and heparin therapy transfusiological methods of immune correction and detoxication should be used for the purpose of controlling the toxogenic immunosuppression, including infusion of Hemodes, native and immune plasma, ultraviolet-irradiated autologous blood, immunoglobulins of different classes and polyimmune preparations.

2. In a deficit of the T-lymphocyte component of immunity, thymic hormone preparations should be used in order to promote differentiation and maturation of T lymphocytes, as well as for an indirect correction of phagocytosis dysfunction.

3. If the syndrome of phagocytosis dysfunction (including all or certain stages of the process) predominates in the immunohematological status, in order to stimulate the metabolism of phagocyting cells preparations of yeast RNA (Na nucleinate) may be used; granulocyte repopulation can be promoted by pyrimidine derivatives (e.g., methyl uracil). Complex therapy includes vitamins E, A, and C and nonvitamin cofactors (lipoic acid, carnitine chloride, potassium orotate) possessing immunomodulatory activity.

4. Immediately after the withdrawal of antibacterial therapy and during the reconvalescence period (3-6 months after surgery) homeopathic immunocorrecting preparations are prescribed, taking into consideration the constitutional parameters of the children, the pattern and localization of the infectious process, and specific features of reactivity.

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The Effect of Serotonin Interaction with Cells

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The study of the mechanisms of serotoninergic immunomodulation is of both particular and general interest, as the effects of serotonin are realized via the neuroendocrine system [2,3,7,17]. It has been shown that the inhibitory action of high doses of serotonin is mediated via the hypothalamo-hypophyseal-adrenal system [8,9], while its stimulatory effect (low doses) is mediated via the vagus nerve [11]. The following facts, besides the ones mentioned above, served as the premises for this study: 1) immunocompetent organs and tissues contain cells bearing heterogenous receptorlike serotonin-binding structures [10-12,22]; 2) the level of specific binding of serotonin by these cells and also by the synaptosomes depends on the dose of antigen introduced [14]; 3) the biological effect of serotonin upon the cells of immunocompetent organs *in vitro* can be further observed *in vivo* following administration of these cells into syngenic recipients and is expressed as opposite changes of the function of amine-treated cells [11]. Thus, there are data concerning opposite regulatory effects of serotonin on the same immunological phenomenon, depending on the targeted action of serotonin on spe-

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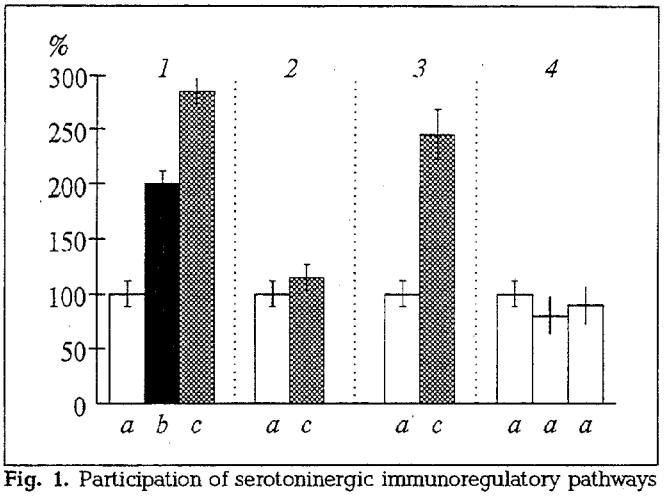

Fig. 1. Participation of serotoninergic immunoregulatory pathways in the realization of the biological effect of serotonin interaction with blood-derived TEC. Recipient mice underwent sham operation (*1*), unilateral vagotomy (*2*), or cross-section of hypophyseal peduncle (*3*) and received intravenously SE only (*a*, 100%), SE plus TEC pretreated with serotonin in a concentration of 10^{-5} M (*b*) or 10^{-7} M (*c*). Comparison of response of operated mice, expressed as percentage of sham-operated mice (*4*).

cific cell types of nontargeted action which occurs with systemic administration. In the latter case, due to extremely high rates of development of the effects of biologically active compounds [2], it is obvious that regional (peripheral) serotonin-responsive structures including those on the cells of immunocompetent tissues, would be the first to interact with serotonin. This led to the assumption that there is a link between the targeted and untargeted action of serotonin.

