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INTERACTION BETWEEN MITOGENIC LECTINS AND PLASMA MEMBRANES OF LYMPHOCYTES

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Mitogenic signals from concanavalin A and phytohemagglutinin were shown to undergo summation in time under conditions when each mitogen separately, if the duration of contact with lymphocytes during the experiment was the same (14 or 20 h), did not induce mitogenesis. The results are discussed from the standpoint of cell–cell interaction between lymphocytes. It is suggested that the ability of these signals to undergo time summation lies at the basis of the nonspecific mechanism of protection against tolerance.

KEY WORDS: mitogens; lymphocytes; plasma membrane.

In order to understand the molecular mechanism of activation of lymphocytes and induction of tolerance, information is needed on the state of the receptor molecules on the surface of the plasma membrane of lymphocytes. A convenient model of interaction between lymphocytes and antigens is provided by the process of nonspecific activation by certain mitogens, mainly lectins of plant origin and of different carbohydrate specificity, which induce RNA, DNA, and protein synthesis and also blast transformation [6].

Because of the nonspecific nature of mitogenic stimuli, it might be expected that mitogens would be "mutually interchangeable," or in other words that signals from different receptors could undergo time summation.

In the investigation described below interaction between two mitogens — concanavalin A (conA) and phytohemagglutinin (PHA) — with lymphocytes from rat lymph nodes was studied.

EXPERIMENTAL METHOD

Purified suspensions of mesenteric lymph node cells from Wistar rats were used. The cells ($2 \cdot 10^6$) were incubated at 37°C in 2 ml of medium No. 199 to which 5% inactivated human blood group AB serum and antibiotics were added. Mitogenesis was induced by addition of conA (from Boehringer Mannheim) or PHA (from Difco) to the culture. The carbohydrate specificity of lectins has been studied sufficiently well, so that it was possible to remove cells binding conA selectively from the surface by washing in medium containing hapten carbohydrates: α-methylmannopyranoside (α-MM) or α-methylglucopyranoside (α-GP) (from Calbiochem). The cells were incubated with the haptens for 20 min at 37°C 14 or 20 h after addition of mitogenic concentrations of conA, after which they were washed three times with culture medium containing the hapten carbohydrate, PHA or conA was added, and culturing continued. Next, 48 h after the beginning of cultivation, 0.5 μCi [^{3}H]thymidine (specific activity 20.6 Ci/mmole) was added to the cultures. After 72 h the cells were treated with cold 5% TCA and the insoluble fraction (DNA) was harvested on membrane filters. The quantity of [^{3}H]thymidine incorporated into DNA was determined by means of the ZhS-106 scintillator on a Mark II counter. The quantitative data on incorporation of [^{3}H]thymidine into lymphocyte DNA consisted of the arithmetic means of measurements in the three to five parallel cultures, expressed as cpm/$2 \cdot 10^6$ cells.

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TABLE 1. Effect of conA and PHA on Incorporation of [^{3}H]-Thymidine into DNA of Lymphocytes from Whose Surface conA Was Removed after Preincubation for 20 h at 37°C in Culture

Substance added to culture at beginning of incubation	Treatment of cells after preincubation with lectins	Agents added after treatment with carbohydrate	Incorporation of [^{3}H]-thymidine, cpm/2·10^6 cells
ConA:	α-GP:		
5 μg	0,4 M	PBS	4 995±450
5 »	0,4 »	ConA 5 μg	12 850±2 150
5 »	0,4 »	PHA 5 μl	8 965±1 870
5 »	PBS	PBS	13 870±1 935
	α-GP		
PBS	0,4 M	ConA 5 μg	2 950±570
PBS	0,4 »	PHA 5 μl	2 730±635
PHA	0,4 »	PBS	8 125±995
PBS	PBS	PBS	1 730±285
Con-A:	α-MM:		
5 μg	0,4 M	—	3 170±830
5 »	Glucose 0.4 M	—	11 385±1 135
5 »	Sucrose 0.3 M	—	13 125±1 650

Legend. Solutions of hapten carbohydrates were made up in phosphate-buffered physiological saline (PBS) immediately before the experiment and sterilized by passage through membrane filters by means of a syring and Millipore attachment.

