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THE ACTION OF PROMETHAZINE (PHENERGAN) IN PROTECTING MICE AGAINST DEATH DUE TO HISTAMINE

BY

B. N. HALPERN* AND D. R. WOOD†

From the Clinique Médicale Propedeutique de l'Hôpital Broussais (Professeur Pasteur Vallery-Radot) et Centre National de la Recherche scientifique

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It is well known that mice are relatively insensitive to histamine. Figures quoted by Guggenheim (1940) indicate that the lethal doses of histamine by subcutaneous injection for the mouse, guinea-pig, and cat are 600–2,000, 3.5–10, and 34 mg./kg. respectively. It has also been shown (Halpern, 1942, 1947a, b; Bovet and Walthert, 1944; Mayer and Broussaud, 1946) that histamine antagonists are unable to protect mice from the lethal effects of histamine and may indeed increase its toxicity. Even promethazine (phenergan), which is able to protect the guinea-pig against 1,500 times the normally lethal dose, cannot protect the mouse against one single lethal dose of histamine. Adrenalectomy increases the sensitivity of animals to histamine as well as to other substances (Dale, 1920; Kellaway and Cowell, 1923; Crivalleri, 1927; Marmoston-Gottesmann and Gottesmann, 1928). According to these authors this increase in the toxicity of histamine in adrenalectomized animals is mainly due to the lack of adrenaline and not to deficiency of cortical hormones.

Thus, in 1920 Dale found that the adrenalectomized cat was more than normally sensitive to the action of histamine. He mentioned several points of "support for the view that secretion of adrenaline by the medulla of the gland is, at least, an important factor in the normal resistance to histamine." Kellaway and Cowell (1923) showed that the altered haemoconcentration reaction to small doses of histamine in adrenalectomized cats is due to medullary defect and can be prevented by adrenaline. According to Wyman (1929) the increased sensitivity of rats to histamine after adrenalectomy is due to deficiency of adrenaline. Ingle's results (1937) show that, although cortical hormones are also concerned, their part is less important than that of adrenaline. Halpern has demonstrated that, like adrenaline, the antihistamine substances, particularly promethazine, have an action on capillary permeability, and this is clearly illustrated in the protection which these substances give against the oedema and haemoconcentration caused by injection of egg-white into normal or adrenalectomized rats (Halpern and Briot, 1950). This action of antihistamine substances on oedema produced by egg-white has also been found

* Present address: Hôpital Broussais, 96, Rue Didot, Paris XIV°, France.
† Present address: Department of Pharmacology, University of Sheffield.

by other workers (Leger and Masson, 1948; Clark and Mackay, 1949). Other findings have also been reported which support this action of the synthetic antihistamine substances on capillary permeability. When histamine or other irritant substance is injected intraperitoneally in rabbits, exudation of fluid occurs into the peritoneal cavity. Evans blue dye injected intravenously will diffuse into this exudate, but the rate of diffusion is much reduced if the animal has previously received promethazine (Halpern, 1948). The passage of fluorescein from the blood into the aqueous humour of the eye is increased by histamine; promethazine opposes this effect (Halpern, Guillaumat, and Cruchaud, 1948). Other experiments indicate that promethazine and other antihistamine substances can reduce or prevent pulmonary oedema due to injected adrenaline in mice and rabbits (Halpern, Cruchaud, Vermeil, and Roux, 1950). It has been shown by Kellaway and Cowell that in adrenalectomized cats histamine even in small doses produces haemoconcentration which is much more considerable and prolonged than in normal cats; when adrenaline is given to these animals the action of histamine is lessened. It has been suggested (Feldberg and Schilf, 1930) that the altered haemoconcentration in adrenalectomized cats after histamine is due to the fact that the capillary wall in these animals is more sensitive and more easily damaged by histamine than in normal animals or animals treated with adrenaline. Our knowledge of the action of promethazine and adrenaline in other experimental syndromes with an obvious alteration of capillary permeability led us to study the effect of these substances in adrenalectomized mice poisoned with histamine in whom the main toxic effect is a change in the capillary permeability. Other factors, such as contraction of the spleen, may play a part in producing the observed haemoconcentration after the injection of histamine, but they are considered to be of less importance than the effect on capillary permeability.

