties of baclofen using a CPA immunosuppression model revealed that the introduction of the drug in doses of 2 mg/kg, 5 mg/kg, and 10 mg/kg had a modulating effect on antibody formation ($p_2<0.05$). In addition, the most pronounced increase of the antibody titre in PHAR was registered in a dose of 10 mg/kg; the studied index was 2.5 times as high as that in the species with an immune pathology while the administration of baclofen in doses of 2 mg/kg and 5 mg/kg increased the index by no more than 60% ($p_2<0.05$). A dose of 20 mg/kg proved to be ineffective: the antibody titre in PHAR was similar to that in the animals with immunosuppression ($p_2<0.05$) (fig. 3).



Experimental groups: 1 - control 1 (physiological saline); 2 - control 2 (CPA); 3 - baclofen (2 mg/kg) + CPA; 4 - baclofen (5 mg/kg) + CPA; 5 - baclofen (10 mg/kg) + CPA; 6 - baclofen (20 mg/kg) + CPA. Notes: Δ and * - $p<0.05$ - reliability of differences as compared to controls 1 and 2 correspondingly (Student's t-criterion with Bonferroni and Newman-Keuls' adjustment for multiple comparisons, single-factor analysis of variance involving the definition of Tukey-Kramer criterion and Scheffe's criterion)

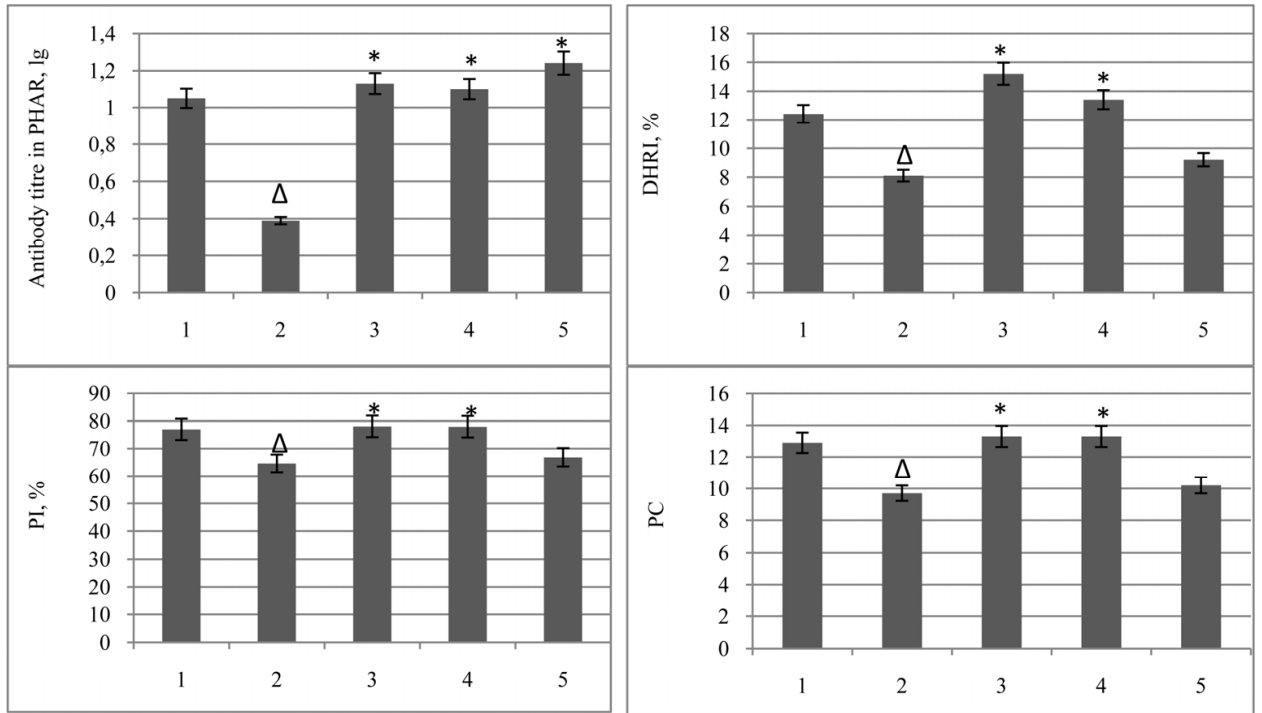
Fig. 3. Effect of baclofen in different doses on PHAR, DHR development and the phagocytic activity of neutrophils in the immunosuppression conditions

As for the cellular link of immunogenesis baclofen was active in doses of 2 mg/kg, 5 mg/kg, and 10 mg/kg. The most significant changes in the DHRI were registered when the drug was administered in doses of 2 mg/kg and 10 mg/kg: the studied index exceeded that in the group of animals with an immune pathology by more than 80% at that. Moreover, baclofen in a dose of 2 mg/kg had a stimulating effect on a cellular immune response increasing the DHRI by 20% as compared to the intact animals. It should be noted that as a dose of

baclofen increased to 20 mg/kg, no corrective action of the drug on DHR development was observed while the CPA’s immunoinhibiting effect intensified (CPA). The results which demonstrate the effect of baclofen in different doses on the indices of the phagocytic activity of neutrophils are of particular interest. We found that regarding this index the drug had a dose-dependent effect: as a dose of baclofen increased from 2 mg/kg to 20 mg/kg its corrective action declined. Thus, when it was administered in a dose of 2 mg/kg phagocytosis activity increased by more than 35% as compared to the immunosuppressed animals ($p_2<0.05$); while in doses of 5 mg/kg and 10 mg/kg this index increased only by 10-20% ($p_2<0.05$); and in a dose of 20 mg/kg a slight increase of CPA immunosuppressive activity was registered (fig. 3). Therefore, proceeding from the analysis results a baclofen dose of 2 mg/kg was chosen for further research as the most active.