This study explores the involvement of the serotoninergic regulatory pathways in the realization of the biological interaction of serotonin with cells.

MATERIALS AND METHODS

The experiments were performed on 445 mice of the CBA strain aged 3-4.5 months, 235 of which served as cell donors. Each group, as a rule, consisted of 7 mice. Animals were challenged with sheep erythrocytes (SE) (10^7 per mouse) and tested 5 days later. At this time a spleen sell suspension was incubated with an equal volume of 3% sheep erythrocyte suspension for 15 min at 37°C and mobile preparations were made, arranged as a drop of cell mixture surrounded by a ring of mineral oil and covered with a glass cover slip. With help of a phase-contrasting device (×1000) the number of rosette-forming cells (RFC per 10^3 cells) was calculated. Operations (sham operation, SO; thymectomy, TE; bilateral adrenalectomy, AE; cross-section of hypophyseal peduncle, SHP; unilateral vagotomy, UVT) were performed under nembutal anesthesia (50 mg per kg body weight) 1 day preceding the immunization. Salt imbalance in the AE mice was compensated with 0.85% NaCl admixed to the water. SHP was performed mechanically via the transauricular route [10], under visual control of the preciseness of destruction. Adherent cells (AC) (including peritoneal cells) were separated on polystyrene plates. T-lymphocyte-enriched cells (TEC) were obtained by removal of B lymphocytes [16]. AC and TEC were incubated in siliconized tubes for 30 min at 37°C with serotonin present in a concentration of 10^{-5} M, which is nearly the saturating concentration [13], or 10^{-7} M. The cells were then washed in the same tubes four times with precooled (+4°C) solution containing 0.85% NaCl and 0.001 M Tris-HCl, pH 7.2, and stored at this temperature until transferred to recipients. In the control experiments the cells were manipulated in a similar way but without the addition of serotonin. For a study of the biological effect of serotonin interaction with serotonin-responsive cell structures, syngenic recipients were inoculated intravenously with a mixture of SE plus TEC, or intraperitoneally with SE plus AC, TEC and AC treated or untreated with serotonin *in vitro*. Serotonin creatinine sulfate (Reanal, Hungary) was used. The results were statistically processed using Student's *t* test. The arithmetic mean values and confidence limits are presented in the figures.

RESULTS

Preceding the statement of results, it is worth pointing out that the effects of the injected cells were not mediated by the serotonin itself, but resulted from the perception and interpretation of the serotonin signal received [11]. As shown in Fig. 1, in SO recipients of syngenic TEC the biological effect of serotonin interaction with these cells could be seen. It

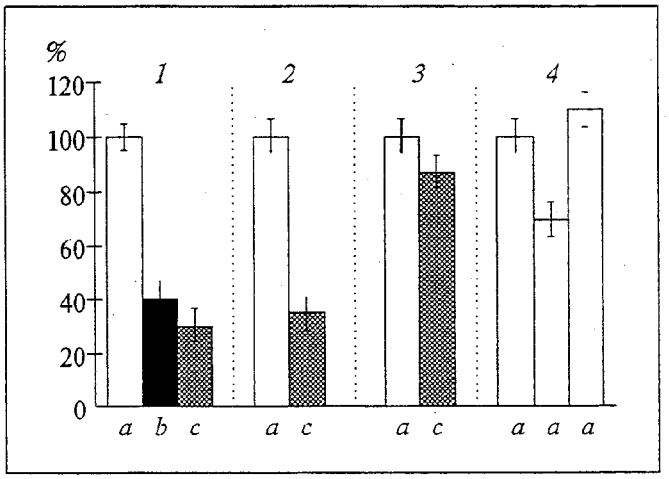

Fig. 2. Participation of serotoninergic immunoregulatory pathways in the realization of the biological effect of serotonin interaction with peritoneal AC. (Designations are the same as in Fig. 1, but cells were injected intraperitoneally.)

