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ATP and Serotonin in Immunomodulation

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The immunomodulatory role of serotonin, a neurotransmitter of the central and peripheral nervous system, was established long ago [3]. Serotonin can be considered as an intersystem mediator because it realizes its effects upon immunogenesis via the neuroendocrine system and because serotonin-reactive target cells are present in the immunocompetent organs and tissues. The interrelationships between the regulatory systems, including the immune system, the variety of functions of the bioactive immunoregulatory substances, and the complex character of immune processes call for a study of the modulator interactions in the regulation of immunological phenomena. Keeping in mind the close morphofunctional relationship between serotonin and adenosine triphosphate (ATP), we proposed a linkage to exist between these agents in the process of immunomodulation. Suffice it to remember the existence of intra- and extracellular serotonin-ATP complexes, localized, specifically, in the platelets, which accompany immunocompetent cells, fix antigen-antibody complexes on the surface, and release serotonin during antigen-antibody interaction and under the influence of ATP. One should also mention the mast cells, which are likewise associated with immunocompetent cells, exert an ATP-induced serotonin release, react to the antigenic stimulus with a change in cAMP level, and can be activated by the antigen-antibody complex [5]. Moreover, ATP itself deserves special interest regarding the possible effects upon immunogenesis, since it is widely used in therapy and has been little studied from the viewpoint of our interests. Exogenous ATP has been shown to promote Ca^{2+} influx to the thymocytes and to inhibit the cytotoxic activity of T lymphocytes [14]. Depending on the origin of immunocompetent cells, ATP induces opposite changes in the cellular DNA level [13] and proliferation rate. ATP also inhibits the activity of blood-derived natural killer cells *in vitro*, induces nonspecific permeability of the membranes of thymocytes, but not of peritoneal cytotoxic lymphocytes [6], and regulates neutrophil functions [16] and Ca^{2+} accumulation in the macrophage membranes. Moreover, the relationship between ATP and the immune system is evidenced by the following: the change in the intracellular ATP level in immunodeficiency states; the specific binding of ATP to the peritoneal cells and blood leukocytes; the presence of ATP receptors on the lymphocytes [4]; the enhanced adenosine deaminase activity in the course of T lymphocyte differentiation; the presence of ecto-ATPase on the surface of lymphocytes, monocytes, and neutrophils; the rise of the ATP concentration in the spleen following immunization.

In this study we attempted to show the immunomodulatory role of ATP and of the ATP-serotonin interaction in the early stages of the induction of the immune response *in vivo*, using the model of rosette-forming cell generation.

MATERIALS AND METHODS

The experiments were carried out on 195 mice of the CBA strain (8-15 animals per group) aged 3-4.5 months. Sheep erythrocytes (SE) were injected intra-

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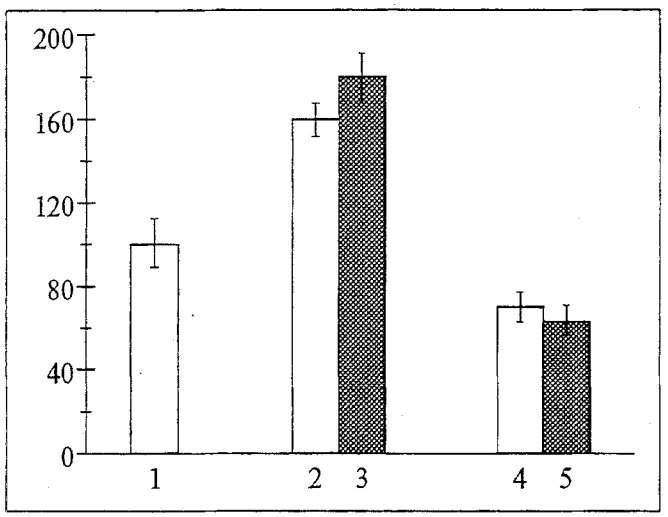

Fig. 1. Immunomodulatory effect of ATP. Mice received intravenously SE only (*1*, control group, 100%), SE + low doses of ATP (*2* and *3*, 1 μg per kg and 4 μg per kg, respectively), and SE = high doses of ATP (*4* and *5*, 35 mg per kg and 140 mg per kg, respectively).