3.3 The immunopreventive and/or immunotherapeutic aspects of the effect of GABA derivatives

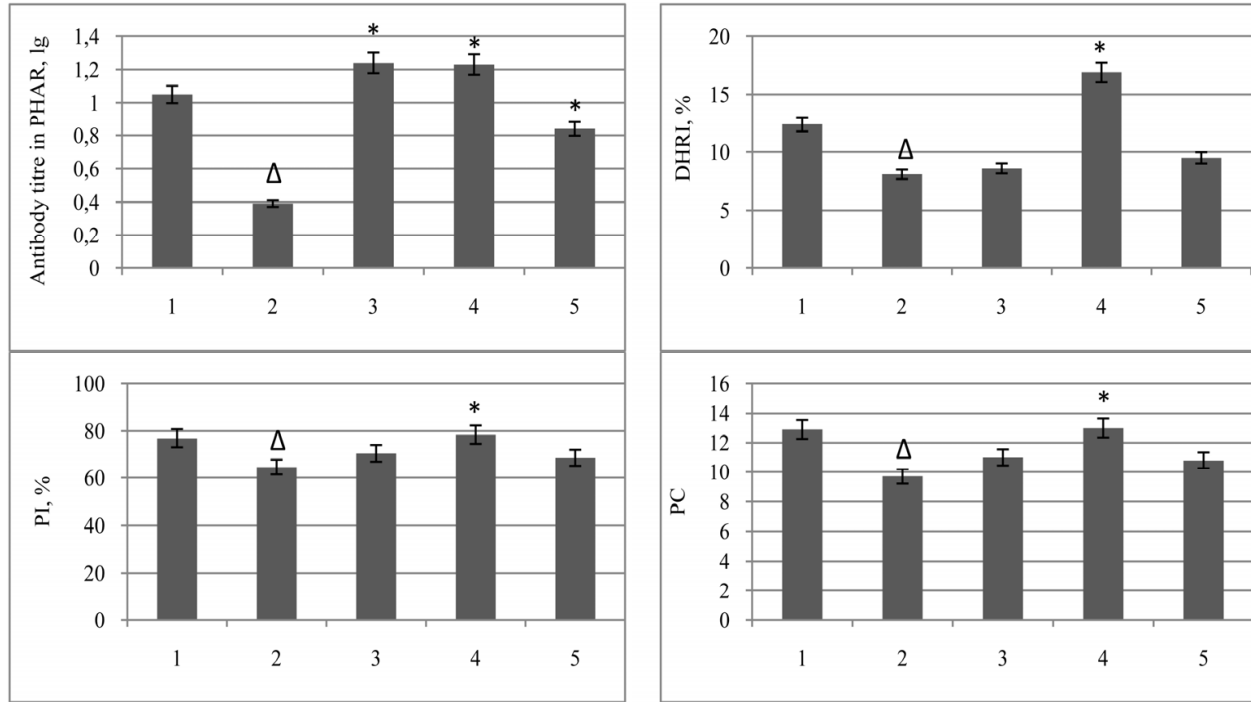
In the second set of experiments we explored the activity of phenotropil, phenibut, and baclofen using the CPA-induced immunosuppression model in a “time-effect” respect to reveal the immunopreventive and/or immunotherapeutic aspects of the action of the drugs (Samotrueva M.A. et al., 2009; Tyurenkov I.N. et al., 2010).



Experimental groups: 1 - control 1 (physiological saline); 2 - control 2 (CPA); 3 - phenotropil (preventive administration) + CPA; 4 - phenotropil (at immunosuppression induction) + CPA; 5 - phenotropil (therapeutic administration) + CPA
Designations: * - $p<0.05$ reliable difference of findings in experimental groups in comparison with control 2; Δ - $p<0.05$ reliable difference in comparison with control 1 (Student t-criterion with Bonferroni correction, Tukey-Kramer one-way ANOVA)

Fig. 4. Effect of phenotropil on the formation of DHR, PHAR and on phagocytic activity of neutrophils when administered at different times in relation to immunosuppression induction

The evaluation of the immunocorrective properties of phenotropil administered intraperitoneally for three times before antigen stimulation and/or immunosuppression induction revealed that the drug proved capable of preventing the disturbances of the cellular and humoral immunity links, as well as of the phagocytic activity of neutrophils induced by CPA. Thus, the DHR index and the level of antired-cell antibodies in the animals of the experimental group were more than 50% higher than the corresponding indices in the immunosuppressed species ($p_2<0.05$) approximating the immune response parameters in the control 1 group ($p_1<0.05$). The number of cells involved in the non-specific body defense (PI) and phagocytosis intensity (PC) in the animals receiving phenotropil before the immunosuppression induction was also restored ($p_2<0.05$) almost reaching the “norm” indices in control 1 (fig. 4).



Experimental groups: 1 – control 1 (physiological saline); 2 – control 2 (CPA); 3 – phenibut (preventive administration) + CPA; 4 – phenibut (at immunosuppression induction) + CPA; 5 – phenibut (therapeutic administration) + CPA

Designations: * – $p<0.05$ reliable difference of findings in experimental groups in comparison with control 2; Δ – $p<0.05$ reliable difference in comparison with control 1 (Student t-criterion with Bonferroni correction, Tukey-Kramer one-way ANOVA)

