EFFECT OF T LYMPHOCYTES ON PROLIFERATION OF RAT AND MOUSE SALIVARY GLAND CELLS INDUCED BY ISOPROTERENOL

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In recent years lymphocytes have been found to have many properties which are not strictly concerned with immunity. For instance, it has been shown that the lymphocytes of animals with regenerating organs, if transplanted syngeneically, can increase the intensity of proliferation of the corresponding types of tissue in intact recipients [1], the participation of lymphocytes in growth of the intact organism [6] and their ability to influence proliferation of fibroblasts and osteoblasts [10, 11] have been demonstrated, and the impossibility of inducing tumors by various viruses and chemical carcinogens in mice with genetically determined absence of the thymus has been observed [12]. In all these cases lymphocytes take part in the regulation of a single process, namely proliferation, and the type of proliferating tissue, moreover, may vary.

This paper describes a study of the ability of lymphocytes to take part in the regulation of isoproterenol-induced proliferation of submaxillary salivary gland cells of rats and mice.

EXPERIMENTAL METHOD

Experiments were carried out on 45 female August rats and on 60 female C57BL/6 and BALB/c mice weighing 15-18 g. Proliferation of the salivary gland cells was induced by a single subcutaneous injection of 10 mg of isoproterenol into rats and 5 mg into mice. The reaction was read as the degree of increase in weight of the submaxillary and parotid glands as a percentage of the weight of the glands in intact animals, and as incorporation of ^{3}H-thymidine into fragments of the glands when incubated *in vitro* [2].

To judge activation of the lymphocytes in the course of induction of salivary gland proliferation by isoproterenol, incorporation of ^{3}H-thymidine into spleen cells of the experimental animals, cultured *in vitro* under the conditions usually used for this purpose [3] was used and the number of activated splenic lymphocytes was determined on the basis of data indicating the lower density of the activated lymphocytes, so that they could be isolated separately by centrifugation in a Ficoll gradient [8]. The number of lymphocytes in the gland tissue itself was determined by isolating lymphocytes from the gland by centrifugation in a Ficoll gradient in the usual way [5]. The function of the isolated lymphocytes was tested by syngeneic transfer into intact animals and recording the weight of the glands 24 h after transplantation of the cells.

To determine whether the lymphocytes were of the T or B series, 2 h before injection of isoproterenol the rats were given an injection of 1 ml of anti-Thy-serum, obtained by immunizing rabbits with rat brain homogenate [7]. To determine the precise characteristics of lymphocytes taking part in isoproterenol-induced proliferation of the gland cells, 2 h before injection of the drug the animals were given an intraperitoneal injection of anti-Ly-1 or anti-Ly-2-serum in a dose of 1 ml; the serum was obtained in the usual way and had a titer of 1:500 in the cytotoxic test with thymocytes [9].

The results were subjected to statistical analysis by Student's test.

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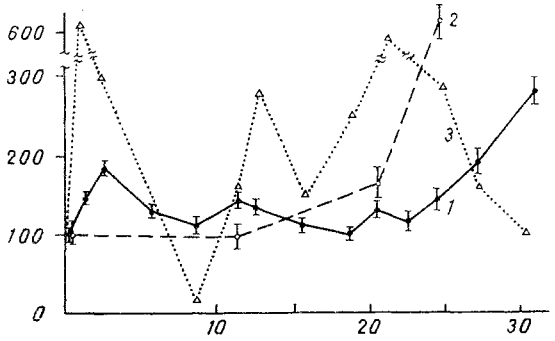

Fig. 1

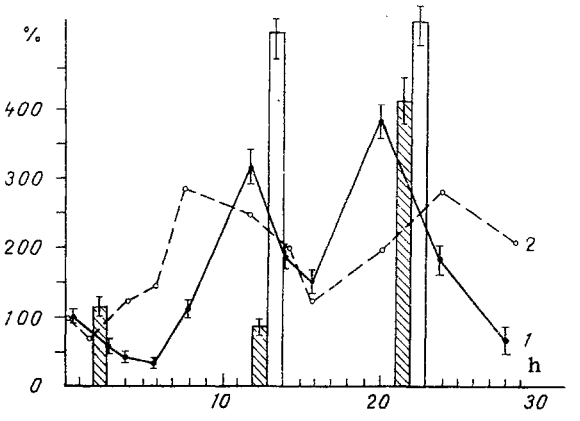

Fig. 2

Fig. 1. Changes in weight of salivary gland and number of lymphocytes contained in it after injection of isoproterenol into rats. Abscissa, time after injection of isoproterenol (in h); ordinate, parameter (in % of data for intact animals); 1) weight of salivary glands; 2) incorporation of ^{3}H-thymidine into salivary gland tissue; 3) number of lymphocytes isolated from salivary gland tissue.

Fig. 2. Proliferation of splenic lymphocytes of rats after injection of isoproterenol. 1) Incorporation of ^{3}H-thymidine into spleen cells; 2) number of activated lymphocytes isolated in Ficoll gradient with density of 1.07. Shaded columns, incorporation of ^{3}H-thymidine into salivary gland lymphocytes isolated in Ficoll density gradient; unshaded columns, incorporation of ^{3}H-thymidine into activated spleen cells. Remainder of legend as to Fig. 1.

EXPERIMENTAL RESULTS

A single injection of isoproterenol in a high dose into rats and mice is known to induce cell proliferation in the submaxillary and parotid glands and to increase their weight [4]. The causes of this reaction are unknown.

