Pathways of the Realization of Immunomodulating Effects of Neurotransmitters

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UDC 612.017.06:612.018.2

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 115, № 4, pp. 401–404, April, 1993
Original article submitted December 8, 1992

Key Words: *serotonin; ATP; interleukin-2; hypothalamo-hypophyseal complex; vagus nerve*

Serotonin [13,15] in high doses suppresses [9] and in low doses stimulates [12] the response to an antigen. The negative control is realized via the hypothalamo-hypophyseal-adrenal complex [10], while the positive control includes other mechanisms [11]. The study of the immunomodulatory capacities of ATP and interleukin-2 (IL-2) revealed (our data, unpublished) a similarity of the effects of serotonin, ATP, and IL-2 with regard to the same immunological phenomenon, and a relationship between serotonin and ATP and IL-2 in immunomodulation. These facts prompted the search for a similarity in the mechanisms underlying the effects of these substances.

The role of ATP as a neurotransmitter of the central and peripheral nervous system [5], and the functional relationship of ATP with the hypophyseal-adrenal complex provide evidence of the possible existence of common regulatory pathways for serotonin and ATP. As for IL-2, the following data point to a probable involvement of the neuroendocrine system in its immunomodulatory functions and permit IL-2 to be considered as an immune system-nervous system transmitter: 1) the expression of IL-2 receptors in the brain; 2) the effect of IL-2 on the function of the pituitary cells [4] and on nerve growth factor activity [8], as well as on the level of several neurotransmitters; 3) the effect of IL-2 on adrenocorticotropic hormone release in the pituitary [3] and opposite effects on the electrical activity of different hypothalamic nuclei [2].

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First it was necessary to determine the pathway of serotoninergic immunostimulation. The autonomic nervous system attracted special interest as, on the one hand, it participates in the regulation of immunogenesis [1] and, on the other, serotonin is its transmitter. The vagus nerve was chosen in view of the following: 1) it is in close morphofunctional contact with the structures containing bodies and terminals of the serotoninergic nerves (medulla oblongata, hippocampus, hypothalamus); 2) it innervates the cardiovascular system and the immunocompetent organs; 3) it contains serotoninergic fibers including afferents with a high threshold of sensitivity [16] that change their polarity following serotonin treatment; 4) it induces a rise of the tonus in response to an antigenic stimulus; 5) it participates in certain effects of low-dose serotonin upon nonimmune processes. Moreover, there is evidence of the possible participation of the vagus nerve also in the stimulatory effects of ATP, which, as mentioned earlier, acts similarly to serotonin. For instance, the representation of ATP in the vagus [7], the participation of the vagus in certain electrophysiological effects of ATP, and the presence of ATP receptors in the nerve endings of the blood vessels have been demonstrated.

The goal of this study was to determine the pathways of the immunomodulatory effects of the transmitters.

MATERIALS AND METHODS

The experiments were carried out on 442 mice of the CBA strain aged 3-4.5 months (each group consisted of at least 7 animals). Sheep erythrocytes (SE) were chosen as the antigen. SE were injected intraperitoneally (premixed with the substances examined -

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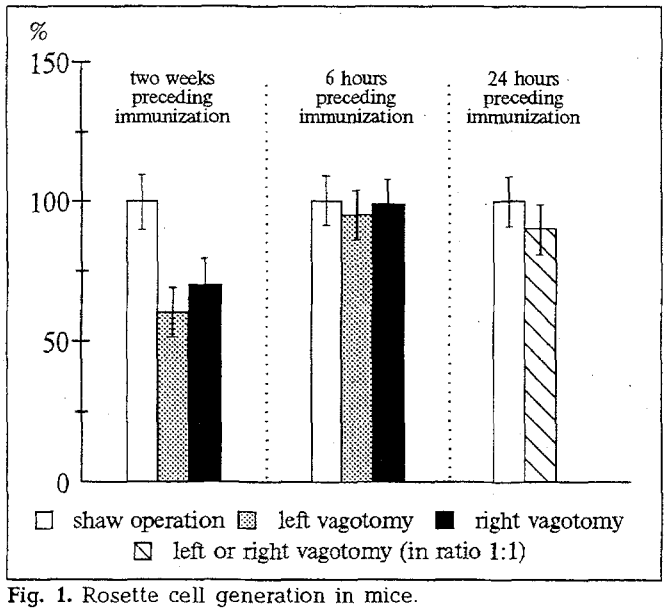

Fig. 1. Rosette cell generation in mice.

