RESTORATION OF THE STRUCTURE OF SPLENOCYTE MEMBRANES OF THYMECTOMIZED ANIMALS BY TACTIVIN AND ITS SUBFRACTIONS

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KEY WORDS: tactivin; splenocyte membranes; immunodeficiency

Immunoregulatory peptides of the thymus have attracted the attention of research workers in various specialties because of their important role in the realization not only of immune processes [6], but also in several biochemical reactions [4]. However, the mechanisms of action of these peptides are unknown.

The aim of this investigation was to study the effect of tactivin and its subfractions on the "microviscosity" of the splenocyte membranes of intact and thymectomized mice.

EXPERIMENTAL METHOD

Male C57BL/6 mice weighing 16-18 g were used. The mice were thymectomized to create a model of secondary immunodeficiency and their splenocytes were used 7-10 days after the operation.

Tactivin and its subfractions were obtained by the method described in detail in [2]. Concanavalin A (con A, from "Calbiochem") was used as activator of T lymphocytes.

The "microviscosity" of splenocyte membranes of intact and thymectomized mice was determined by the spin probes method. As spin probe for this task we used 5-doxylstearic acid ("Sigma") in a final concentration of $5 \cdot 10^{-5}$ M. The cell concentration in the ampul of the EPR-spectrometer was 10^7 in 100 μl of medium 199. EPR spectra were recorded on an E-4 radiospectrometer ("Varian," USA) at 37°C under the following conditions: power of shf radiation 10 mW, amplitude of hf modulation 1.0 G, scanning speed of magnetic field 15-25 G/min, with time constant 1.0 sec. The parameter of orderliness (S), characterizing the state of "microviscosity" of the membranes, was calculated by the equation in Fig. 1.

EXPERIMENTAL RESULTS

It was shown previously that the molecular heterogeneity of tactivin is responsible for the functional diversity of the molecules present in this preparation [2]. In this connection it was considered important to study the effect not only of tactivin), but also of its subfractions, on splenocyte membranes. All subfractions were studied in four immunological tests (Table 1), selected so as to study the action of peptides on T lymphocytes in different stages of development.

Induction of Thy-1^+ antigen on the surface of a bone marrow cell is one of the earliest stages of T-cell differentiation [7]. The fact that the total preparation is most active in this test will be noted (Table 1). Consequently, this stage of T-cell differentiation depends on interaction between several immunoregulatory peptides (a complex).

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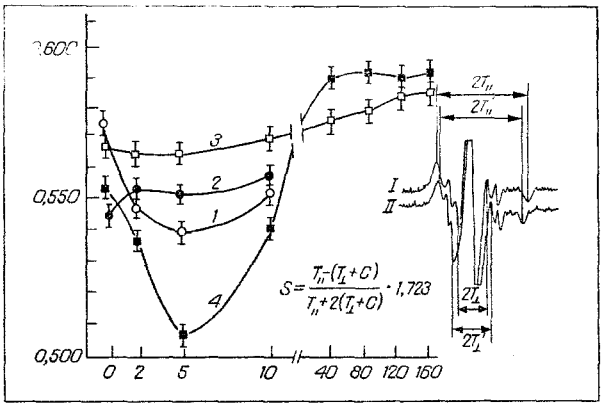

Fig. 1. Dependence of change in parameter of orderliness (S) of 5-doxylstearic acid in splenocyte membranes of intact (1) and thymectomized (2) mice on time of action of con A (5 μg/ml): of intact (3) and thymectomized (4) mice on time of action of tactivin (5 μg/ml). Values of S of 5-doxylstearic acid in splenocyte membranes of intact (S_i = 0.572 ± 0.008) and thymectomized (S_{te} = 0.550 ± 0.007) mice. EPR spectra of 5-doxylstearic acid in mouse splenocyte membranes before (I) and after (II) action of con A. Formula for calculating parameter of orderliness; in which T_{II} denotes half the distance between the external extrema of the spectrum and T_I half the distance between the internal extrema of the spectrum: C = [1.463 − 0.053(T_{II} − T_I)], G.

