THE ANTAGONISM AND SYNERGISM OF HISTAMINE AND ANTIHISTAMINES IN MICE

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BOVET and Waltheret[1] and Halpern and Ducrot[2] were the first to report that although antihistamines protect most species against the lethal effect of histamine; a synergistic effect is observed between these substances in mice. This phenomenon was studied in detail by Mayer and Brousseau,[3] using tripelennamine hydrochloride and diphenhydramine hydrochloride. This study was designed to further investigate the above phenomenon.

Besides the aforementioned toxic effect of histamine, we also used its body-temperature decreasing effect as a means of evaluation, since mice, although highly resistant to the toxic action of histamine, are relatively sensitive to its temperature decreasing effect.[4]

Swiss, albino, female mice of 25 $\pm$ 3 g., of our own colony were used. They were maintained on a Rockland complete rat diet and water (*ad lib.*). The animals were kept in air-conditioned quarters at 21° C. and all work was done at this temperature. Body temperature was measured by the thermoelectric method using copper-constantan thermocouples. Readings were made from a potentiometer when the thermoelectric current was counterbalanced as indicated by a galvanometer. A constant temperature water bath at 37 $\pm$ 0·05° was used for the reference junction. Histamine diphosphate U.S.P. and the following antihistamines were used: β-dimethylaminoethyl benzhydryl ether hydrochloride (diphenhydramine hydrochloride; benadryl); *NN*-dimethyl-*N'*-2-pyridyl-*N'*-*p*-methoxybenzyl-ethylenediamine maleate (mepyramine maleate; neoantergan); 2-(*N*-benzylanilinomethyl)-imidazoline (antazoline; antistine); *N*-dimethylamino-ethyl-phenothiazine hydrochloride (3015RP); *NN'*-dimethyl-*N'*-2-pyrimidyl-*N'*-*p*-methoxybenzylethylenediamine hydrochloride (thonzylamine hydrochloride; neohetramine); *NN*-dimethyl-*N'*-2-pyridyl-*N'*-2-thenyl-ethylenediamine hydrochloride (thenylpyramine hydrochloride; histadyl); 2-methyl-9-phenyl-2:3:4:9-tetrahydro-1-pyridindene (phenidamine; thephorin); *N*-methyl-*N'*-4-chlorobenzhydrylpiperazine hydrochloride (chlorcyclizine; di-paralene); 1-phenyl-1:2-pyridyl-3-dimethylaminopropane (prophenpyridamine; trimeton); *N*-ethylpyrolidinyl-phenothiazine hydrochloride (pyrathiazine hydrochloride; pyrrolazote); 2-(10-phenothiazinyl) *iso*propyltrimethylammonium benzenesulphonate (thiazinamium; 3554RP); *NNN'N'*-tetramethyl-*NN'*-bis (β-(10-phenothiazinyl)) ethyl pentamethylene diammonium dibromide (3550RP); diethylaminocarbethoxy-bi*cyclo*hexyl hydrochloride (33536 Merrell). All

preparations were made with normal saline solution and concentrations were calculated as the base.

In determining acute toxicity antihistamines were injected subcutaneously, followed in 15 minutes by the injection of histamine either subcutaneously, intraperitoneally, or intravenously. The animals were then observed for a period of 24 hours.

In the temperature experiments the normal rectal temperature of mice previously fasted for 24 hours was measured three times within 20 minutes prior to the subcutaneous injection of the histamine and antihistamines. The mean of these readings was considered as the normal rectal temperature. The injection of histamine followed the antihistamine injections by 15 minutes. Temperature readings were taken every 10 minutes during the experimental period.

Toxicity. According to preliminary experiments on about 200 mice the intravenous LD100 of histamine in our strain of mice is 277 mg./kg. and the intravenous LD50 is 192 mg./kg., as calculated according to the method of Behrens.[5] We confirmed the results of Mayer and Brousseau[3] and Table 1 extends the available data as observed by us on some other antihistamines. None of the antihistamines protected mice against an intravenous or intraperitoneal LD100 dose of histamine. When an LD50 dose of histamine was given