details see below) in a dose of 5×10^6 per mouse. The immune response was assessed on the fifth day. For this purpose a spleen cell suspension was incubated for 15 minutes with an equal volume of 3% SE suspension at 37°C, after which a mobile preparation was made (a drop of suspension to be examined was placed on a glass slide, surrounded with a ring of mineral oil, and covered with a glass cover slip). The number of rosette-forming cells (RFC) was counted under the microscope using a phase-contrast device (×1000). The operations were carried out under nembutal anesthesia (50 mg per kg body weight) one day before the immunization (except in cases specified elsewhere). Mechanical cross-section of the hypophyseal peduncle (HPC) was performed transauricularly [12] under visual control. Unilateral vagotomy (UVT) was performed under the microscope. Control animals underwent a sham operation (SO). Serotonin-creatinine sulfate (Reanal, Hungary) in doses of 1.5 μg and 50 mg per kg body weight, adenosine-5'-triphosphate disodium salt (ATP) (Reanal, Hungary) in doses of 1.0 μg and 35 mg per kg body weight, and recombinant human interleukin-2 (Institute of Organic Synthesis, Latvian Academy of Sciences) in doses of 2×10^{-3} IU and 200 IU per mouse were used. These doses, according to previous data, exhibit pronounced opposite effects on rosette formation (RF): low doses stimulate it and high doses inhibit it. The statistical analysis of the results was performed using Student's *t* test. The figures show the arithmetic mean values and confidence levels.

RESULTS

In the first experiment the mice were divided into groups with left and right vagotomy (LVT, RVT) and were immunized at different times after the operation. This was due to the intolerance of bilateral section, the probability of functional asymmetry, the alteration of the peripheral portion of the dissected nerve, and the state of the denervated immunocompetent organ depending on the time interval after the operation. It was found (Fig. 1) that the changes occurring two weeks post-operation led to the inhibition of RF in the spleen, while operations performed 6 to 24 hours before immunization had no effect on the number of RFC. This was equally true for LVT and RVT mice. Therefore, the subsequent operations were performed one day prior to immunization.

The second experiment showed the following: 1) the SO mice exhibited, as before [12], dose-dependent opposite effects, while in the HPC mice the inhibitory effect of high-dose serotonin was abrogated, but the stimulating effect of low-dose serotonin was preserved (Fig. 2, *1a*, *1b*, *2a*, *2b*); 2) the inhibitory effect was retained and the stimulating effect abrogated in theUVT mice (Fig. 2, *3a*, *3b*); 3) double-operated (UVT+HPC) animals exhibited a blocking of both effects (Fig. 2, *4a*, *4b*); 4) an operation per se produced no effect on the immune reaction. In a special experiment the abrogation of stimulation was shown to occur in both the LVT and the RVT group, and therefore the mice were not further divided according to this feature. Thus, our assumption proved to be correct and it became obvious that: 1) the vagus nerve is a hitherto unknown channel transmit-

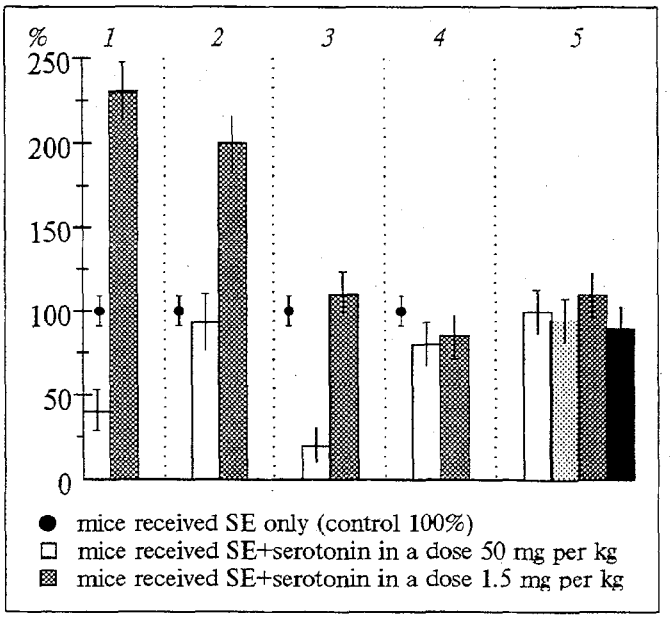

Fig. 2. Experiment to establish serotonin immunostimulatory pathway. *1*) sham-operated animals; *2*) hypophyseal peduncle cross-section; *3*) unilateral vagotomy; *4*) both operations; *5*) comparison of RFC number in control group (sham operation) and groups of operated animals.

