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Inhibition by antihistamines of the vascular permeability increase induced by bradykinin

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1. Mepyramine and triprolidine hydrochloride have a marked inhibitory effect on the local increase of vascular permeability induced by bradykinin.

2. The anti-bradykinin effect of both antihistamines was species dependent, being evident in rabbits, rats and mice but not in guinea-pigs.

In the course of investigating the possible pharmacological mediators of passive cutaneous anaphylaxis induced in rabbits with heat labile homocytotropic antibody (Zvaifler & Becker, 1966) we noticed that antihistamines were effective in inhibiting the local increase in vascular permeability induced by bradykinin. Because of this rather unexpected and interesting finding we investigated the effect of antihistamines on the vascular permeability response not only in rabbits but also in guinea-pigs, rats and mice. The present paper describes our results.

Methods

Animals

Mice: ICR or Bagg strain males weighing 20–30 g; guinea-pigs: Walter Reed Hartley strain, albino female weighing 800–1,100 g; rats: Walter Reed strain albino male weighing 150–250 g; rabbits: albino rabbits of both sexes weighing 2,000–4,500 g. All animals were reared at Walter Reed Army Institute of Research Animal Farm.

Drugs

Mepyramine maleate was a gift from Merck Sharpe and Dohme, Rahway, New Jersey. Triprolidine hydrochloride was obtained from Burroughs Wellcome Co., Tuckahoe, N.Y. Histamine dihydrochloride was purchased from Fisher Scientific Co., Fair Lawn, New Jersey. Synthetic bradykinin was obtained from Calbiochem, California. Pontamine sky blue was a gift from E. I. Dupont Co., Wilmington, Delaware. Chlorpheniramine maleate was obtained from Schering Corp., Bloomfield, New Jersey. All drugs were dissolved in saline (NaCl 0.85%) and the pH adjusted to 7.0 when necessary. The concentration of all drugs used are given in terms of the base when acid salts were employed.

Measurements of the effect of antihistamines on the permeability increasing action of bradykinin and histamine

Rabbits. Rabbits were injected with 1 ml. of a 5% solution of pontamine sky blue and immediately after that were intradermally injected with 0.2 ml. saline containing either histamine, 500, 100, 10, 1 and 0.5 μg; or bradykinin, 2, 0.2, 0.1, 0.02 and 0.01 μg. Twenty minutes later the diameters of the blue spots were recorded, after which the animals received a subcutaneous injection of mepyramine (5 mg/kg or 0.5 mg/kg) or an intravenous injection of triprolidine (0.5 mg/kg). Thirty minutes after mepyramine or 5 min after triprolidine, the same amount of each permeability increasing agent was injected on the other side of the dorsal skin and the diameters of the lesions read 20 min afterwards.

Rats. Rats were injected with 1 ml. of a 0.5% solution of pontamine sky blue and then received an intradermal injection of 0.1 ml. saline containing 20, 2 or 0.2 μg of histamine; or 1.0, 0.2 or 0.04 μg of bradykinin. This same treatment was repeated on the other side of the dorsal skin 5 min after the intravenous injection of mepyramine either 5 mg/kg or 0.5 mg/kg or triprolidine 0.5 mg/kg or chlorpheniramine maleate 6 mg/kg. The diameters of the blue spots were measured 30 min after injection of the permeability increasing agents both before and after the injection of the antihistamines.

Mice. Mice were stained with an intravenous injection of 0.5 ml. of a 0.5% pontamine sky blue and immediately after that were injected with 0.05 ml. saline containing 20, 2 or 0.2 μg of histamine or 1, 0.02 or 0.005 μg of bradykinin. Twenty minutes later the sizes of the blue areas were measured and the animals were injected with mepyramine (0.5 mg/kg or 5 mg/kg) or triprolidine (0.5 mg/kg). Five minutes later they were injected again with the same doses of histamine or bradykinin on the other side of the skin. The area of the skin of the mice was too small to allow comparison in the same animal of the effects of the inhibitor on the behaviour of histamine and of bradykinin; therefore, unlike the other species tested, the effect of an inhibitor on histamine was determined on one group of mice and its effect on bradykinin on another group. This might have contributed to the somewhat erratic results noted in the experiments with mice.