venously or intraperitoneally in a dose of 5×10^6 cells per mouse. The immune response of the organism was checked 5 days later. For this purpose, a spleen cell suspension was incubated with an equal volume of SE 3% suspension (15-20 SE per spleen cell) at 37°C for 15 min, followed by vigorous stirring with a Pasteur pipette, and a mobile preparation was made consisting of a drop of suspension placed on a glass slide, surrounded with a circle of mineral oil and covered with a cover slip. The preparation was examined under the microscope with a phase-contrast device (magnification ×1000), and the number of rosette-forming cells (RFC) per 1000 cells was determined. The mobility of the cells in the preparation made it possible to monitor the true SE adsorption on the lymphocytes. Serotonin-creatinine sulfate and adenosine-5'-triphosphate disodium salt (Reanal, Hungary) were used in the experiments. The pulse nature of the action of the agents was ensured by the rapid expression of the effects of serotonin [8] and ATP, as well as by their administration simultaneously with the antigen. In this way, possible physiological situations were experimentally modelled. The agents were mixed with the SE suspension directly before injection separately for each mouse or 1 hour before injection, in order to permit the formation of serotonin-ATP complexes. The data were statistically processed using Student *t* test. The arithmetic mean values and 95% confidence levels are shown in the figures.

RESULTS

As ATP can produce opposite biological effects depending on its concentration, the assumption has arisen that ATP can not only modulate the immune response, but also produce the opposite effect. The ATP doses were chosen on the basis of the optimal values of serotonin doses for producing an opposite effect [7] and the optimal ATP/serotonin ratios in the produced complexes. It follows from the results that the perception of antigenic information by the organism is changed against the background of a raised ATP level (Fig. 1). ATP, like serotonin, produced dose-dependent opposite effects: a stimulating effect when given in low concentrations and an inhibitory effect in high concentrations. A four-fold increase of doses failed to yield appreciably different results, and therefore the lower doses were subsequently used.

Explaining the mechanisms of exogenous ATP action upon immunogenesis is not a simple matter. First of all, ATP may interact with the corresponding receptors on the lymphocytes and thus produce the immunobiological effects described. The effect of ATP may result from its capacity to inhibit cAMP generation [17], as the latter is known to modulate immunogenesis. Further, as exogenous ATP is extremely rapidly metabolized by exoenzymes, ATP administration is likely to induce (especially in large doses) the formation of adenosine (AD). As in the case of ATP, the cells of immunocompetent organs possess AD-specific receptors [12]. The participation of AD seems to be very likely, the more so that it is known to inhibit antibody production and to play a role in the control of immune functions. The opposite effect of ATP and AD on some processes [4] also favors the assumption regarding the AD-linked

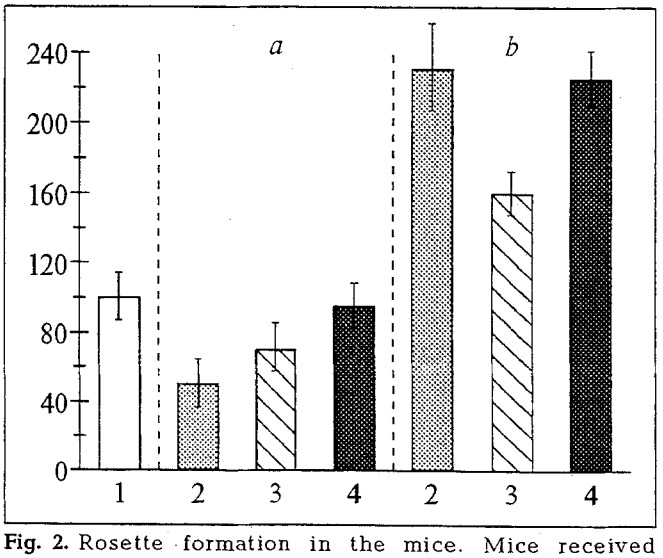

Fig. 2. Rosette formation in the mice. Mice received intravenously SE only (*1*, control group, 100%), SE + serotonin (*2*), SE + ATP (*3*), and SE + serotonin–ATP complex (*4*). The agents were used in high (*a*) or low (*b*) concentrations (serotonin, 50 mg per kg and 1.5 μg per kg: ATP, 35 mg per kg and 1 μg per kg).