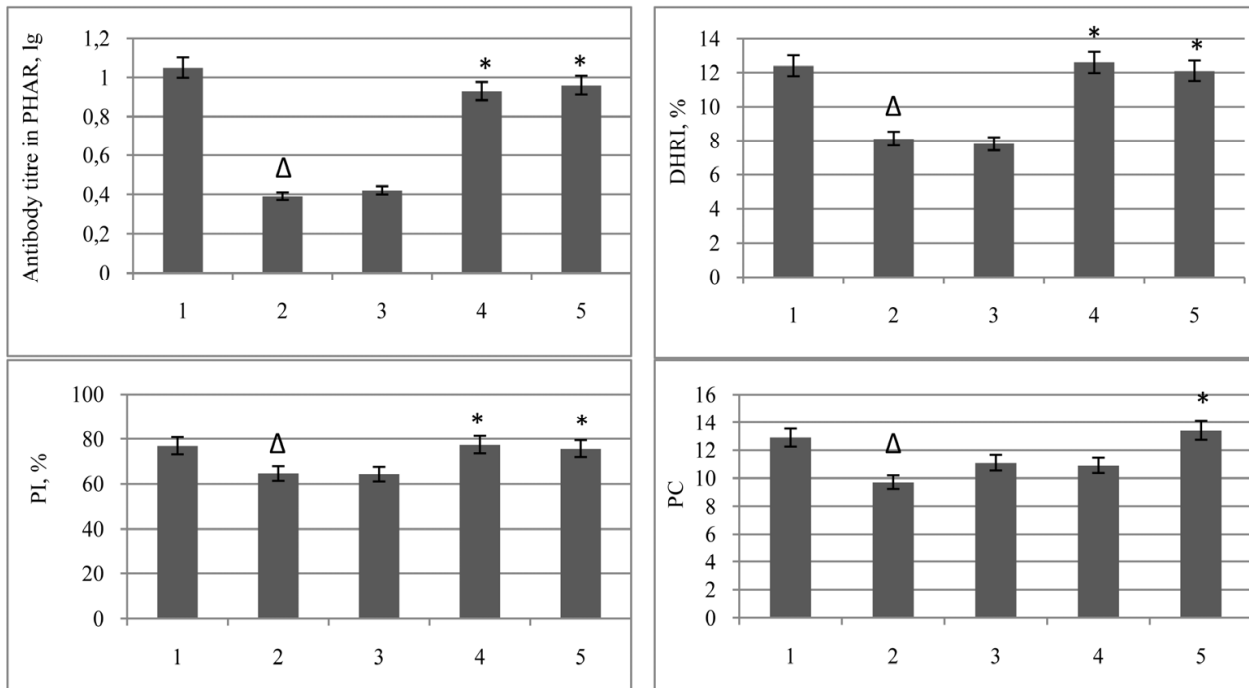
Fig. 5. Effect of phenibut on the formation of DHR, PHAR and on phagocyric activity of neutrophils when administered at different times in relation to immunosuppression induction

The introduction of phenotropil after the immunization and/or immunosuppression induction contributed to the restoration of only the humoral link of immunogenesis: the level of serum antibodies in the experimental animals was more than 60% higher than that of the immunosuppressed species ($p_2<0.05$). No restoration of the cellular immunity link and phagocytic activity of neutrophils affected by phenotropil administered, when immunosuppression had already developed, was observed: DHR index, PI, and PC

remained similar to the indices of the animals with an immune system pathology ($p_2<0.05$) (fig. 4).

Thus the therapeutic effect of phenotropil, once immunosuppression has been induced, is only manifested in relation to the humoral link of immune reactivity. However if the drug is administered prior to antigen stimulation and / or immunosuppression induction, this allows avoidance of immunological insufficiency development manifested by suppression of the activity of all links of immunogenesis, which indicates that phenotropil displays a therapeutic immunocorrective action.

Studying the time-effect aspect of the properties of phenibut demonstrated that the drug displays an ability to eliminate the immunosuppressive effect of CPA upon its administration during the induction phase of immunogeneis (that is, on the first day of antigen stimulation), and simultaneously with induction of immunopathology: the indices of cellular and humoral immune response in mice exceeded similar indices more than twice ($p_2<0.05$) both in immunosuppressed animals and in intact ones (fig. 5).



Experimental groups: 1 – control 1 (physiological saline); 2 – control 2 (CPA); 3 – baclofen (preventive administration) + CPA; 4 – baclofen (at immunosuppression induction) + CPA; 5 – baclofen (therapeutic administration) + CPA

Designations: * – $p<0.05$ reliable difference of findings in experimental groups in comparison with control 2; Δ – $p<0.05$ reliable difference in comparison with control 1 (Student t-criterion with Bonferroni correction, Tukey-Kramer one-way ANOVA)

Fig. 6. Effect of baclofen on the formation of DHR, PHAR and on phagocytic activity of neutrophils when administered at different times in relation to immunosuppression induction

As for humoral immunoreactivity, phenibut showed both an immunoprotective and immunotherapeutic effect: a reliable increase in the antibody level more than twice in comparison with control 2 ($p_2<0.05$) (fig. 5) upon preventive administration of the

substance (three days prior to immunopathology induction) and upon its introduction to the productive phase of immunogenesis (three days after immunization and immunopathology induction). Phagocytic count and phagocytic index, parameters of nonspecific resistance, were also sensitive to the corrective effect of phenibut, but the effect of the drug was only manifest upon its administration on the day of the antigen and cytostatic exposure; at that time the indices of phagocytosis were virtually the same as in intact animals ($p_2 < 0.05$).

Thus the obtained results permit a conclusion that phenibut is capable of preventing the development of disturbances in all the components of the immune system under study, but this only occurs upon its administration on the day of immunization and of exposure to an immunopathology inductor.

An assessment of baclofen impact on the immunity status of animals with immunosuppression by cyclophosphamide upon administration at different times in relation to immunization and pathology simulation showed that the drug was only capable of displaying an immunocorrective effect either upon simultaneous administration with an immunodepressant agent or after the onset of a lesion. In the before-mentioned groups of animals the DHR index, antibody titer and the number of neutrophils participating in phagocytosis exceeded reliably the same parameters in animals with an immunity disorder ($p_2 < 0.05$) achieving background values of immune response in control 1 (fig. 6).

Thus the findings obtained in the course of studying the temporal dependence of immunocorrective effects of baclofen indicate that the drug exerts an immunotherapeutic effect while administration of baclofen for prevention of immunity disturbances in conditions of CPA-induced immunosuppression turned out to be ineffective.