Autologous rosettes reveal a less mature class of lymphocytes. At this stage one tactivin subfraction, namely F.1, is sufficient at this stage, and the amount of it required to achieve the immunological effect is two orders of magnitude less. However, in the test of restoration of sensitivity of the splenocytes of thymectomized mice to the action of azathioprine, fraction B.1 was the most active. Meanwhile, in the Thy-I$^+$-antigen induction test, it was inactive, but in the test of restoration of the number of autologous rosettes, it exhibited low activity. The greatest increase in the titer of serum thymic activity (STA) was observed under the influence of the F.1 fraction. These data indicate that each step of differentiation and of formation and maturation of T-lymphocytes takes place under the action of a definite immunoregulatory peptide or complex of thymic substances, as in the case with induction of Thy-I$^+$-antigen. These facts provided the basis for testing the effect of tactivin and its subfractions, differing in physicochemical parameters and biological activity, on splenocyte membranes of intact and thymectomized animals.

In this investigation we used the spin probes method, for it yields information not only about the orientation and mobility of the fatty-acid chains of phospholipids in the membrane, but also about fine structural changes taking place in the microenvironment of the probe under the influence of various biologically active substances [10].

The first experiments had the aim of determining the "microviscosity" of plasma membranes of splenic lymphocytes of intact and thymectomized mice. As the data in Fig. 1 show, the "microviscosity" of splenocyte membranes of thymectomized animals had lower values of the parameter of orderliness (S_{te}) than the control (S_i). This probably indicates that thymectomy leads to structural changes in plasma membranes of lymphocytes.

Investigations have shown that in the early stages of interaction of the mitogen con A with the plasma membrane of lymphoid cells changes take place in the EPR spectrum that indicate reduction of orderliness of the phospholipid bilayer of the cell membrane [9, 11]. In other words, one of the first stages of the normal response of the lymphocyte to the mitogen must be changes in the phospholipid bilayer of the plasma membrane.

The question can rightly be asked, will the same structural changes be observed in membranes of splenic lymphocytes of thymectomized mice under the influence of con A? The results of an experiment are given in Fig. 1. They show that disturbances caused by thymectomy in the plasma membrane have the result that the specific T-cell activator does not cause structural changes in splenocyte membranes of thymectomized mice (curve 2), whereas the value of the parameter S of the spin probe falls in the early stages of action of the activator in membranes of splenic lymphocytes of intact animals (curve 1). Initiation of structural changes in splenocyte membranes of intact mice and the absence of any such changes in the case of thymectomized

TABLE 1. Molecular Masses and Activity in Biological Tests of Tactivin and Its Subfractions

Substance	Molecular mass, kD	Biological tests				
		induction of Thy-1$^+$-antigen of bone marrow cells of TE-mice*	restoration of sensitivity of spontaneous E-RFC of spleen of TE-mice to azathioprine*	restoration of number of autologous RFC in spleen of TE-mine*	log of titer of STA**	STA, conventional units
Tactivin	1—6	0,06	0,4	1,0	8	++
B.1	4,5±1,5	n.a.	0,02	2,5	4	+
C.1	4,5±1,5	30,0	0,02	0,05	2	+
C.3	1,5±0,1	n.a.	0,05	0,04	4	+
D.1	4,5±6,0	n.a.	n.a.	0,01	8	++
E.3	1,5±0,1	n.a.	n.a.	0,03	4	+
F.1	3,0—6,0	n.a.	n.a.	0,03	16	+++

Legend. *) minimal quantity of preparation (in μg) required to obtain 50% effect, **) 5 μg of preparation was injected into each TE-mouse and STA was determined 20 h after injection by method described in [8]. n.a.) substance had no biological activity in this test.

animals under the influence of con A may evidently be due to the presence of specific receptors on the surface of the splenocyte membranes of intact mice and their absence or defectiveness on cell membranes of thymectomized animals.