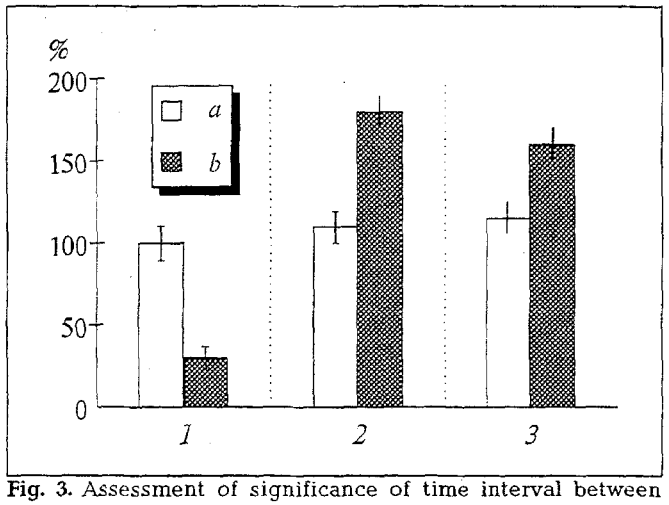

Fig. 3. Assessment of significance of time interval between bilateral AE and administration of SE only (*a*) or SE plus serotonin, 50 mg per kg body weight (*b*). Sham-operated mice (*1*); mice subjected to AE 3 weeks preceding (*2*) or 1 day (*3*) preceding injections.

manifested itself in an increase of the RFC number, which was especially high when donor cells were pretreated with 10^{-7} M serotonin (Fig. 1, *1, b, c*), i.e., the lower concentration. The efficiency of the small concentration is perhaps explained by the sufficiency of serotonin interaction with just some of the receptors in order to produce the maximal effect. Proceeding from this, we used the optimal concentration of serotonin in the cell transfer experiments with SHP and UVT recipients. It turned out that the recipients with SHP-related impaired integrity of the down-regulation pathway, like SO animals, also exhibited the biological effect resulting from serotonin pretreatment of donor TEC (Fig. 1, *2, a, c*). On the other hand, UVT-induced disturbance of the integrity of the serotoninergic immunostimulating pathway interfered with the expression of the biological effect (Fig. 1, *3, a, c*). The operations per se produced no effect on the reaction (Fig. 1, *4*). These results provide evidence that the TEC-targeted serotonin effect on immunogenesis and the nontargeted effect mediated via the vagus nerve are interlinked. In other words, one may assume that among the TEC there are cells directly participating in the immunostimulatory action of serotonin and apparently promoting the recruitment of the nonspecific regulatory system in the modulation of a specific process. While accepting that there might be other explanations of the described phenomenon, we would prefer to hypothesize the participation of amine-binding cells in the performance of the corresponding afferent signal perceived by the autonomic nervous system, which in turn provides evidence that the immune system possesses features intrinsic in the sensory organs.

By analogy with the described experiments, we followed up the realization of the biological effect of serotonin on AC in SO, UVT, and SHP recipients. AC pretreated with different doses of serotonin produced an effect of similar magnitude in SO recipients. The effect was expressed in the reduction of the number of RFC in the spleen cell population as compared to the SO recipients of untreated AC (Fig. 2, *1, a, b, c*). The suppressor function of transferred cells was realized in UVT mice (Fig. 2, *2c*), but was abolished in SHP recipients as a result of preceding alteration in the integrity of the down-regulation pathway of serotoninergic immunomodulation (Fig. 2, *3c*). The surgical procedures per se had no effect on the RFC levels (Fig. 2, *4*). Thus, additional evidence is obtained regarding the linkage of three processes: 1) the targeted effect of serotonin on a specific cell population, i.e., AC; 2) a serotonin-induced suppressor function of these cells, and 3) a nontargeted inhibitory effect of serotonin mediated via the hypothalamus, exercising control over the peritoneal macrophages [18-20].