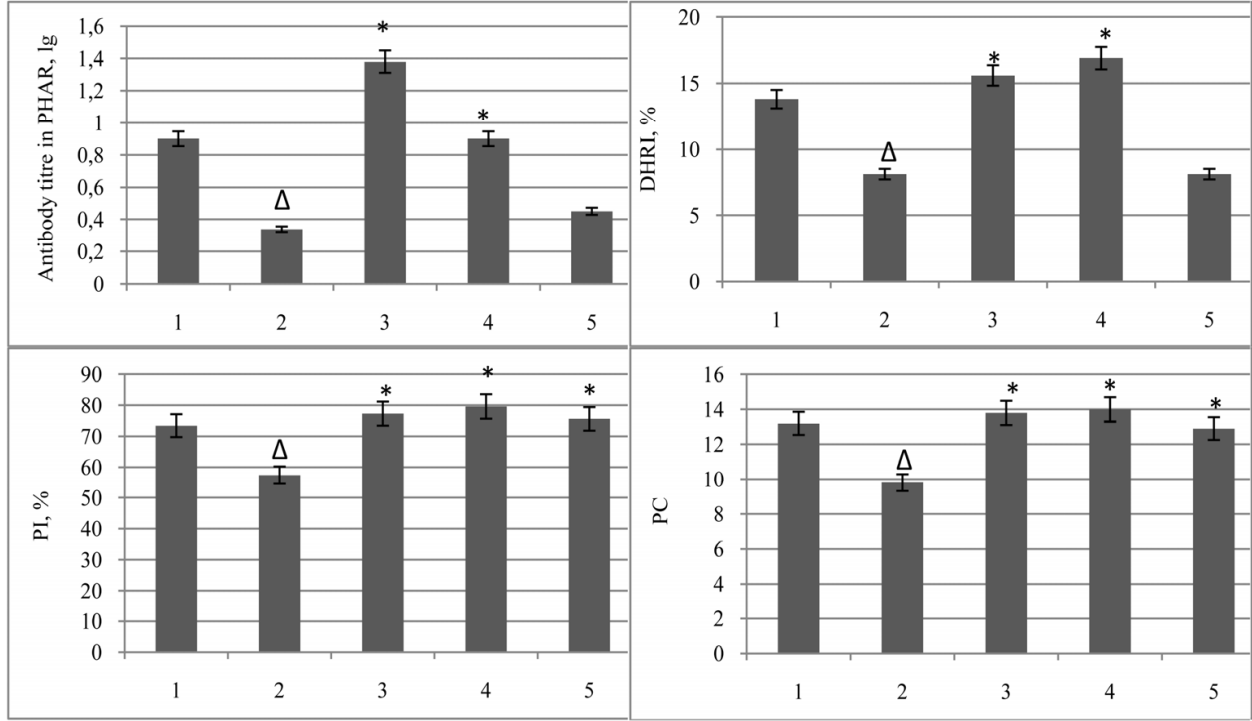
3.4 The immunomodulating activity of GABA derivatives when introduced during a peroral course of treatment

The third series of experiments was devoted to studying the extent of immunomodulating effects of phenotropil, phenibut and baclofen upon their peroral administration over a 14-day course of therapy.

In conditions of immunosuppression, peroral administration of phenotropil and phenibut in a course led to a restoration of cellular immunoreactivity: the index of delayed-type hypersensitivity exceeded the corresponding values in control 2 ($p_2 < 0.05$) twice achieving the values displayed by intact animals. Baclofen showed no effect in relation to the studied parameter (fig. 7).

An estimation of the effect of substances of these experimental series on the humoral link of immunogenesis showed that phenotropil displayed the most effect in conditions of immunosuppression exerting a stimulating effect on the process of sheep erythrocyte-specific antibody formation. Thus the hemagglutinin titer in the reaction of passive hemagglutination exceeded that of control 2 animals 3.8 times ($p_2 < 0.05$) and 1.6 times – that of intact animals ($p_2 < 0.05$). Administration of phenibut also promoted a more than two-fold elevation of the level of anti-erythrocytic antibodies in comparison with the animals receiving the CPA immunodepressant ($p_2 < 0.05$). As for baclofen, it showed no activity in relation to the humoral link of immunogenesis in conditions of immunosuppression (fig. 7).

The nonspecific link of immunogenesis was also sensitive to the corrective effect of the substances under study. Thus phenotropil, phenibut and baclofen caused an intensification of phagocytosis (of phagocytic index and phagocytic number as well) in comparison with these parameters in immunosuppressed animals, and the intensity of phagocytosis virtually achieved the background values in control 1 ($p_2<0.05$) (fig. 7). Therefore, the findings of the study of the effect of substances on the values of immune response upon peroral administration for 14 days indicated that the most activity upon administration in a course was shown by phenotropil and phenibut; these drugs eliminated the CPA-induced suppression of the cellular, humoral and nonspecific links of the immunity.



Experimental groups: 1 - control 1 (physiological saline); 2 - control 2 (CPA); 3 - phenotropil (25 mg/kg) + CPA; 4 - phenibut (25 mg/kg) + CPA; 5 - baclofen (2 mg/kg) + CPA
Designations: Δ - $p<0.05$ reliable difference in comparison with control 1 (Student t-criterion with Bonferroni correction and Newman-Keuls test for multiple comparisons); * - $p<0.05$ reliable difference in comparison with control 2 (Student t-criterion with Bonferroni correction and Newman-Keuls test for multiple comparisons)

Fig. 7. Effect of GABA derivatives after a 14-day peroral administration on the formation of PHAR, DHR and on phagocytic activity of neutrophils in peripheral blood of animals with CPA - induced immunosuppression

3.5 Influence of GABA derivatives on leukopoiesis and on lymphoproliferative and biochemical processes in the immunocompetent organs in conditions of immunosuppression

In the fourth series of experiments we studied the effect of phenotropil, phenibut and baclofen on leukopoiesis and on lymphoproliferative processes in immunocompetent organs (thymus, the spleen) in conditions of immunosuppression. In these series the

substances under study were introduced intraperitoneally for five days in the most active doses (first administration two days prior to CPA). Animals’ blood sampling was done on the next day after the last administration of the substances under study (Samotrueva M.A. et al., 2008; Tyurenkov I.N. et al., 2009; Samotrueva M.A. et al., 2011).

An assessment of the effect of drugs under study on the total number and qualitative composition of leukocytes in peripheral blood showed that under the impact of CPA the administration of phenotropil resulted in an increase of the total leukocyte number up to background values of control 1($p_2<0.05$). Neither phenibut nor baclofen caused any significant change in the total leukocyte number in comparison with the group of immunosuppressed animals ($p>0.05$) (tab. 1).