The significance of differences between the quantity of thymidine incorporated into DNA when the cell surface was acted upon in different ways was calculated by Student's t-test.

EXPERIMENTAL RESULTS

Analysis of the dependence of the degree of mitogenesis on the concentration of conA and PHA showed significant differences between the state and interaction of the conA and PHA receptors in the plasma membrane of the lymphocytes. Effects of conA (induction of mitogenesis) were characterized by a pointed maximum, whereas the intensity of DNA synthesis induced by PHA remained unchanged over a wide range of concentrations. Binding of an additional number of receptors, recorded on the addition of supraoptimal concentrations of conA, led to inhibition of mitogenesis, whereas an increase in the number of PHA receptors immobilized by the lectins did not affect mitogenesis [2].

The composition of the carbohydrate residues of the glycoprotein receptors participating in reception of mitogenic stimuli from conA and PHA also differed, for treatment of the cells with α-MM or α-GP after preincubation for 14 or 20 h led to marked inhibition of mitogenesis induced by conA but did not affect mitogenesis induced by PHA. Inhibition was specific, for sucrose in general does not affect mitogenesis, and glucose, a weak inhibitor, was ineffective even in a concentration of 0.4 M (Table 1).

On treatment with hapten carbohydrates practically all the bound conA was removed from the surface [4].

To study the possibility of summation of signals from different receptors in time the lymphocytes were incubated initially with conA, after which the conA was removed from the surface of the cells by hapten carbohydrates and cultivation continued in the presence of freshly added conA or of PHA.

As the results in Table 1 and Fig. 1 show, replacement of the removed conA by PHA led to induction of DNA synthesis. Each of the mitogens separately, given the same duration of contact with the lymphocytes, did not induce mitogenesis. Addition of PHA after removal of conA at the 14th or 20th hour of incubation led to induction of DNA synthesis by a much lesser degree than after the readdition of conA. It is important to note that the procedure of washing to remove conA did not itself affect the lymphocytes, for on readdition of conA the same degree of incorporation of thymidine into DNA was attained as in cultures of lymphocytes stimulated with conA which had not been treated with hapten carbohydrates.

The lesser degree of incorporation of thymidine into DNA on replacement of the conA by PHA is evidence that not all cells, if activated beforehand by conA molecules, can bind with

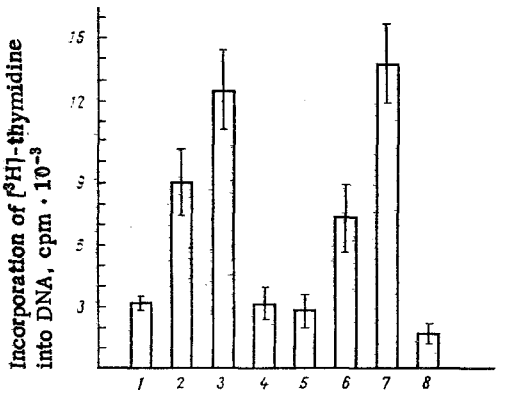

Fig. 1. Effect of conA and PHA on incorporation of [^{3}H]thymidine into DNA of lymphocytes preincubated with conA for 14 h at 37°C in culture. 1) ConA removed from surface of lymphocytes by washing in 0.4 M α-GP; 2 and 3) 5 µl PHA and 5 µg conA respectively was added to lymphocytes washed to remove conA; 4 and 5) 5 µl PHA and 5 µg conA respectively added to lymphocytes incubated in absence of mitogen for 14 h; 6 and 7) stimulation of lymphocytes by PHA and conA respectively; 8) spontaneous transformation of lymphocyte (in absence of mitogen).

and be activated by conA molecules. The population of PHA-reactive cells in rat lymph nodes is evidently smaller than the population of conA-reactive cells. The heterogeneity of the population of lymph node cells in the processes of transformation induced by the mitogen is also revealed by a parameter such as the "minimal time required for activation by mitogens." As the results show, removal of conA after preincubation for 14 or 20 h with conA did not completely abolish incorporation of thymidine, and DNA synthesis in such cultures took place at a significantly higher level than that observed during spontaneous blast transformation of the lymphocytes (in the absence of mitogens).