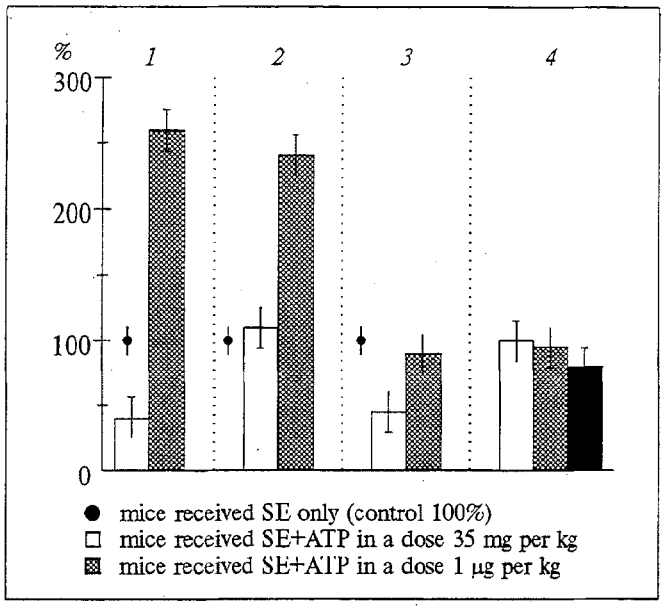

Fig. 3. Experiment to establish ATP immunomodulating pathways. *1*) sham-operated animals; *2*) hypophyseal peduncle cross-section; *3*) unilateral vagotomy; *4*) comparison of RFC number in control group (sham operation) and groups of operated animals.

ting the immunostimulatory effect of serotonin; 2) the opposite effects of serotonin are mediated via autonomous routes. Now it was to be established whether, besides the analogy in the serotonin and ATP effects, there is also a similarity in the pathways of realization of the effects. Analysis of the results showed that: 1) in agreement with earlier results (unpublished), SO mice exhibited dose-dependent oppositely directed effects of ATP (Fig. 3, *1a*, *1b*); 2) the inhibitory effect of high-dose ATP was abrogated and the stimulating effect of low-dose ATP was retained in HPC mice (Fig. 3, *2a*, *2b*); 3) UVT mice preserved the inhibitory effect but not the stimulating effect (Fig. 3, *3a*, *3b*); 4) an operation per se produced no effect on the immune reaction (Fig. 3, *4*). Thus, the realization of both the serotonin and ATP immunomodulatory effects implies the recruitment of the same systems of physiological regulation. As for the abrogation of stimulation due to section of one of the vagus nerves, because the triggering of the neuronal systems depends on the magnitude of the information flux, it is possible that in UVT mice the number of sensitive fibers falls below the threshold of signal perception nd transmission.

According to our data (unpublished), the combined introduction of serotonin and ATP produce an additive or synergic effect. This suggested that the expression of the immunostimulatory effect of each of these two agents requires their combined action (probably in the form of complexes). In this case it only remained to assume that the introduction of one of the agents into the organism should induce the release of the other. And since UVT abrogated the stimulatory effects of both, it became obvious that the ATP-serotonin interaction in immunomodulation is not restricted to the periphery, but requires the participation of the nervous system. If this is indeed the case, then UVT should not have abrogated the stimulation induced by the combined administration of serotonin and ATP. This idea was experimentally confirmed when each of the agents separately stimulated RF (Fig. 4, *a*, *1-3*), UVT abrogated the stimulation in both cases (Fig. 4, *b*, *2*, *3*) but the effect of UVT was overcome by a simultaneous injection of ATP and serotonin (Fig. 4, *b*, *4*). UVT also failed to abrogate the stimulation when animals received the agents in the following schemes (Fig. 4, *b*, *5*, *6*): 1) SE+serotonin followed by ATP two seconds later; 2) SE+ATP followed by serotonin in the same time interval (two syringes were used in this experiment; the needles were plunged simultaneously). It follows from these results that: 1) serotonin and ATP given in low doses are of equal importance in their interaction in the course of immunomodulation; 2) the period required for their interaction is very short; 3) in response to serotonin or ATP administration a release of the needed free partner of this pair is ensured by the vagus nerve.

It remained to check the assumption regarding the similarity in the pathways of realization of the IL-2 effect on RF to those of serotonin and ATP. It follows from identical trials differing only in the SE doses (Fig. 5, *a*, *b*) that: 1) SO mice (Fig. 5, 1*a*, *1b*, *1c*) exhibited, as in earlier experiments (unpublished), opposite effects, presumably due to high-