Experimental groups	Total leukocyte number, $\times 10^9/L$
Control 1 (physiological saline)	10.8 ± 0.7
Control 2 (CPA, 150 mg/kg)	$8.3 \pm 0.7 \Delta$
Phenibut (25 mg/kg) + CPA (150 mg/kg)	8.9 ± 0.3
Phenotropil (25 mg/kg) + CPA (150 mg/kg)	$10.4 \pm 0.4^*$
Baclofen (2 mg/kg) + CPA (150 mg/kg)	9.6 ± 1.1

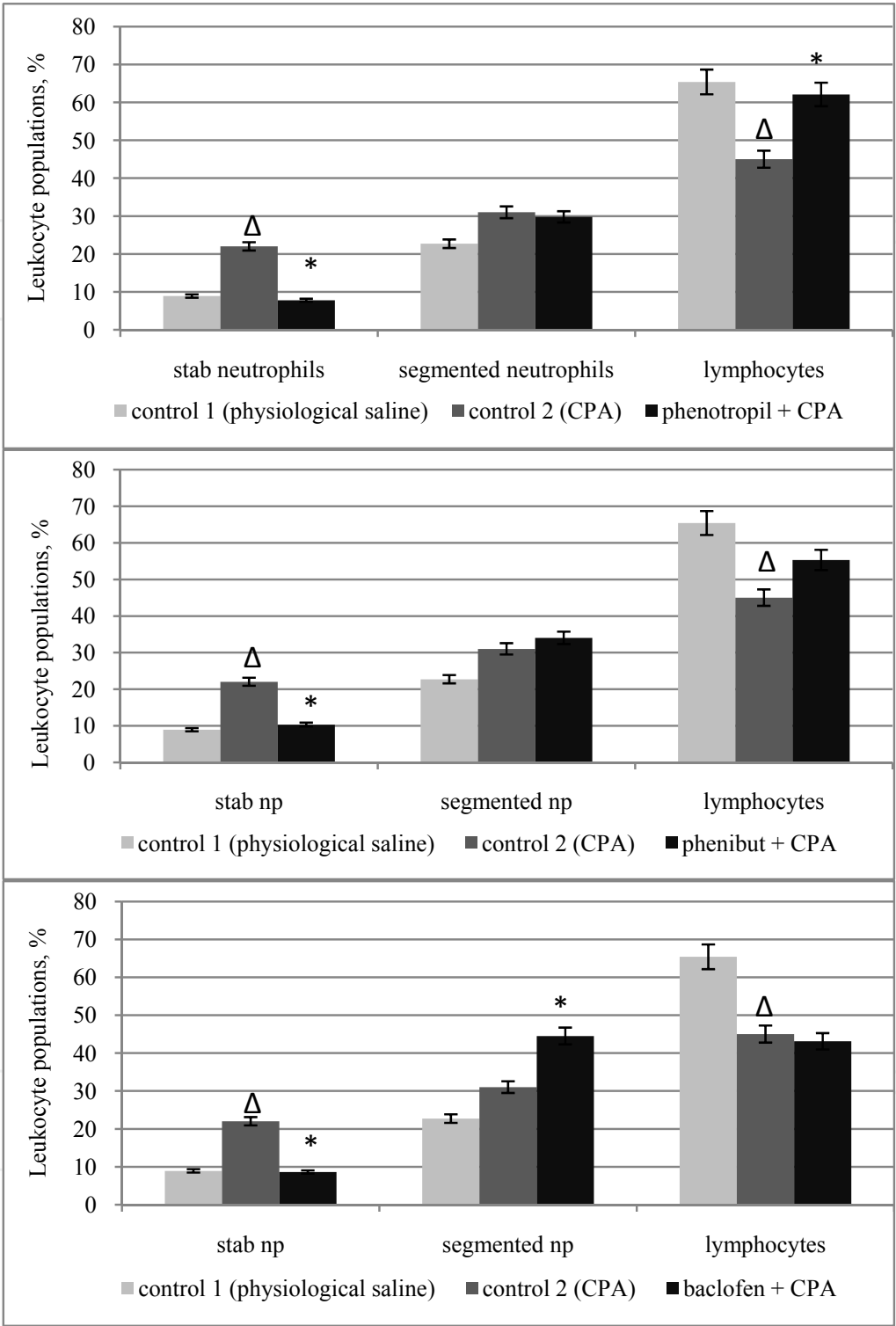
The degree of credibility Δ – $p<0.05$ – in relation to control 1 and * – $p<0.05$ – in relation to control 2 (Student t-criterion with Bonferroni correction for multiple comparisons)

Table 1. Effect of GABA derivatives on total leukocyte count in animals with CPA -induced immunosuppression

In animals with CPA -induced immunological insufficiency a restoration of leukocyte population composition was only noted under the impact of phenotropil administration when a relative number of leukocytes was noted to increase; the content of lymphoid cells in peripheral blood of mice of experimental groups considerably exceeded this parameter in control 2 animals ($p_2<0.05$). Administration of baclofen was accompanied by an elevation of the number of mature segmented neutrophils ($p_2<0.05$), while the level of lymphocytes remained within the range of values typical of immunosuppressed animals ($p>0.05$). No significant changes in the leukogram were noted under the impact of phenibut ($p>0.05$) (fig. 8).