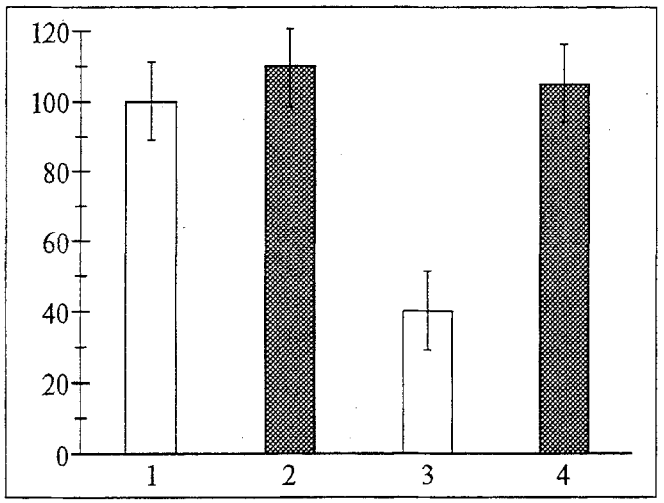

Fig. 3. Immune response to antigen challenged intraperitoneally. Administration of SE only (*1*); of mixture consisting of SE, serotonin (50 mg per kg), and ATP (35 mg per kg) prepared for each animals separately, directly before injection (*2*); SE + serotonin followed by ATP with an interval of 2 seconds (*3*); SE + ATP followed 2 sec later by serotonin (*4*).

realization of some ATP effects. Yet in other situations ATP and AD may produce similar effects, whose direction can be opposite depending on the dose [15]. This is explained by the existence of high- and low-affinity receptors, owing to which each of these substances can regulate several functions in opposite fashion. Perhaps the participation of both adenine nucleotides in the observed effects should not be ruled out.

Due to the analogous effect of serotonin and ATP, one might expect that their simultaneous action should yield a synergistic effect. This was to be ascertained in the next experiment. The compounds were introduced in the form of complexes produced using the optimal doses of unidirectional effects. The corresponding dose for serotonin was 1.5 μg per kg body weight (stimulatory effect) and 50 mg per kg body weight (inhibitory effect); and for ATP 1.0 μg and 35 mg, respectively (the above-mentioned optimal complex-forming ratios were taken into account).

The analysis of the results showed that the combined administration of low doses of modulators (Fig. 2, *b*) resulted in neither additiveness nor synergism of the effects. A stimulation was observed, equal to that attained by the injection of serotonin alone. Hence it follows that the formation of a serotonin-ATP complex *in vivo* is probably necessary for the manifestation of an immunostimulatory effect of each of them. AS for high doses of the agents, complex formation blocked their inhibitory effect (Fig. 2, *a*). This phenomenon could be most simply explained by a shielding or occupation of the structures essential for the realization of each factor's suppressive effect, occurring due to the complex formation. Possibly, it is precisely the cells that are unable to react with the complex, which are responsible for the inhibitory action of both modulators, unlike the cells responsible for the enhancing action, which need the preformation of complexes and/or combined action of serotonin and ATP in the form of two signals. The first type of cells is apparently represented by a subpopulation of serotonin-binding cells of peritoneal exudate which lose this capacity in the presence of ATP, while some serotonin-binding blood leukocytes, that react with serotonin irrespective of ATP presence of absence, may belong to the second cell type.

Before continuing the experiments it was checked whether there is a difference between the effects of preformed complex and of the mixture prepared ex tempore before injection. The effects were the same, and therefore the latter variant was further used.

The grounds for the third experiment were the data regarding the ATP-mediated blocking of serotonin binding, unexplained by competition for the binding sites (as a serotonin excess did not prevent the specific ATP binding to the corresponding structures). A hypothesis arose regarding an unequal combined effect of these substances, depending on the proper sequence of them in the consecutive administration. The methodological peculiarity was in that two syringes were used at the same time. The needles of both syringes were plunged simultaneously, so that all animals underwent just a single injection procedure. However, the contents of the syringes were introduced with a time interval: first a mixture of one of the agents with SE, and after two seconds the second agent (both in high doses). It follows from Fig. 3 that administration of SE + serotonin followed by ATP resulted in the manifestation of an inhibitory action of serotonin on RFC generation. On the other hand, an abrogation of the inhibitory effect could be seen when mice received SE + ATP followed by serotonin. This situation resembled the one where all three components were administered simultaneously. We may therefore assume that ATP-serotonin complex formation may act as one of the mechanisms for the neutralization of too high levels of serotonin, i.e., transforming it into a form inactive regarding the structures which respond to a high amine concentration and realize its immunosuppressive effect.