In view of the demonstration that promethazine can, like adrenaline, prevent the development of haemoconcentration and oedema produced by the injection of egg-white in rats, we have studied its effects on the action of histamine in normal and adrenalectomized mice. We have already reported in a preliminary communication (Halpern and Wood, 1950) that promethazine restores the sensitivity of mice to histamine to normal levels after it has been increased by adrenalectomy.

METHODS

Mice weighing about 20 g. were used, unselected for sex. The acute toxicity of histamine was first investigated. Normal mice and mice adrenalectomized two days previously were given histamine intraperitoneally. The dose of histamine dihydrochloride per 20 g. body weight was injected in 1 ml. of 0.9 per cent (w/v) NaCl. Bilateral adrenalectomy was performed under light ether anaesthesia and the animals allowed full access to normal diet with plenty of fluid. On the day of the operation only, the animals received a subcutaneous injection of 250 μg. deoxycortone acetate. No additional salt was supplied. In some animals, promethazine or adrenaline was injected subcutaneously thirty minutes before the injection of histamine. Haemoconcentration was measured in the earlier experiments by the change in red cell count and later by the change in haemoglobin concentration. Blood samples were obtained by puncture of the cavernous sinus through the inner angle of the eye, before injection and again about 20–30 min. after the injection of histamine. In the normal mice, after injection of 25 mg. histamine dihydrochloride per 20 g. body weight, blood samples were also taken at 90 and 120 minutes.

RESULTS

Effect of adrenalectomy on acute toxicity of histamine

The acute toxicity of histamine in mice is increased between 50 and 100 times after adrenalectomy. Thus the LD50 for histamine dihydrochloride in normal mice is rather less than 50 mg. per 20 g., and in adrenalectomized mice it is about 0.5 mg. per 20 g. (Table I*a*, *b*; Fig. 1A).

Effect of promethazine on toxicity of histamine

Although it did not increase the resistance of normal mice to histamine, the previous injection of 0.4 mg. promethazine per 20 g. did significantly increase the resistance of the adrenalectomized mouse to histamine; the toxicity of histamine was reduced approximately to normal. In adrenalectomized mice, 0.5 mg. histamine kills 13 of 21 animals (61 per cent), and after promethazine some 50 times the dose of histamine is required to produce about the same mortality (Table I*c*, *d*; Fig. 1B).

TABLE I

INCREASED SENSITIVITY OF ADRENALECTOMIZED MICE TO HISTAMINE, ITS RESTORATION TO NORMAL BY PROMETHAZINE, AND ITS REDUCTION BY ADRENALINE

Previous treatment	Dose of histamine diHCl mg./per 20 g. mouse, i.p.	Mortality	
		Observed	%
(*a*) Normal mice	10	0/10	0
	25	0/10	0
	50	9/12	75
(*b*) Adrenalectomized mice	0.2	0/3	0
	0.25	2/10	20
	0.50	13/21	61
	1.0	7/9	78
(*c*) Normal mice 30 min. after 0.4 mg. promethazine, s.c.	50	7/11	64
(*d*) Adrenalectomized mice 30 min. after 0.4 mg. promethazine, s.c.	5	0/5	0
	10	0/5	0
	25	2/13	15
	50	6/11	55
(*e*) Adrenalectomized mice 30 min. after 20 μg. adrenaline, s.c.	0.5	0/4	0
	5	1/11	9
	10	9/10	90
	20	8/9	89
	25	4/4	100

Effect of adrenaline on toxicity of histamine in adrenalectomized mice

If 0.5 mg. histamine is injected 30 min. after a subcutaneous injection of 20 μg. adrenaline hydrochloride none of the adrenalectomized mice die, and it requires between 5 and 10 mg. of histamine to kill as many animals as were killed by 0.5 mg.

in the absence of adrenaline or promethazine. The protection given to adrenalectomized mice is considerable, but less than that given by promethazine (Table I*e*; Fig. 1B).