Guinea-pigs. The animals were first injected intravenously with 0.5 ml. of a 10% solution of pontamine sky blue and immediately after that intracutaneously with 0.2 ml. of saline containing 20, 10, 5, 2.5, 1.25 or 0.63 μg of histamine or 16, 8, 4, 2, 1 or 0.5 ng of bradykinin. Twenty minutes later the diameters of the blue spots were read and the animals were injected intravenously with triprolidine (1 mg/kg). Five minutes later they were injected again with the same doses of histamine and bradykinin and the diameters of the blue spots read 20 min later.

Results

Rabbits. As is evident from Fig. 1, over the range of concentrations used there is a linear relationship between the logarithm of the dose of either histamine or bradykinin injected and the average diameter of the blued spot. This is essentially the same result found by Carr & Wilhelm (1965). The slope of the dose response curves given by histamine and by bradykinin are essentially unchanged following the administration of the antihistamine. This allows a comparison of the change

in activity of the two permeability increasing agents followed treatment with antihistamine. As can be seen from Fig. 1, the ratio of the doses of histamine which are equiactive before and after the administration of triprolidine is 43, whereas the ratio of equiactive doses of bradykinin is 14. This implies that triprolidine is approximately one-third as active as an antibradykinin than as an antihistamine in inhibiting the permeability increasing effects of these agents.

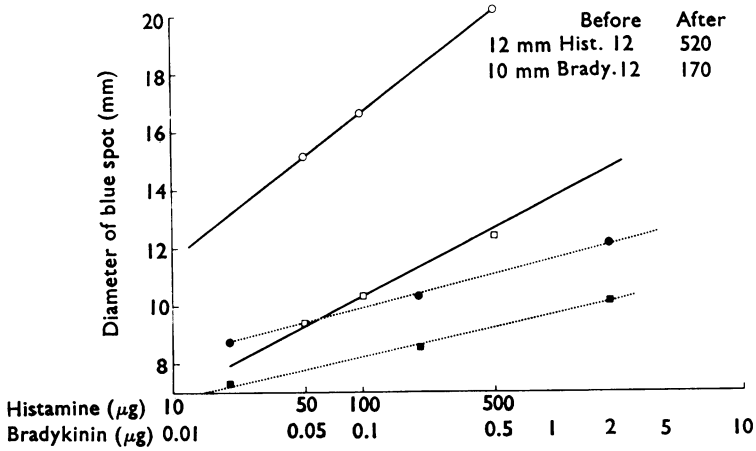

FIG. 1. Inhibition by triprolidine (0.5 mg/kg) of permeability increasing effect in rabbit skin of bradykinin as well as histamine. Each point is the average from three rabbits. The insert in the figure gives the diameters of the blue spots and the corresponding equiactive doses. Histamine before the injection of triprolidine ○——○; after triprolidine □——□; bradykinin before injection of triprolidine ●- - -●; after triprolidine ■- - -■.

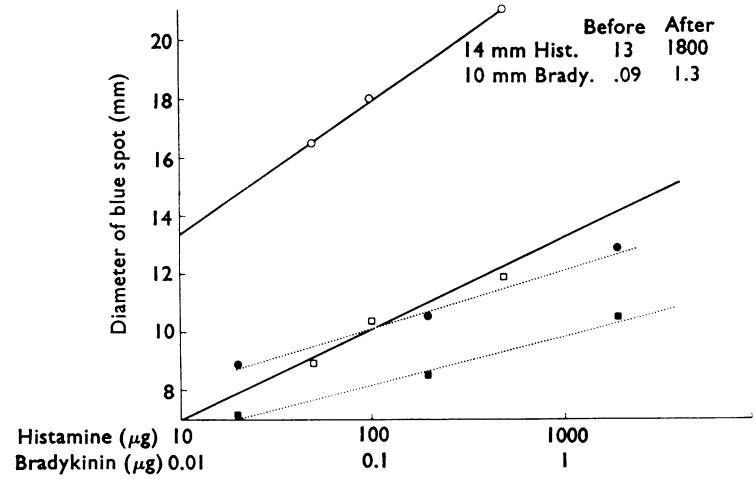

FIG. 2. Inhibition by mepyramine (0.5 mg/kg) of permeability increasing effect in rabbit skin of bradykinin and histamine. Each point is the average from three rabbits. The insert in the figure gives the diameter of the blue spots and the corresponding equiactive doses. Histamine before the injection of triprolidine ○——○; after triprolidine □——□; bradykinin before the injection of triprolidine ●- - -●; after triprolidine ■- - -■.