REFERENCES

1. G. Burnstock, *Circulat. Res.*, **46**, Suppl. 1, 175-182 (1980).
2. S. Chiba, T. Ohhashi, and T. Azuma, *Clin. Exp. Pharmacol. Physiol.*, 7, № 2, 195-198 (1980).
3. M. L. Cohen, R. W. Fuller, and K. S. Wiley, *J. Pharmacol. Exp. Ther.*, **218**, 421-425 (1981)

4. N. J. Cusack, S. M. O. Hourani, and L. A. Welford, *Adenosine and Adenine Nucleotides: Physiology and Pharmacology*, Ed. D. M. Paton, London (1988), pp. 93-100.
5. B. M. Czarnetski, *Behring Inst. Mitt.*, № 68, 82-91 (1981).
6. F. Di Virgillo, V. Bronte, and P. Zanovello, *J. Immunol.*, **143**, № 6, 1955-1960 (1989).
7. L. S. Eliseeva, *Fiziol. Zh. SSSR*, **73**, № 8, 1084-2089 (1987).
8. L. S. Eliseeva, *Byull. Sib. Otd. Akad. Nauk SSSR*, № 5-6, 90-94 (1988).
9. L. S. Eliseeva and L. E. Stefanovich, *Biokhimiya*, **47**, № 5, 810-813 (1982).
10. L. S. Eliseeva, L. E. Stefanovich, and V. S. Popova, *Tsitologiya*, **24**, № 10, 1174-1180 (1984).
11. C. El-Mostassim, N. Bernad, J.-C. Mani, and J. Dornand, *Biochem. Cell Biol.*, **67**, № 9, 495-502 (1989).
12. R. B. Gilbertsen, *Agents and Actions*, **22**, № 1-2, 91-98 (1987).
13. J. L. Gordon, *Biochem. J.*, **233**, № 2, 309-319 (1986).
14. A. A. Hakim, *Cell and Biol.*, **32**, № 2, 141-148 (1986).
15. Y. Katayama and K. Morita, *J. Physiol.*, **408**, 373-390 (1989).
16. S. T. McGarrity, A. H. Stephenson, and R. O. Webster, *J. Immunol.*, **142**, № 6, 1986-1994 (1989).
17. F. Okajima, Y. Tokumitsu, Y. Kondo, and Ui. Michio, *J. Biol. Chem.*, **262**, № 28, 13483-13490 (1987).

The Immunodepressive Effect of Transcerebral Laser radiation

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A new physical agent - low-energy laser radiation (LLI) in the infrared [IR] band - has gained acceptance in modern physical therapy due to its various, for example, immunomodulative, effects [5,6]. Such investigations provide a basis for the use of laser in the correction of hormonal or immune disorders and offer wide opportunities for creating new therapeutic methods. It is now proven that the state of the endocrine and immune systems can be changed under the local influence of physical factors on the endocrine glands [1,7].

The aim of the present investigation was to study the effect of IR LLI on the immune response and on the state of the hypothalamic-hypophyseal-adrenal and endogenous opioid systems for transcerebral radiation of rabbits.

Russian Scientific Center of Rehabilitation and Physical Therapy (Presented by A. D. Ado, Member of the Russian Academy of Medical Sciences)

MATERIALS AND METHODS

The experiments were carried out on male rabbits weighing 2.8-3.2 kg. Ten times a day the animals were exposed to laser radiation from a contact radiator of LITA apparatus (radiation wavelength 0.89 μ, mean pulse power 25 W). The radiation dose received by the animals during one exposure was 0.08 J in the first group and 2.1 J in the second. The animals of the third group were exposed to continuous irradiation from AMLT-01 apparatus without a magnetic attachment (total output power of 7 mW). The radiation dose was 2.1 J per day. The control

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