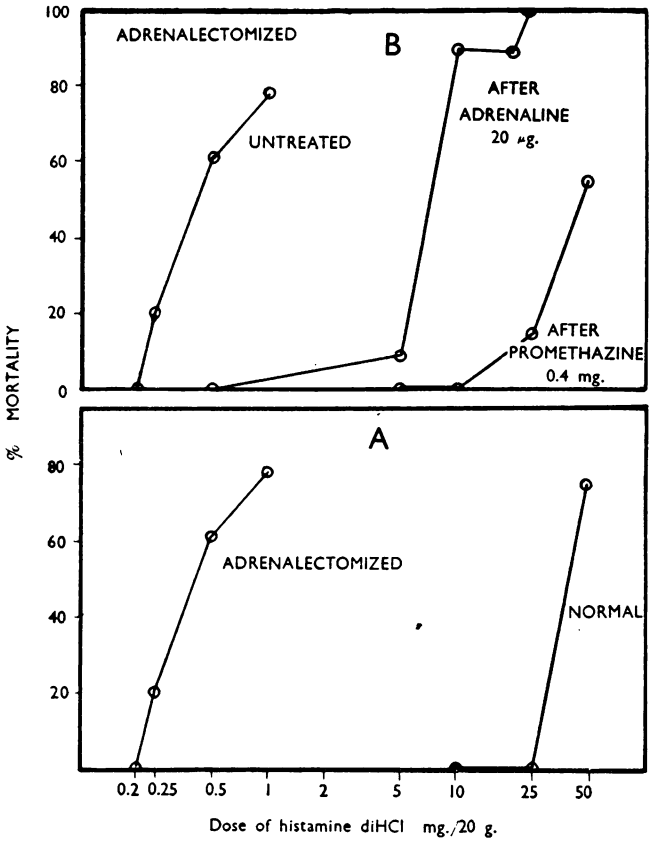

FIG. 1.—(A) Effect of adrenalectomy on toxicity of histamine in mice. (B) Reduction of toxicity of histamine in adrenalectomized mice by subcutaneous injection 30 min. previously of 20 μg. adrenaline or 0.4 mg. promethazine (phenergan). Ordinates: percentage mortality. Abscissae: log. dose of histamine dihydrochloride (mg./20 g.).

Effect of promethazine on haemoconcentration due to histamine

In normal mice the non-lethal dose of 25 mg. histamine caused haemoconcentration of about 32 per cent. This effect was very obvious after only 0.25 mg. in the adrenalectomized mouse. After promethazine even 25 mg. histamine caused only a slight haemoconcentration in adrenalectomized mice, which were also saved from death (Table II).

It will be seen from Table III that the haemoconcentration which occurred after the injection of 25 mg. histamine/20 g. in normal mice usually persisted for at least 2 hours, although only 1 out of 10 animals died.

Effect of adrenaline on haemoconcentration due to histamine

As with promethazine, the haemoconcentration caused by histamine in adrenalectomized mice is much reduced if adrenaline is given earlier. This effect of

TABLE II

EFFECTS OF PROMETHAZINE AND ADRENALINE ON HAEMOCONCENTRATION PRODUCED BY HISTAMINE IN ADRENALECTOMIZED MICE

Second blood sample taken 20–30 min. after histamine injection. Haemoconcentration estimated by change in red cell count or haemoglobin concentration. Number of animals in parentheses.

