β-ADRENORECPTORS ON THE SURFACE MEMBRANES OF LYMPHOCYTES AND MACROPHAGES

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The effect of β-adrenergic stimulators adrenalin and isoproterenol and of the β-adrenergic blocker propranolol on lymphocytosis and on the macrophage adhesion inhibition reaction was studied in vivo and in vitro on sensitized and intact guinea pigs and Wistar rats. Adrenalin and isoproterenol were shown to inhibit the reaction of the antigen with both sensitized and intact cells. Propranolol abolishes the protective effect of adrenalin and isoproterenol and restores the sensitivity of the cells to antigen.

KEY WORDS: β-adrenoreceptors; adrenalin; isoproterenol; propranolol.

The modulating effect of adrenergic drugs on immunologic reactivity of lymphocytes and macrophages is of considerable theoretical and practical interest.

It has recently been shown that adrenergic drugs which increase the concentration of cyclic AMP inhibit the reaction of immune rosette formation, lower the cytotoxicity of lymphocytes and chemotaxis of leukocytes, and depress the macrophage migration reaction [1, 3-6, 8, 9]. It has been suggested that the action of these drugs on immunocompetent cells is effected through specific adrenoreceptors located on their surface membranes. However, this problem requires experimental study.

The object of the present investigation was to study the presence of β-adrenoreceptors on the surface membranes of lymphocytes and macrophages, by using specific β-adrenomimetic and adrenolytic agents for this purpose.

EXPERIMENTAL METHOD

The specific immunologic reactivity of the above-mentioned cells was investigated by the lymphocytolysis test [2] and the macrophage adhesion inhibition (MAI) test [7]. The adrenomimetic agents used were adrenalin, which stimulates mainly β-adrenoreceptors, and isoproterenol (isadrine), a highly specific β-adrenomimetic. To inhibit β-adrenoreceptors propranolol (obsidan) was used. For the lymphocytolysis test, lymphocytes isolated from the mesenteric lymph nodes of sensitized and unsensitized guinea pigs were used. The animals were sensitized subcutaneously with 0.4 ml of normal horse serum (NHS). The isolated lymphocytes were filtered through fat-free cotton wool, centrifuged at 2000 rpm for 10 min, and suspended in medium 199, in which their number was adjusted to $1 \cdot 10^6$ cells/ml. The following dilutions were used: the specific antigen $1 \cdot 10^{-4}$, adrenalin $1 \cdot 10^{-6}$, isoproterenol $1 \cdot 10^{-6}$, and propranolol $1 \cdot 10^{-6}$. All the above-mentioned substances were added to the tubes in a volume of 2 drops. The results of the test were read as the percentage of dead cells stained with trypan blue.

For the MAI test peritoneal cells of sensitized and unsensitized Wistar rats were used. The animals were sensitized with NHS with adjuvant, by injecting 1.5 ml NHS and 0.5 ml adjuvant subcutaneously into them. The adjuvant used consisted of adsorbed pertussis-diphtheria-tetanus vaccine (APDT). The cells were washed with Hanks' solution, centrifuged at 2000 rpm for 40 min, and suspended in medium 199. The test antigen in this case was used in a dilution of $1 \cdot 10^{-2}$, adrenalin $1 \cdot 10^{-5}$, isoproterenol $1 \cdot 10^{-6}$, and propranolol $1 \cdot 10^{-5}$. The results of the test were read as the percentage of cells not adhering to the walls of the tubes. In some experiments the adrenergic drugs were injected intraperitoneally and the peritoneal cells were subsequently used in vitro.

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TABLE 1. Effect of Adrenergic Drugs on Lymphocytolysis

Substance	Sensitized animals			P	Unsensitized animals			P
	number of experiments	% of dead cells	change in reaction in %		number of experiments	% of dead cells	change in reaction in %	
—	9	20,6	—	—	3	18	—	—
AG	9	36,6	45	<0,001	3	23	21	>0,1
AG + adrenalin	9	25,2	31	<0,001	4	19,5	15	>0,1
AG + adrenalin + propranolol	9	37,2	32	<0,001	4	25	22	>0,1
AG + isoproterenol	9	26,6	27	<0,001	4	17,6	28	>0,1
AG + isoproterenol + propranolol	8	32,9	19	<0,001	4	24	27	>0,1

Note. Here and in Tables 2 and 3: Ag = antigen.