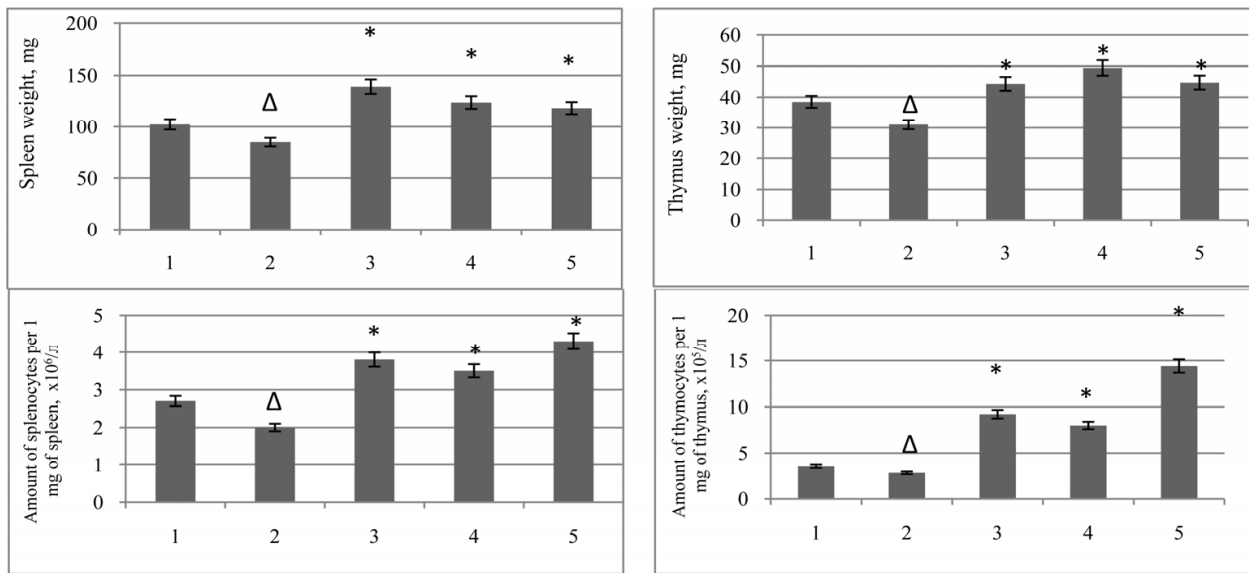
Thus phenotropil displayed the most activity in relation to the leukogram parameters by promoting a restoration of leukopoiesis processes which was manifested in an elimination of the inhibiting effect of CPA on the lymphoid hematopoietic lineage.

The results of studying the effects of phenotropil, phenibut and baclofen on lymphoproliferative and biochemical processes in immunocompetent organs in conditions of CPA -induced immunosuppression indicate that the drugs produce a corrective effect on the morphometric parameters which was evident from the increase in thymus and spleen weight, a restoration of their cellular composition ($p_2<0.05$) (fig. 9-11).



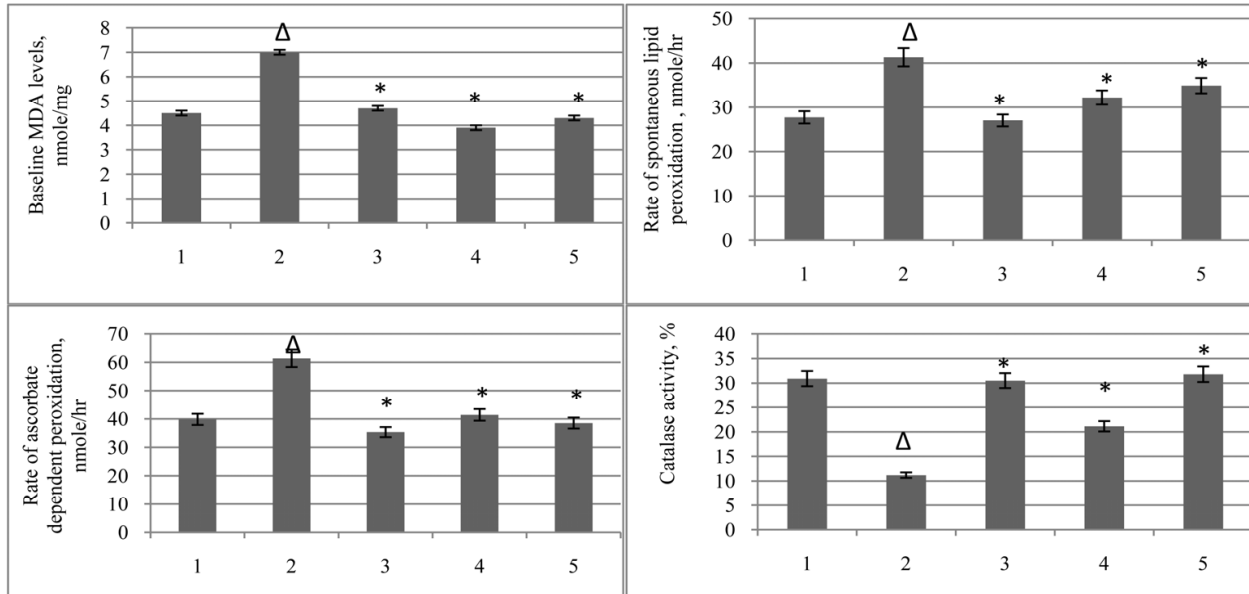
Designations: Δ - p<0.05 reliable difference in comparison with control 1 (Student t-criterion with Bonferroni correction and Newman-Keuls test for multiple comparisons); * - p<0.05 reliable difference in comparison with control 2 (Student t-criterion with Bonferroni correction and Newman-Keuls test for multiple comparisons)

Fig. 8. Effect of GABA derivatives on various leukocyte populations in the leukogram in animals with CPA -induced immunosupression