TABLE I

Antihistamine injected 15 minutes preceding histamine injection	Dose mg./kg. subcutaneously of antihistamine	Histamine intravenously deaths/animals		Histamine intraperitoneally deaths/animals 1500 mg./kg.
		277 mg./kg. (LD100)	192 mg./kg. (LD50)	
Control	0·0	18/18	8/18	14/15
Mepyramine maleate	0·1	10/10	6/10	
" "	0·5	10/10	4/12	
" "	1·0	10/10	9/10	
" "	2·0	11/12	10/10	
" "	5·0	10/10	9/10	
" "	10·0	8/8	8/8	10/10
" "	75·0	6/6	4/4	
Thonzylamine hydrochloride	20·0	10/10		
Phenidamine	20·0	10/10		10/10
Prophenpyridamine	20·0	10/10		10/10
Promethazine hydrochloride	20·0	10/10		10/10
Thenylpyramine hydrochloride	20·0	10/10		
Antazoline	20·0	10/10		10/10
Pyrathiazine hydrochloride	20·0	10/10		
33536 Merrell	20·0	9/10		
3015 RP	20·0	10/10		4/4
3550 RP	20·0	10/10		
Thiazinamium	20·0	10/10		

after a dose of mepyramine maleate larger than 1 mg./kg. a synergistic effect was evidenced. The tests using subcutaneous doses of histamine (Table II) showed wider variation than the intravenous or intraperitoneal tests. However, it seems that definite protection is afforded by 5 mg. of mepyramine maleate/kg.

Effect on body temperature. Figure I shows the composite results of all experiments using different dose levels of histamine as well as mepyramine maleate. This figure is constructed on temperatures recorded 20 minutes after injection of histamine. Each point represents the

TABLE II

Histamine subcutaneously mg./kg.	No pretreatment deaths/animals	Pretreated with 5mg./kg. of mepyramine maleate 15 minutes before histamine deaths/animals
100	4/10	0/10
200	19/30	0/30
300	6/10	0/10
600	7/10	3/10
1000	12/20	3/20
2000	27/30	19/30
LD50	230 mg./kg.	1560 mg./kg.

average of 8 mice. Figure 2 gives the entire course of the reaction following histamine 50 mg./kg. and varied doses of mepyramine maleate. Similar graphs obtained with other dosage levels of which Figure 1 was constructed are not given because of the limitation of space.

From the data of Figure 1 the following conclusions may be drawn: (1) Small doses of histamine (10 and 25 mg./kg.) and small doses of mepyramine maleate (0·5 and 1·0 mg./kg.) act synergistically or additively. Larger doses of histamine and the same small doses of mepyramine maleate are antagonists; (2) by increasing the dose of mepyramine maleate an increased protection occurs against all doses of histamine and maximal protection is obtained with about 5 mg./kg. of mepyramine maleate; (3) upon further increasing the dose of mepyramine maleate (25 mg./kg.) the protection against all doses of histamine decreases.

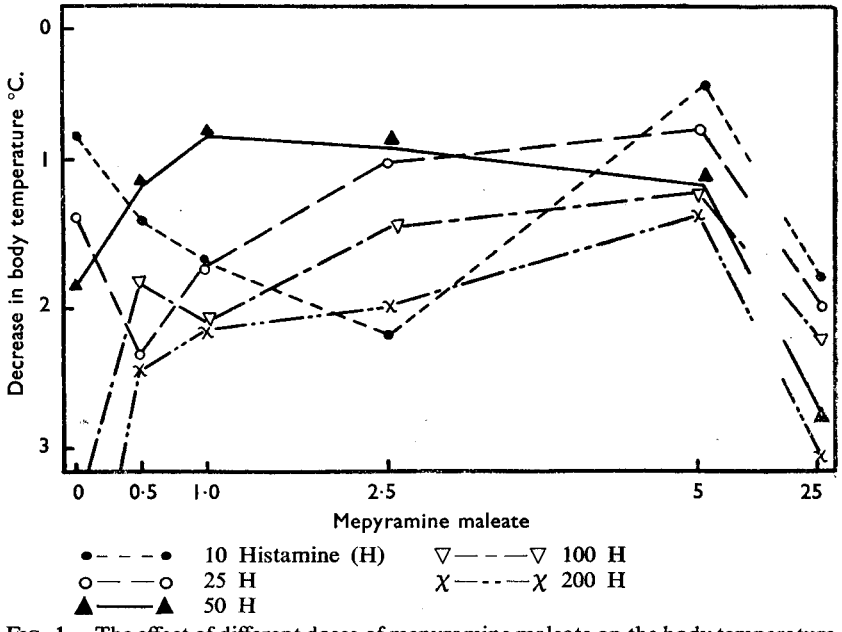

FIG. 1. The effect of different doses of mepyramine maleate on the body temperature-decreasing effect of varied doses of histamine. All doses in mg./kg. of base. Each point is average of 8 mice. Temperature recorded 20 minutes after injection of histamine.

