ROLE OF MONOAMINE OXIDASE INHIBITION IN FORMATION OF HYPERSENSITIVITY OF DELAYED TYPE

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The monoamine oxidase inhibitor iproniazid, if given repeatedly to guinea pigs in a dose of 50 mg/kg, inhibits the formation of hypersensitivity of delayed type to bovine serum albumin. The same effect is given by a single dose of 100 mg/kg iproniazid administered 24 h before sensitization. The inhibitory action was found during testing on the 7th day after sensitization. Later (9th and 12th days) hypersensitivity of delayed type was more marked in the experimental animals than in controls.

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Previous observations [1, 2] have shown that changes in metabolism of biogenic amines such as 5-hydroxytryptamine (serotonin) and noradrenalin can influence the course of the immune response.

The object of this investigation was to study the effect of iproniazid, a monoamine oxidase (MAO) inhibitor, on the formation of hypersensitivity of delayed type (HDT).

EXPERIMENTAL METHOD

Experiments were carried out on male guinea pigs weighing 400-500 g. The animals were sensitized by injection of 12.5 μg bovine serum albumin into the plantar pads of all 4 limbs in a volume of 0.1 ml together with Freund's complete adjuvant. The cutaneous reaction of HDT was evoked on the 7th day by intradermal injection of the same dose of bovine serum albumin in 0.1 ml physiological saline (as a control, the same volume of physiological saline was injected into the animal's other flank). The reaction was estimated by size (diameter of induration in millimeters) and intensity after 2, 4, 6, 24, and 48 h.

TABLE 1. Hypersensitivity of Delayed Type in Guinea Pigs Receiving Iproniazid

Dose (in mg/kg)	Day of injection	Size of reaction after 18-24 h	P
10	0—5	9,7±4,7	>0,2
Control	—	12,5±2,8	
25	0—5	6,3±1,6	>0,2
Control	—	8,8±2,1	
50	0—5	2,2±0,7	<0,05
Control	—	9,7±2,5	
50	0—3	5,5±2,0	>0,2
Control	—	8,5±2,0	
50	(—3)—0	0,3±0,3	<0,001
Control	—	11,5±2,1	

The three degrees of hyperemia were equated with indices: a strong (+ + +) corresponded to 1.0, moderate (+ +) to 0.66, weak yet distinct (+) to 0.33, and doubtful (±) to 0.17 [8]. By multiplying the estimated size of the response by the index corresponding to the degree of hyperemia, a single index was obtained to reflect both characteristics of the reaction.

To transfer HDT, the regional lymph glands were taken from sensitized guinea pigs 24 h after skin testing. Cells obtained from them, after appropriate treatment, were injected intraperitoneally into intact guinea pigs; each animal received $(85 \pm 15) \cdot 10^6$ living cells. In parallel experiments blood was taken from sensitized guinea pigs to discover whether transfer of the response by serum was possible.

The time of appearance of the reaction usually 6 h or more after skin testing) and also reproduction of the reaction when trans-

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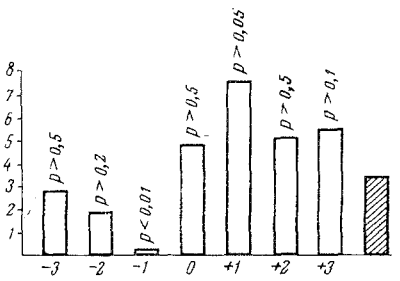

Fig. 1. Hypersensitivity of delayed type after injection of 100 mg/kg iproniazid at different times before and after sensitization. Abscissa, days of injection of iproniazid; ordinate, size of reaction; shaded column represents control.

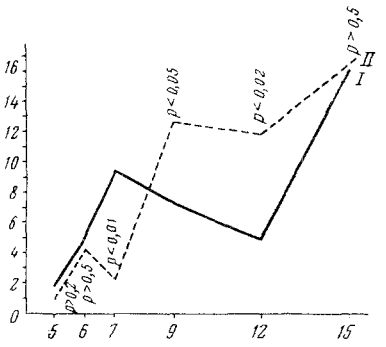

Fig. 2. Dynamics of hypersensitivity of delayed type after a single injection of iproniazid on day 1 of the experiment. I) Control; II) experiment; abscissa, days of skin testing after sensitization; ordinate, size of reaction.

ferred by lymph gland cells, under negative result if transferred by serum of sensitized guinea pigs, indicated the development of a reaction of delayed type.