Whereas UVT could equally alter both the afferent and efferent information pathways, SHP interrupted the efferent pathway only. As was shown in earlier investigations, this process passed through the adrenals, and the disengagement of these with AE resulted in the opposite effect [9] due to reorientation to the hypothalamo-hypophyseal-thymus axis [12]. Figure 2, *3c* shows the effect of AC which required integrity of the hypothalamic-hypophyseal complex for realization of the serotonin-induced suppressor function in the AE and TE recipients. Since first we used mice 2-3 weeks post-operation and later findings showed the expedience of minimizing the time interval between operation and immunization, we conducted an appropriate check. As can be seen in

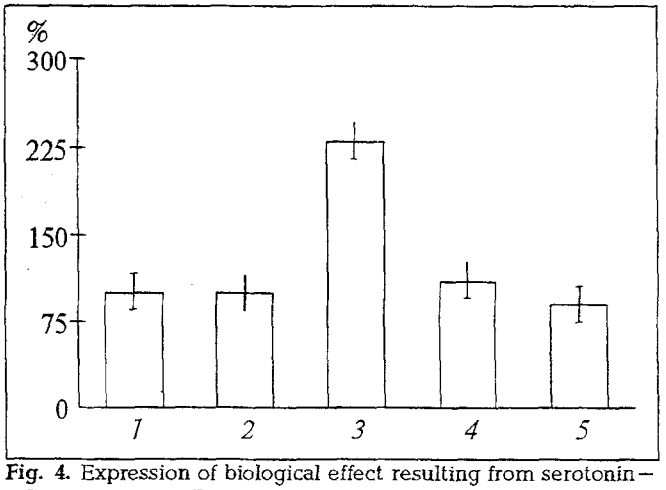

Fig. 4. Expression of biological effect resulting from serotonin-AC interaction. AE mice received SE only (*1*, control group, 100%), SE plus untreated AC (*2*), SE plus serotonin-pretreated AC (*3*); AE+SHP mice received SE plus serotonin-pretreated AC (*4*); AE+TE mice received SE plus serotonin-pretreated AC (*5*).

Fig. 3, regardless of the time interval, AE failed to alter the level of RFC, and the usual inhibitory effect of high-dose serotonin in SO recipients (Fig. 3, *1b*) switched to the opposite effect (Fig. 3, *2b*, *3b*), in agreement with the earlier findings [9,10]. This enabled us to continue to perform AE one day before immunization.

The analysis of the findings obtained in the next experiment disclosed the following. Transfer of untreated AC to AE mice did not influence the level of RFC, as in the case of unoperated recipients (Fig. 4, *2*). AE as well as SHP preceding cell transfer prevented the expression of a suppressor effect of serotonin-treated cells. This pointed to the existence of a macrophage-hypothalamic-hypophyseal-adrenal axis, similar to the lymphocyte-hypothalamic-adrenal axis [6]. However, unlike SHP, AE not only abolished the effect but also replaced it by the opposite one (Fig. 4, *3*). This phenomenon is apparently related to the ability of the hypothalamus and adrenals to control the same process in different manners [4]. Withdrawal of adrenal negative control presumably led to the preponderance of stimulating regulatory mechanisms. Once again the targeted action of serotonin concurred with the nontargeted action (Fig. 3, *4*). Based on the assumption that serotonin-treated transferred cells function as a source of afferent information, one could assume the need for two signals (afferent and efferent) for realizing the effect of these cells. The revealed abolition of the stimulatory effect of serotonin-pretreated AC (Fig. 4, *4*, *5*) in recipients having undergone a double operation (AE+SHP or AE+TE) serves as evidence that the mentioned stimulatory influence is realized via the hypothalamic-hypophyseal-thymus axis. Thus, the pathways of targeted and nontargeted serotonin effect once more concurred, as the stimulatory effect of systemic administration of high-dose serotonin to AE mice was also abolished with superimposed SHP or TE [10,12]. The results presented provide proof for these assumptions. Probably, in situations where the immunomodulatory influence of serotonin begins at the periphery (an increase in its level due to endogenous processes of exogenous stimuli), both serotonin itself and cells whose receptors have received and interpreted its signal represent inner components of the organism which serve as sources of afferent information. Depending on the strength of their action and the sensitivity threshold of the perceiving structures, they become an object of one or another regulatory system.

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