Tactivin is known to lead to differentiation of T-cells and to the appearance of specific receptors on the membrane surface [1, 5]. Meanwhile tactivin has no effect on mature lymphocytes. The question arises, will tactivin in the absence of con A cause changes in the structural organization of lymphocyte membranes in the mouse spleen. To answer this question, changes in the parameter of orderliness of the spin probe in splenocyte membranes of intact and thymectomized animals in the presence of tactivin were studied. The experimental results are given in Fig. 1. Clearly during the first 5-6 min of action of tactivin the value of S of the spin probe in splenic lymphocyte membranes of thymectomized mice decreased (curve 4). These changes in the structural organization of the plasma membrane may be connected with the fact that under the influence of tactivin, expression of receptors takes place on the splenocyte cell membrane. It is important to emphasize that tactivin caused no changes in the parameter of orderliness of the spin probe in splenocyte membranes of intact mice (curve 3). Moreover, with an increase in the dose of tactivin and in the duration of incubation, no changes were observed. It must be pointed out that increasing the incubation time of the splenocytes of thymectomized animals with tactivin led to significant changes in the structural organization of the membranes. For instance, after incubation with tactivin for 40 min, the value of the parameter of orderliness of the spin probe in splenocyte membranes of thymectomized mice rose sharply, to reach or even surpass somewhat its value in intact animals (Fig. 1, curve 4). With a longer incubation time (160 min) of the cells with tactivin, the value of the parameter remained virtually unchanged and did not differ significantly from the control tests (Fig. 1, curves 3 and 4).

As was pointed out above, the action of con A on intact splenocytes leads to structural changes in the membranes, but no such changes take place in the cells of thymectomized animals. The question arises, will structural changes be observed under the influence of con A in splenocyte membranes of thymectomized mice after their incubation with tactivin? The experimental results are given in Fig. 2. The graphs show that con A led to a decrease in values of the parameter S of the spin probe in splenocyte membranes of thymectomized mice preincubated with tactivin for 80 min (curve 3). The same changes under the influence of the mitogen were also observed in cell membranes of intact animals (curve 1). Meanwhile, in lymphocyte membranes not incubated with tactivin, no such changes were observed (curve 2).

As already pointed out, tactivin is an immunoregulatory preparation consisting of a mixture of thymus peptides. The question remains unanswered: is the whole complex of peptides constituting this preparation necessary for structural changes at the membrane level, or are only certain of its subfractions sufficient. For these experiments subfractions of tactivin differing both in molecular mass and in immunobiological properties were chosen: C.3, D.1, and F.1 (Table 1). As Fig. 3 shows, a single injection of tactivin or of its subfractions into thymectomized animals led to a fall in the value of the parameter of orderliness of the spin probe in splenocyte membranes of thymectomized mice (S_T, $S_{C.3}$, $S_{D.1}$, $S_{F.1}$). It can be tentatively suggested that this increase in "microviscosity" of the membranes is connected with expression of receptors on the cell surface under the influence of tactivin and its subfractions. It was shown above that splenocytes of thymectomized animals preincubated in vitro with tactivin acquired the ability to respond to con A (see Fig. 2, curve 3). Is the same effect preserved after injections of tactivin or its subfractions into thymectomized mice. As will be clear from Fig. 3, a decrease in the parameter of orderliness of the spin probe was observed in splenic lymphocyte membranes from thymectomized animals receiving tactivin, evidence of structural changes

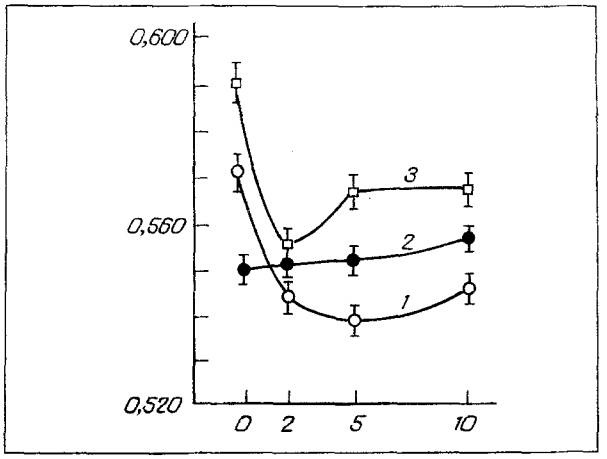

Fig. 2. Dependence of change in parameter of orderliness (S) of 5-doxylstearic acid in splenocyte membranes of intact (1) and thymectomized mice before (2) and after (3) incubation with tactivin (5 μg/ml) on duration of action of con A (5 μg/ml).