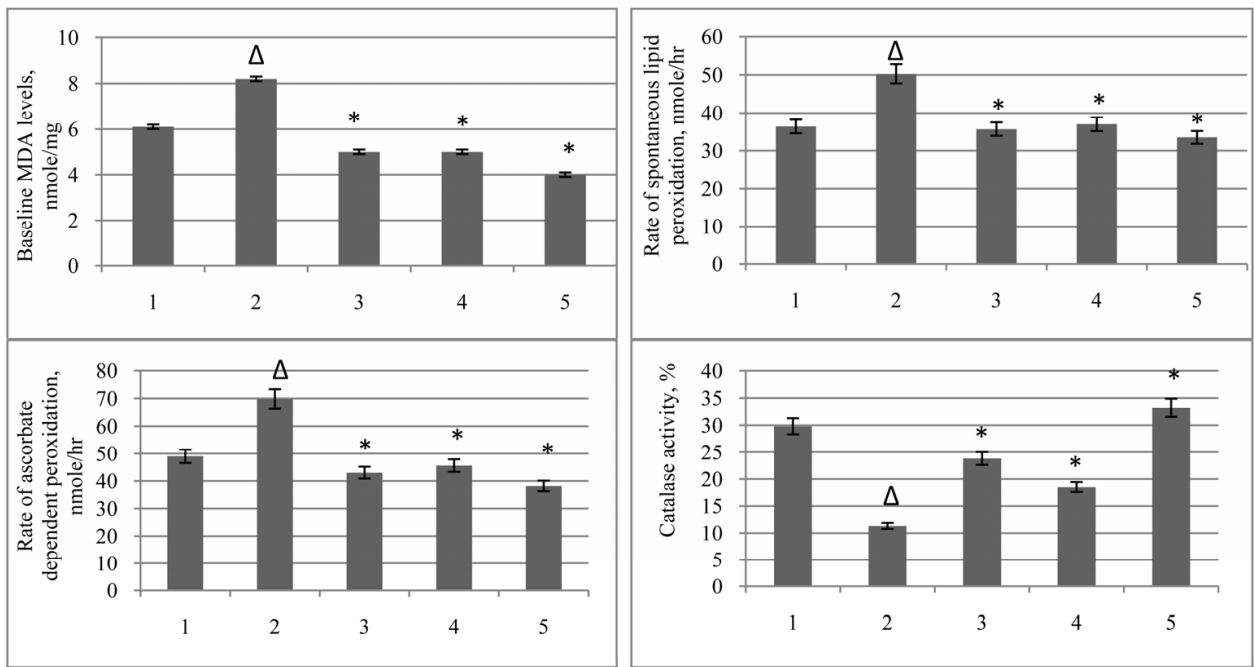
Experimental groups: 1 – control 1 (physiological saline); 2 – control 2 (CPA + physiological saline); 3 – phenotropil (25 mg/kg) + CPA; 4 – phenibut (25 mg/kg) + CPA; 5 – baclofen (2 mg/kg) + CPA
Designations: Δ – p<0.05 reliable difference in comparison with control 1 (Student t-criterion with Bonferroni correction and Newman-Keuls test for multiple comparisons); * – p<0.05 reliable difference in comparison with control 2 (Student t-criterion with Bonferroni correction and Newman-Keuls test for multiple comparisons)

Fig. 9. Effect of GABA derivatives on the weight and cellularity of immunocompetent organs in animals with CPA-induced immunosuppression



Experimental groups: 1 – control 1 (physiological saline); 2 – control 2 (CPA); 3 – phenotropil (25 mg/kg) + CPA; 4 – phenibut (25 mg/kg) + CPA; 5 – baclofen (2 mg/kg) + CPA
Designations: Δ – p<0.05 reliable difference in comparison with control 1 (Student t-criterion with Bonferroni correction and Newman-Keuls test for multiple comparisons); * – p<0.05 reliable difference in comparison with control 2 (Student t-criterion with Bonferroni correction and Newman-Keuls test for multiple comparisons)

Fig. 10. Effect of GABA derivatives on lipid peroxidation and catalase activity in the spleen of animals with CPA -induced immunosuppression



Experimental groups: 1 – control 1 (physiological saline); 2 – control 2 (CPA); 3 – phenotropil (25 mg/kg) + CPA; 4 – phenibut (25 mg/kg) + CPA; 5 – baclofen (2 mg/kg) + CPA
Designations: Δ – p<0.05 reliable difference in comparison with control 1 (Student t-criterion with Bonferroni correction and Newman-Keuls test for multiple comparisons); * – p<0.05 reliable difference in comparison with control 2 (Student t-criterion with Bonferroni correction and Newman-Keuls test for multiple comparisons)

Fig. 11. Effect of GABA derivatives on lipid peroxidation and catalase activity in the thymus of animals with CPA-induced immunosuppression

4. Conclusion

We would like to highlight the importance and urgency of the problem under discussion. Immune imbalance underlies the pathogenesis of CNS diseases (depression, disorder of cerebral circulation, epilepsy, multiple sclerosis, Alzheimer’s disease, schizophrenia, etc.), and one of the causes of these conditions is dysregulation of GABA-ergic system as one of the key factors of neuro-immune interactions. An analysis of our own data and those from literature permits a conclusion that a realization of immunoactive properties of GABA-ergic substances is mediated by both central and direct impact on the corresponding receptors of effector cells in the immune system. The pronounced immunomodulating effect of phenotropil, phenibut and baclofen that we showed in CPA-induced immunosuppression widens the range of their possible administration not only in CNS diseases that are often accompanied by immune status disturbances, but also in the immune system diseases accompanied by suppression of certain immunogenesis links.

1. GABA derivatives – phenotropil, phenibut and baclofen – reduce the manifestations of immunosuppression induced by CPA administration.
2. Phenotropil at a dose of 25 mg/kg, 50 mg/kg and baclofen at a dose of 2 mg/kg produce the most pronounced immunomodulating effect in conditions of CPA-induced immunosuppression.
3. Immunotropic effects of phenotropil and phenibut are most pronounced upon their preventive administration prior to CPA-induced immunosuppression, while the effect

of baclofen is most pronounced upon its therapeutic administration when the immune system disease is already in progress.

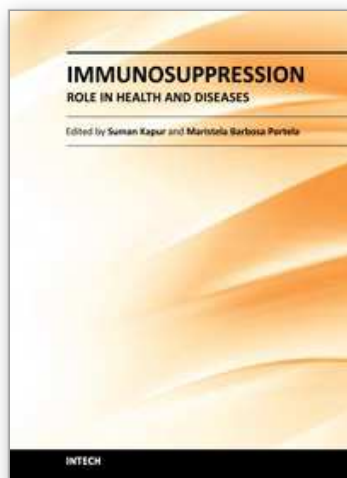
4. All the substances studied in this work can restore lymphoproliferative processes and normalize the parameters of lipid peroxidation in the immunocompetent organs (thymus and spleen); while only phenotropil was able to eliminate the disturbances of leukopoietic processes due to immunosuppression.
5. The immunomodulating effect of phenotropil, phenibut and baclofen established in our study as well as their neurotropic effects established by many researchers, allow an inclusion of GABA derivatives into a new class of neuroimmunomodulating agents.