Iproniazid was injected subcutaneously in doses of between 10 and 100 mg/kg daily for several doses or once only. In the case of repeated daily injections of iproniazid, administration of the drug began to be given from day 0 (day of sensitization) to day 5 or day 3 after sensitization inclusive, while single injections of the drug were given at various times either before the day of sensitization (days -1, -2, -3) or after sensitization (days 1, 2, 3). Special experiments were carried out to rule out the effect of the last injection of iproniazid (when given repeatedly) directly on manifestation of the reaction. For this purpose a single dose of iproniazid was given before intradermal injection of the antigen.

EXPERIMENTAL RESULTS

Repeated daily injection of various doses of iproniazid into guinea pigs showed that the minimal effective dose was 50 mg/kg, and if given from day 0 to day 5 of the experiment it reduced both the size and intensity of the HDT (Table 1) on day 7 of the experiment, when the reaction in the control animals reached a maximum. The use of the same dose from the day of sensitization, but on fewer days (0, 1, 2, 3), had no significant effect on the reaction. Meanwhile, injection of 50 mg/mg iproniazid for 4 days, but before sensitization (days -3, -2, -1, 0), caused almost total inhibiton of the reaction at these times. These experiments thus suggest that the action of iproniazid is dependent on the times of its injection relative to the day of sensitization. To test this hypothesis a special series of experiments were carried out in which a single injection of 100 mg/kg iproniazid was given at various times both before and after sensitization. These experiments showed that a single injection of the drug on various days after sensitization caused no changes in the reaction compared with the control animals, whereas a single injection of iproniazid before sensitization on day -1 (Fig. 1) considerably inhibited HDT when tested on day 7.

When given as a single dose of 100 mg/kg, iproniazid inhibits MAO by 90-100% after 2-3 h, but the serotonin level rises during this period by only 20-30%, and is doubled after 14-24 h. Iproniazid causes prolonged inhibition of MAO, and the serotonin level in different tissues remains elevated to 150% for more than 4 days [3-7].

Next, using results indicating the most effective injection of 100 mg/kg iproniazid on day -1, the dynamics of HDT development associated with MAO inhibition was studied. Skin tests were carried out at various times: on days 5, 6, 7, 9, 12, and 15 after sensitization. In the animals of the control series the reaction reached a maximum on day 7 (Fig. 2), after which the HDT diminished, so that by day 15 of the experiment hypersensitivity of immediate type was observed in all the animals.

In guinea pigs receiving iproniazid as a single dose on day -1, in contrast to the control, maximal inhibition of HDT was observed on day 7 of the experiment; on day 9 not only was no inhibition of the reaction observed but, on the contrary, it was maximal in degree. On day 15 hypersensitivity of immediate type was observed in both groups of animals.

Transfer of the reaction by lymph gland cells was successfully accomplished in control animals on days 5, 6, and 7 of the experiment, while in guinea pigs receiving iproniazid lymph gland cells were capable of transferring HDT at later periods (days 7 and 9). On day 15 transfer of the reaction was possible only by serum in both groups of animals, indicating the addition of hypersensitization of immediate type.

The time of appearance of the reaction, which was characteristic of both delayed and immediate type, agreed with results obtained during transfer of the reaction by lymph gland cells and serum. Whereas until day 9 inclusive a skin reaction developed in all observed guinea pigs 6 h or more after the reacting injection of antigen; on the 12th day a reaction was found in 40% of the control animals after only 2 h. In guinea pigs treated with iproniazid,however, the reaction still appeared on this day after 6 h or more. On the 15th day in both the experimental and control animals the reaction was recorded 2 h after skin testing.

The results illustrating the character of development of HDT in the animals which received iproniazid point to delay in the times of formation of HDT against the background of iproniazid action and as the result of prolongation of the state of HDT.

MAO inhibition raises the level of both serotonin and catecholamines. The results of earlier investigations [1] revealed inhibition of HDT by administration of serotonin or of its precursor, 5-hydroxytryptophan, whereas adrenomimetric substances and 3, 4-dihydroxyphenylalanine, precursor of the catecholamines did not inhibit but, in fact, slightly stimulated the immune responses [2]. The analogy between the action of serotonin, its precursor 5-hydroxytryptophan, and the MAO inhibitor iproniazid suggest that the effect of delay in the formation of HDT in guinea pigs associated with MAO inhibition is probably due to elevation of the endogenous serotonin level.

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