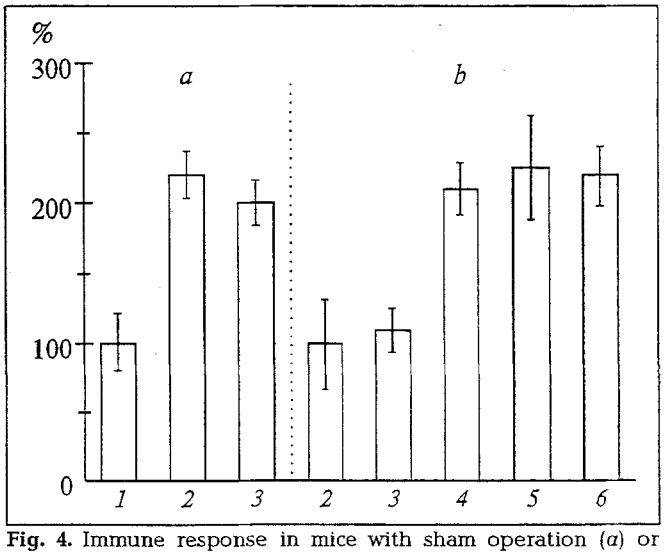

Fig. 4. Immune response in mice with sham operation (*a*) or unilateral vagotomy (*b*). Animals received SE only (1: control, 100%), SE plus serotonin, 1.5 μg per kg (2), SE plus ATP, 1.0 μg per kg (3), SE plus serotonin plus ATP (4), SE plus serotonin followed by ATP after 2 sec (5), or SE plus ATP followed by serotonin after 2 sec (6).

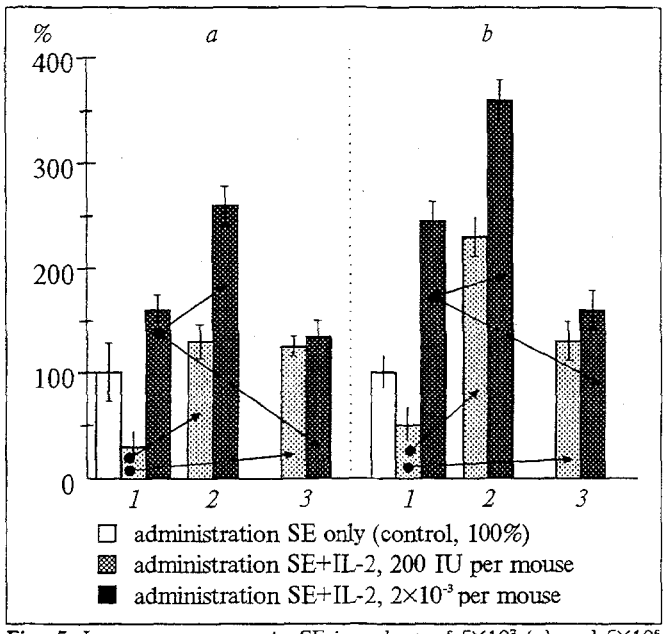

Fig. 5. Immune response to SE in a dose of 5×10^7 (*a*) and 5×10^6 (*b*) in mice after sham operation (*1*), hypophyseal peduncle cross-section (*2*), and unilateral vagotomy (*3*).

and low-affinity IL-2 receptors (high doses of IL-2 induced suppression, and low doses stimulation; 2) HPC not only abolished the suppressive effect but changes it to the opposite effect (Fig. 5, *2b*) and increased the stimulating effect (Fig. 5, *2c*); 3) UVT transformed the suppressive effect in a way similar to HPC (Fig. 5, *3b*), whereas the stimulating effect was reliably diminished (Fig. 5, *3c*), yet was not abolished as in the experiments with serotonin and ATP injection. A comparison of the results in the SO and UVT groups shows that the effects are more pronounced against the background of a weaker antigenic stimulus, this trend being especially noticeable in the case of a stimulatory effect. The switch of the effect to the opposite one for the same dose of modulator was already a known phenomenon to us, as serotonin given in an RF-inhibiting dose had induced a dramatic rise of this index in mice with adrenalectomy-associated derangement of the regulatory pathway. The resemblance between these situations was moreover reinforced by an increase of the stimulating effect in these mice [12]. Returning to the last experiment, it is worth mentioning that the hypothalamo-hypophyseal complex and vagus nerve participate in the realization of both (opposite) effects of IL-2. This is consistent with: a) the ability of neurons to respond to vagus stimulation with either a rise or a fall of activity; b) the morphofunctional relationship of this nerve to the hypothalamus, which contains areas of formation of reactions of opposite directions (anterior hypothalamus) and of unidirectional reactions (posterior hypothalamus). The last fact leads to the assumption that the effects of IL-2 are connected with the anterior hypothalamus, and those of serotonin and ATP with the posterior hypothalamus. The restructuring mechanisms of IL-2 action in the derangement of the regulatory pathways remain unclear. The complex nature of the processes and the need for the participation of reciprocal regulatory systems ensuring transmitter balance, altered by our interventions, can be seen here. The explanation of the switchings occurring is further complicated by the marked differences in the doses of agents inducing opposite effects, implying the participation of high- and low-affinity receptors of both the immune and the neuroendocrine system in the events discussed.

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