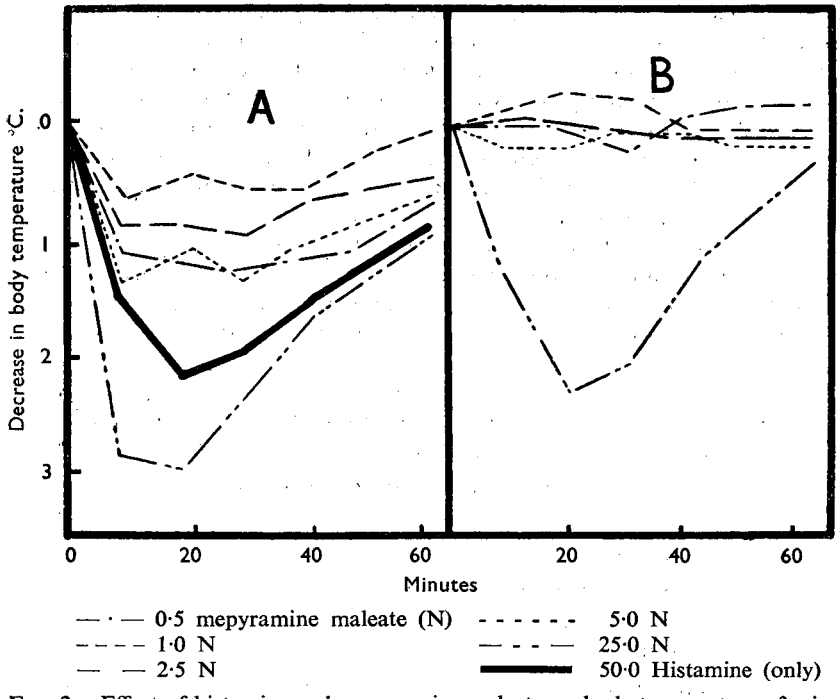

FIG. 2. Effect of histamine and mepyramine maleate on body temperature of mice. All doses in mg./kg. base. Each line is average of 10 mice.

A. 50 mg./kg. Histamine and varied doses of mepyramine maleate.

B. Mepyramine maleate (only).

The body temperature-reducing effect of the different doses of mepyramine maleate alone is set forth in the right portion of Figure 2 wherein it is to be noted that of the doses used only 25 mg./kg. of mepyramine maleate is able to elicit a significant decrease of body temperature.

The observations of other investigators concerning the synergism of histamine and antihistamines in mice from the point of view of intravenous toxicity have been confirmed by our findings. However, a protection was evidenced against toxic doses of histamine given subcutaneously. Loew[6] advanced the hypothesis that because of the great resistance of mice towards histamine, the deciding factor in the death caused by intravenous injections of this substance may be its acidity. As antihistamines are also of acid character, the synergism between these two substances may be interpreted on this basis. It is possible that upon subcutaneous injection, because of the slower absorption and greater efficiency of the neutralizing mechanisms (*a*) acidity plays only a minor role; (*b*) lower levels of histamine are produced against which the protection afforded by the antihistamines is sufficient.

Parfentjev and Goodline[7,8] and Halpern and Roux[9] found that if the sensitivity of mice toward histamine is increased by the injection of

pertussis vaccine, antihistamines exert definite protection against a toxic dose of histamine. Halpern and Wood[10] described that antihistamines protect mice in a similar manner against the lethal effect of histamine, if the histamine sensitivity of these animals is increased by adrenalectomy.

According to our findings, the body temperature-decreasing effect of histamine, an effect to which mice are relatively sensitive, is antagonised by certain doses of antihistamine. Above and below these optimal doses the degree of protection is decreased.

The antagonism or synergism of histamine and antihistamines in mice seems to be related to the high degree of natural resistance of these animals to histamine. Nevertheless there is no difference in principle between the reaction of mice and other species towards these substances.

Summary

1. Mepyramine maleate acts synergistically with toxic doses of histamine upon intravenous injection.

2. 11 other antihistamines failed to protect mice under the same conditions.

3. Of 6 antihistamines tested, none gave protection against toxic doses of intraperitoneally injected histamine.

4. Mepyramine maleate protected mice against subcutaneous toxic doses of histamine.

5. The body temperature-decreasing effect of histamine is antagonised by mepyramine maleate in certain optimal doses, below and above which a synergistic or additive effect, or at least less protection is evidenced.

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