TABLE 2. Effect of Adrenalin and Propanolol on Inhibition of Macrophage Adhesion

Substance	Sensitized animals			P	Unsensitized animals			P
	number of experiments	% of dead cells	change in reaction, in %		number of experiments	% of dead cells	change in reaction, in %	
—	11	17	—	—	4	15	—	—
AG	11	29		<0,001	11	20		>0,1
AG + adrenalin	11	14	51,8	<0,001	11	11	45	>0,1
AG + adrenalin + propranolol	10	28	50	<0,001	11	17	35	>0,01
AG + propranolol	11	27	6	<0,001	11	19	5	>0,1

TABLE 3. Effect of Intraperitoneal Injection of Adrenalin and Propranolol on Macrophage Adhesion Reaction

Substance	Sensitized animals		P	Unsensitized animals		P
	number of experiments	% of non-adherent cells		number of experiments	% of non-adherent cells	
—	14	17		3	15	
AG	14	29	<0,001	3	20	>0,01
AG + adrenalin	7	15	<0,001	5	9,5	$0,05<P<0,1$
AG + propranolol	7	25	>0,1	4	19	>0,1
AG + adrenalin + propranolol	8	31	<0,001	4	18	>0,1

EXPERIMENTAL RESULTS

The observations showed (Table 1) that specific lymphocytolysis was increased under the influence of the antigen by 45% compared with the control. After the addition of adrenalin to the system the number of dead cells was reduced by 31%, and after addition of isoproterenol by 27%. The addition of propranolol, which specifically blocks β-adrenoreceptors, to the reacting system reduced the action of adrenalin and isoproterenol, as was shown by a decrease of 32 and 19% respectively in the number of nonadherent cells.

In experiments on unimmunized animals (Table 1) the same patterns were observed in the action of these catecholamines and of their inhibitor, propranolol, on structural changes in the lymphocytes under the influence of antigen, except that these reactions were weaker in intensity for they took place in a nonimmune system.

The results of the experiments to study the inhibition of macrophage adhesion are given in Table 2.

It will be clear from Table 2 that adrenalin potentiated the adhesive properties of the macrophages. The number of nonadherent cells under the influence of adrenalin was reduced by 51.8%. The addition of propranolol to the system abolished the action of adrenalin. The number of nonadherent cells in this case was increased by 50%. Propranolol itself had no significant effect on the immune response. Similar results were obtained on macrophages of unsensitized animals.

Intraperitoneal injection of adrenergic drugs into sensitized and unsensitized rats with subsequent assessment of their response to the antigen in vitro showed that adrenalin under these conditions also potentiated the reaction of adhesion of macrophages of both sensitized and unsensitized animals. Intraperitoneal injection of adrenalin simultaneously with propranolol restored the response of the macrophages to the antigen (Table 3).

The results of these experiments thus indicate that adrenomimetic drugs modify the reactivity of immunocompetent cells to specific antigen. Under the influence of adrenalin and isoproterenol the lymphocytes of the sensitized and unsensitized animals acquired increased resistance to the modifying action of the antigen. These drugs also had a similar action on macrophages. The property of adhesion, as one of the main features of the cells of this type, was sharply increased by the action of adrenalin and isoproterenol. As these experiments showed, propranolol, a specific inhibitor of β-adrenoreceptors, abolished the protective effect of adrenalin and isoproterenol and restored the sensitivity of the cells to the antigen. The percentage of damaged lymphocytes and of nonadherent macrophages was increased up to the control values. These data show that the action of adrenalin and isoproterenol on these immunocompetent cells leading to changes in their sensitivity to the antigen does not take place on the whole surface membrane of the cells, but only on certain parts of it, namely the adrenoreceptors. Blocking these receptors by drugs capable of specifically blocking β-adrenoreceptors makes the cell insensitive to adrenomimetics. The discovery of β-adrenoreceptors on the surface membranes of lymphocytes and macrophages is evidence, on the one hand, of definite functional and structural features of these cells and, on the other hand, it is a reflection of the important role of adrenoreceptors in the neurogenic regulation of the function of specific immunity.

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