According to the available data [1], the process of blast transformation of rat lymph node cells induced by conA is a process of the cell–cell type, like reactions in a mixed culture of lymphocytes. With this indirect mechanism of activation it is suggested that conA binds with the surface of the "stimulating" cell and changes its surface so that it is recognized as "foreign" by the surface of the "responding" cell, which is to undergo transformation.

The discovery of the "mutual interchangeability" of the receptors is evidence that there is no strictly localized region of "recognition" or "modification" on the surface of the responding and stimulating cells.

The functional significance of this phenomenon of signal summation from different receptors may be cumulation of signals under conditions when, because of the lateral redistribution of antigen-specific receptors, they disappear from the cell surface [7]. Since, in place of the lost receptors, new ones capable of repeated interaction with the same antigen are synthesized [3, 5], the total time required for activation of the lymphocytes is not increased.

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EFFECT OF ESTRADIOL ON PROLACTIN SECRETION BY ADENOHYPOPHYSEAL CELLS OF INTACT AND OVARIECTOMIZED RATS IN PRIMARY MONOLAYER CULTURES

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Estradiol directly stimulated prolactin secretion by adenohypophyseal cells of intact rats in monolayer culture. Complex relations between estradiol and certain other regulators of the function of the pituitary lactotrophs were found. Changes in the synthesis and secretion of prolactin in animals with disturbed function of their gonads were shown to continue for 3 or 4 days during culture of adenohypophyseal cells *in vitro*.

KEY WORDS: culture of the adenohypophysis; prolactin; estradiol.

The pituitary hormone prolactin plays an important role in the regulation not only of lactation, but also of the function of the gonads. An increase in prolactin secretion due to the development of a pituitary adenoma or other factors is accompanied by hypogonadism in both women and men [8]. Meanwhile, the serum prolactin level varies depending on the estrogen levels. Experiments on rats have shown that ovariectomy is followed by a decrease in the prolactin concentration in the pituitary gland, and replacement therapy with estradiol restores the secretory activity of the lactotrophs [4]. Meanwhile many problems concerning the mechanism of action of estrogens on prolactin secretion have not been sufficiently thoroughly discussed in the literature.

The object of this study was to investigate the direct effect of estrogens on lactotroph function in a primary culture of the adenohypophysis, and the nature of their interaction with other regulators of prolactin secretion. Another aim was to compare the secretory activity of the lactotrophs *in vitro* during culture of cells obtained from experimental animals after ovariectomy, with or without replacement therapy with estradiol.

EXPERIMENTAL METHOD

Experiments were carried out on sexually mature and immature female Wistar rats weighing 150-200 g. Overiectomy was performed through a midline laparotomy incision on the animals anesthetized with pentobarbital. Seven days after the operation the rats were given daily subcutaneous injections of 100 μg of an oily solution of estradiol dipropionate for 5 days. The completeness of ovariectomy and the action of estradiol were verified by examination of vaginal smears.

Cultures of adenohypophyseal cells from control, ovariectomized (12 days after the operation), and estradiol-treated rats were set up by the method described previously [1]. The cells were grown in sloping tubes 16 mm in diameter in medium No. 199 with the addition of antibiotics, 20% fetal calf serum for the first 2 days and 10% during the subsequent days of growth, in an atmosphere of 5% CO_2 and 90% air. Each tube contained 1 ml of culture medium. The experiments were carried out on 3- and 4-day cultures. After a change of medium, 24 h

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