Treatment	Average haemoconcentration per cent ± S.E.
Normal mice:	
25 mg. histamine diHCl	33 ± 3.4 (12)
Adrenalectomized mice:	
Without previous treatment	
0.25 mg. histamine diHCl	43 ± 12.3 (6)
0.5 mg. histamine diHCl	65 ± 7.4 (9)
After 0.4 mg. promethazine	
25 mg. histamine diHCl	13 ± 4.2 (10)
After 20 μg. adrenaline HCl	
0.5 mg. histamine diHCl	8.5 ± 3.6 (8)
5 mg. histamine diHCl	25 ± 6.1 (12)
10 mg. histamine diHCl	47 ± 5.9 (10)

TABLE III

HAEMOCONCENTRATION IN NORMAL MICE AFTER THE SUBCUTANEOUS INJECTION OF HISTAMINE DIHYDROCHLORIDE (25 MG./20 G.)

Number	g. Haemoglobin/100 ml. blood				Maximal increase per cent	Observation
	Before histamine	30 min. after	90 min. after	120 min. after		
1	12.5	14.3	15.6	13.3	25	Survival
2	11.8	16.2	15.4	14.3	37	Survival
3	10.8	12.5	13.0	10.0	20	Survival
4	11.8	15.4	14.7	14.1	30	Survival
5	12.5	16.3	16.5	—	—	Death
6	11.8	16.3	16.4	17.5	48	Survival
7	11.2	15.8	13.7	12.5	41	Survival
8	12.7	16.7	16.0	15.4	31	Survival
9	12.7	16.3	14.0	13.0	29	Survival
10	12.5	15.6	16.5	14.4	32	Survival
				Average	32.4	

adrenaline is not so marked as that of promethazine with the doses used here. The haemoconcentration due to 0.5 mg. histamine in adrenalectomized mice is much less (8.5 per cent) after adrenaline than in those animals not given adrenaline (65 per cent). In normal mice 25 mg. histamine causes about the same degree of haemoconcentration as 0.25 mg. does in the untreated adrenalectomized rat and about the same as between 5 and 10 mg. of histamine in the adrenalectomized mouse previously given adrenaline (Table II).

Discussion

Earlier evidence that it is the deficiency of adrenaline which makes the capillaries of adrenalectomized animals more sensitive to histamine is supported by our results in which injected adrenaline prevented or reduced the haemoconcentration due to histamine in adrenalectomized mice. It seems probable that the adrenaline acts directly by reducing the sensitivity of the capillaries to the action of histamine, which increases their permeability. The same explanation could account for the similar reduction by promethazine of the haemoconcentration produced by histamine. This is in agreement with the previously cited evidence for such an action on capillary permeability—for example, reduction of the oedema and haemoconcentration produced by the injection of egg-white in rats, and protection against pulmonary oedema. The protection against the haemoconcentration occurring after histamine in adrenalectomized mice parallels roughly the protection which promethazine and adrenaline also give against death due to histamine. It is probable that the protective action of promethazine against the lethal effect of histamine, seen only in adrenalectomized and not in normal mice, is due to its ability to prevent the loss of fluid from the circulation which results from quite small doses of histamine. In normal mice the capillaries are much less sensitive to histamine, and its toxicity may be related to some other effect which is not antagonized by promethazine; the latter drug may actually increase the mortality.

Summary

1. The acute toxicity of histamine in mice is increased 50–100 times by adrenalectomy.

2. Haemoconcentration, caused by the action of histamine on capillary permeability, is produced in adrenalectomized mice by very much smaller doses than are required in normal mice.

3. Promethazine and adrenaline can each protect adrenalectomized mice against death due to histamine. At the same time they also reduce or prevent the haemoconcentration due to histamine. This effect may be explained by an action of these substances on the capillaries, whereby they are made less sensitive to the effect of histamine.

4. It is probable that it is this effect of promethazine on capillary permeability which explains its activity in protecting adrenalectomized mice, but not normal mice, from death due to histamine.

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