5. References

- [1] Alexandrovsky U.A., Chekhonin V.P. Clinical immunology of borderline mental disorders. – Moscow: GEOTAR-Media, 2005. – 256 p.
- [2] Alford L. Findings of interest from immunology and psychoneuroimmunology // *Man. Ther.* – 2007. – Vol. 12. – N 2. – P. 176-80.
- [3] Arkadyev V.G., Makarenko A.N., Mironiuk U. M. Experimental reproduction of medium and severe immunosuppression with cyclophosphane administration // *Vestnik KNU. Biology series.* – 2003. – Vol. 39. – P. 51-52.
- [4] Arushanian E.B. Medicamentous amelioration of cognitive brain function. – Stavropol: SGMA Publishers, 2004. – 401 p.
- [5] Devoyno L.V., Iliuchenok R.U. Neuromediator systems in psychoneuromodulation: dopamine, serotonin, GABA, neuropeptides. – Novosibirsk, ZE RIS, 1993. – 273 p.
- [6] Fleshner M., Laudenslager M.L. Psychoneuroimmunology: then and now // *Behav. Cogn. Neurosci. Rev.* – 2004. – N 3. – P. 114-130.
- [7] Freund G.G. Psychoneuroimmunology // *Preface Immunol. Allergy Clin. North. Am.* – 2009. – Vol. 29. – N 2. – P. 15-16.
- [8] Irwin M.R. Human psychoneuroimmunology: 20 years of discovery // *Brain Behav. Immun.* – 2008. – Vol. 22 (2). – P. 129-139.
- [9] Khaitov R.M., Gushin I.S., Pinegin B.V. Methodological recommendations on studying immunotropic effects of pharmacological agents // *Handbook of experimental preclinical study of new pharmacological agents* / Ed. by R.U. Khabriev. – Moscow, 2005. – P. 501-514.
- [10] Korneva E.A. Introduction to immunophysiology. – St Petersburg: ELBI, 2003. – 48 p.
- [11] Kryzhanovsky G.N., Magaeva S.V., Makarov S.V., Sepiashvili R.I. Neuroimmunopathology. – Moscow: Meditsina, 2003. – 438 p.
- [12] Samotrueva M.A., Ovcharova A.N., Tyurenkov I.N. Evaluation of the immunocorrecting activity of phenibut // *Vestnik novykh meditsinskih technologii.* – 2008. – Vol. 15. – N 3. – P. 168-169.
- [13] Samotrueva M.A., Ovcharova A.N., Tyurenkov I.N. Phenibut and its derivatives' influence on the cell section of the immune response in immunodeficiency // *European Journal of Natural History.* – 2008. – N 5. – P. 40-41.
- [14] Samotrueva M.A., Tyurenkov I.N., Luzhnova S.A., Magomedov M.M., Kuleshevskaya N.R., Serezhnikova T.K. Experimental learning of dose-related influence of phenotropil on humoral link of immunogenesis // *European Journal of Natural History.* – 2010. – N 3. – P. 61-62.

- [15] Samotrueva M.A., Tyurenkov I.N., Serezhnikova T.K., Grazhdantseva N.N., Khlebtsova E.B., Berestovitskaia V.M., Vassilieva O.S. Immunomodulating properties of phenotropil derivatives // *Farmatsia*. – 2011. – N 1. – P. 28-30.
- [16] Samotrueva M.A., Tyurenkov I.N., Teply D.L., Luzhnova S.A., Magomedov M.M. Extent of immunocorrective effects of phenotropil upon its administration at different times in relation to immunosuppression induction // *Meditinskaja immunologia*. – 2009. – N 6. – Vol. 11. – P 567-570.
- [17] Samotryeva M.A., Teply D.L., Tyurenkov I.N. Ways to realization of neuro-immune-endocrine interactions // *Estestvennye nauki*. – 2009. – N 4. – Vol. 29. – P. 112-130.
- [18] Tyurenkov I.N., Galimzianov H.M., Teply D.L., Samotrueva M.A., Luzhnova S.A. Experimental study of immunocorrective effects of phenotropil in the dose-effect aspect // *Immunologia*. – 2009. – N 5. – P. 302-305.
- [19] Tyurenkov I.N., Samotrueva M.A. Comparative study of immunocorrective activity of phenibut and its organic salts in experimental immunodeficiency // *Bull. Exp. Biol. Med.* – 2009. – N 5. – P. 606-668.
- [20] Tyurenkov I.N., Samotrueva M.A., Kuleshevskaja N.R., Serezhnikova T.K. Experimental substantiation of phenibut administration as a modulator of immune response // *Farmatsia*. – 2010. – N 4. – P. 42-44.
- [21] Tyurenkov I.N., Samotrueva M.A., Ovcharova A.N. Baclofen influence on the cellular parts of immune reaction // *Experimentalnaja i klinicheskaja farmakologia*. – 2008. – N 3. – P. 43-45.

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Immunosuppression - Role in Health and Diseases

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A need for a book on immunology which primarily focuses on the needs of medical and clinical research students was recognized. This book, "Immunosuppression - Role in Health and Diseases" is relatively short and contains topics relevant to the understanding of human immune system and its role in health and diseases. Immunosuppression involves an act that reduces the activation or efficacy of the immune system. Therapeutic immunosuppression has applications in clinical medicine, ranging from prevention and treatment of organ/bone marrow transplant rejection, management of autoimmune and inflammatory disorders. It brings important developments both in the field of molecular mechanisms involved and active therapeutic approaches employed for immunosuppression in various human disease conditions. There was a need to bring this information together in a single volume, as much of the recent developments are dispersed throughout biomedical literature, largely in specialized journals. This book will serve well the practicing physicians, surgeons and biomedical scientists as it provides an insight into various approaches to immunosuppression and reviews current developments in each area.

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