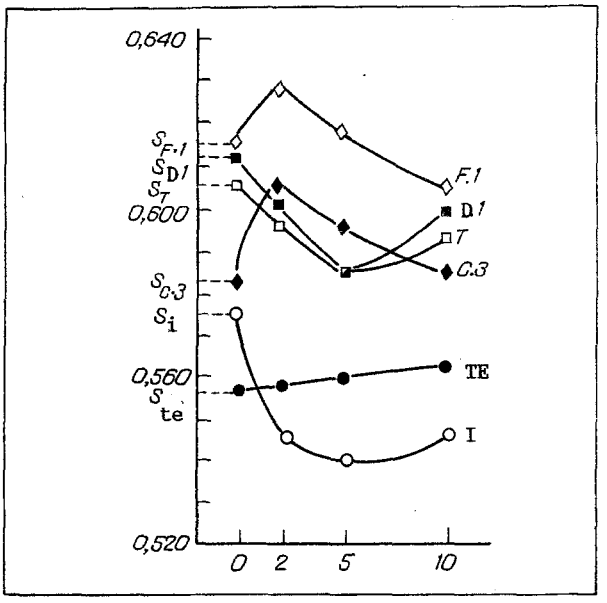

Fig. 3. Dependence of change in parameter of orderliness (S) on 5-doxylstearic acid in splenocyte membranes of intact (I) and thymectomized (TE) mice before and after injection of tactivin (T), and its subfractIons D.1, C.3, and F.1 on duration of action of con A (5 μg/ml). Values of S of 5-doxylstearic acid in splenocyte membranes of intact (S_i = 0.577 ± 0.004) and thymectomized mice before (S_{te} = 0.557 ± 0.003) and after injection of tactivin (S_T = 0.607 ± 0.003) and subfractions C.3 ($S_{C.3}$ = 0.583 ± 0.004), D.1 ($S_{D.1}$ = 0.612 ± 0.005) and F.1 ($S_{F.1}$ = 0.615 ± 0.003).

having taken place in the membrane under the influence of con A (curve T). Subfraction D.1 restored the response to the mitogen in the same way as the whole preparation (Fig. 3, curve D.1). The fact will be noted that in the first 2-3 min of action of con A the "microviscosity" of the membranes of thymectomized mice receiving subfractions C.3 and F.1 increased, after which it fell to regain its original level (curves C.3 and F.1). Thus tactivin and subfraction D.1 restore the structure of the membranes,

and under these circumstances an adequate response of the splenocyte is observed to con A, whereas subfractions C.3 and F.1 act in the opposite direction. During the first minutes the "microviscosity" is not reduced, as is usually the case, but increased.

The investigation showed that thymectomy leads to a disturbance of the structure of mouse splenocyte membranes. Under the influence of tactivin the "microviscosity" of the membranes is restored. Moreover, subfractions of tactivin possess the opposite kind of action, confirming the hypothesis of functional heterogeneity of tactivin, postulated previously [2].

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EFFECT OF DEOXYCORTICOSTERONE ON 5′-NUCLEOTIDASE AND ADENOSINE DEAMINASE ACTIVITY IN THE RAT HYPOTHALAMUS AND HIPPOCAMPUS

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Most hormones, including corticosteroids, have a considerable influence on the state of brain function, and modify metabolism in its functionally different structures. Corticosteroids affect synaptic transmission, by modifying reception and release, biosynthesis, and conversion of mediators and modulators actually in the neuron or in its synaptic endings. The study of the character of changes in metabolism of synaptic mediators is particularly important in the limbic system of the brain, whose individual structures are responsible, on the one hand, for antagonistic control of biosynthesis and secretion of hormones of the hypophyseoadrenal system, and on the other hand, for the realization of hormonal influences on functions of the CNS. An important place in the mechanism of these neuroendocrine relations is occupied by biogenic amines: catecholamines, serotonin, etc. [3]. Meanwhile the functional role of other possible chemical mediators of neurotransmission, such as adenosine, in neuroendocrine responses of limbic structures has not been adequately studied.

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