In the present experiments, in agreement with data in the literature, an increase in ^{3}H-thymidine incorporation into fragments of rat or mouse salivary glands in culture was observed 16 h after injection of isoproterenol into the animals (Fig. 1). The maximal increase in weight of the gland occurred 30 h after injection of the drug in rats and 22-24 h after injection in mice. Sharp changes in the number of lymphocytes isolated from the gland were observed: only 2 h after injection of isoproterenol intensive migration of lymphocytes was observed into the gland tissue, with a 6-10-fold increase in their number compared with the control (intact animals). Later the number of lymphocytes fell below the background level and rose again immediately before the beginning of proliferation of the salivary gland cells. Yet another peak of the number of lymphocytes in the gland was observed at the time of maximal proliferation of its cells.

Marked changes in the number of activated lymphocytes, which were of lower density, also were observed in the spleen of the experimental animals. An increase in incorporation of ^{3}H-thymidine into the spleen cells also was observed, indicating induction of proliferation of splenic lymphocytes in the course of isoprotoerenol-induced proliferation of mouse and rat salivary glands (Fig. 2). The time of formation of the peaks of activated lymphocytes in the spleen was related reciprocally to the time of formation of peaks of lymphocytes in the salivary gland tissue; accumulation of activated lymphocytes in the spleen was accompanied by their active proliferation with the formation of two peaks: one preceding development of proliferation and the other coinciding with development of proliferation of the salivary gland cells.

Function of the isolated lymphocytes was tested by syngeneic transplantation (Table 1). Injection of $5 \cdot 10^6$ cells intravenously into syngeneic recipients caused an increase in weight of the salivary gland of the intact animals if cells of peak I or II, isolated from the salivary gland were injected, and to a decrease in its weight if cells of peak III were injected into animals treated with isoproterenol.

Induction of cell proliferation in rat salivary gland by isproterenol and the increase in weight of these glands could be observed after preliminary injection of anti-Thy-serum,

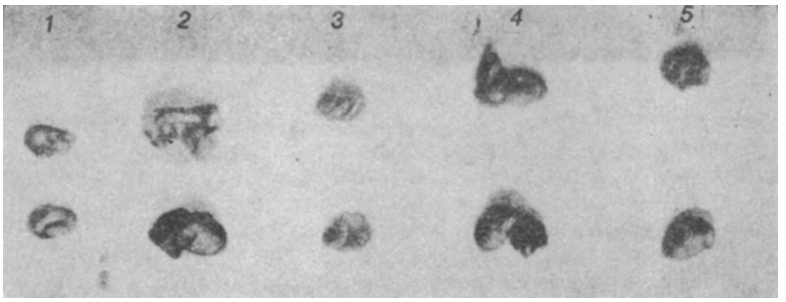

Fig. 3. Rat salivary glands after injection of isoproterenol. 1) Intact rat; 2) 36 h after injection of drug; 3) injection of isoproterenol preceded by injection of anti-Thy-serum; 4) intact rats after injection of $5 \cdot 10^6$ lymphocytes obtained from salivary gland of rats 2 h after injection of isoproterenol; 5) animals receiving injections of isoproterenol and of $5 \cdot 10^6$ lymphocytes obtained from salivary glands of rats 25 h after injection of isoproterenol.

TABLE 1. Effect of Lymphocytes Isolated from Rat Salivary Glands at Different Times after Injection of Isoproterenol on Weight of Salivary Glands after Syngeneic Transplantation

Source of transplanted cells	Recipients	Weight of gland, % of intact control
No transplantation	Intact	100.0 ± 7.5
Animals 2 h after injection of isoproterenol	Same	146.2 ± 2.5*
Animals 16 h after injection of isoproterenol	Same	154.2 ± 4.7*
Animals 24 h after injection of isoproterenol	Receiving isoproterenol 6 h before cell transplantation	139.7 ± 4.9†
No transplantation	Receiving isoproterenol	208.5 ± 5.4†

Legend. Weight of gland recorded 30 h after injection of cells or of isoproterenol. *$P < 0.01$, †$P < 0.001$.

which is toxic for T lymphocytes but not for B lymphocytes, into the animals (Fig. 3). Experiments on mice showed that the increase in weight of the gland could be prevented by the use of anti-Ly-1 serum, whereas anti-Ly-2 serum increased the weight of the gland by 35-40%.

Isoproterenol-induced activation of proliferation of salivary gland cells is thus mediated by Ly-1-positive T lymphocytes, which respond first to the stimulus, infiltrate the gland tissue during the first hours of action of the drug, then migrate into the spleen, where they proliferate, and again migrate into the gland tissue, triggering its proliferation. In the course of active proliferation of the gland cells a second lymphocytes population, capable of inhibiting the proliferative process, is activated. Accumulation of this population in the spleen and in the gland tissue evidently stops the proliferation process.

Salivary gland cells, lymphocytes stimulating proliferation of the gland cells, and lymphocytes inhibiting such proliferation, functioning together, thus constitute a single system acting together in intimate unity and determining the degree of proliferative power of a tissue in the body. It seems that the functioning of this system may also be extended to other types of tissues, which would make it possible to speak of a single system for cellular regulation of proliferation of the body tissues, represented by a regulatory system of lymphocytes stimulating and inhibiting proliferation of widely different tissues. Since this system is oriented toward maintaining proliferative homeostasis of the tissues themselves, toward preservation of what is proper to the body itself, it differs qualitatively from the immune system, which is oriented toward recognition and elimination of what is "foreign."

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