Although the decreases in activity of both bradykinin and histamine following the injection of triprolidine were of the same order of magnitude, the distinct difference in the slope of the dose-response curves made the effect of triprolidine on the activity of histamine more evident on inspection of the rabbit's skin than its effect on bradykinin.

Figure 2 shows that mepyramine also inhibits the permeability increasing effect of bradykinin as well as histamine. The ratio of equiactive doses of histamine before and after mepyramine is 140, compared with 14 for the dose-ratio given by bradykinin. This implies that mepyramine is one-tenth as active as an antibradykinin than as an antihistamine in inhibiting the permeability increasing effects of these agents.

Rats. Table 1 shows that mepyramine either 5 mg/kg or 0.5 mg/kg was active in decreasing the effect of bradykinin on rat skin. The dose response curves are not sufficiently well determined to allow the potency of mepyramine as an antihistaminic to be compared with its antibradykinin activity.

Janoff (1966) has reported that chlorpheniramine maleate in a dose of 6 mg/kg had no effect on the response of rat skin to bradykinin. Because of this, chlorpheniramine (6 mg/kg) was used to duplicate our previous experiments with mepyramine. It is apparent from Table 1 that chlorpheniramine also has antibradykinin activity, although chlorpheniramine appears distinctly more effective against histamine than against bradykinin. We can provide no explanation for the difference between our results and Janoff's, but it may be due to a difference in the strain of rats. In this connection it is of interest, however, that Zweifach (1967) reported that systemic administration of chlorpheniramine to rats did depress the permeability increasing effects of bradykinin.

Mice. Table 2 shows that in five out of six experiments there was an inhibition of the permeability increasing effect of bradykinin. In the second experiment

TABLE 1. *Effect of mepyramine or chlorpheniramine on the permeability increasing activity of histamine and bradykinin in rat skin*

Exp.	No. of animals	Inhibitor	Permeability increasing agent (μg)	Diameter of lesions (mm) Before inhibitor	After inhibitor
1	6	Mepyramine 5 mg/kg	Histamine 20	10·9±1·7*	6·5±2·1
			2	9·7±1·7	6·0±2·0
			0·2	6·9±2·2	4·3±2·3
			Bradykinin 1	9·2±1·4	4·0±2·6
			0·2	6·8±1·1	3·8±3·0
			0·04	5·7±3·3	2·6±2·3
2	6	Mepyramine 0·5 mg/kg	Histamine 20	12·2±1·9	8·5±2·4
			2	10·9±1·4	7·3±1·5
			0·2	7·6±0·9	4·3±3·0
			Bradykinin 1	9·5±0·3	7·0±1·2
			0·2	8·2±1·0	6·2±1·7
			0·04	6·5±1·2	3·8±2·6
3	4	Chlorpheniramine 6 mg/kg	Histamine 20	12·2±2·0	5·0±3·5
			2	9·6±0·75	0
			0·2	7·9±0·85	0
			Bradykinin 1·0	9·1±1·5	6·7±2·4
			0·2	7·6±2·7	4·0±2·8
			0·04	7·0±2·7	2·3±0·2

*Mean and standard deviation.

mepyramine 5 mg/kg had no detectable effect. When the same concentration of mepyramine was given to another group of six ICR mice on another occasion (experiment 2a) there was a very evident inhibition of the permeability increasing activity of bradykinin. We have no explanation for the aberrant results of experiment 2.

TABLE 2. *Effect of mepyramine and triprolidine on permeability increasing activity on mouse skin of bradykinin and histamine*

Exp.	Treatment	Permeability increasing agent (μg)	Diameter of lesions (mm) Before inhibitor	After inhibitor
1	Four ICR mice injected i.v. with mepyramine 0·5 mg/kg	Bradykinin 1·0	6·4±1·0*	3·9±2·7
		0·2	4·7±1·0	2·3±2·7
		0·04	3·1±2·3	0·6±1·3
2	Four ICR mice injected i.v. with mepyramine 5 mg/kg	Bradykinin 1·0	6·9±0·9	6·9±1·5
		0·2	4·7±0·6	2·5±2·1
		0·04	4·3±0·6	5·3±1·6
2a	Six ICR mice injected i.v. with mepyramine 5 mg/kg	Bradykinin 1·0	7·3±1·0	2·7±2·1
		0·2	5·6±0·9	0
		0·04	3·3±1·0	0
2b	Five ICR mice injected i.v. with mepyramine 5 mg/kg	Histamine 20·0	11·9±1·2	0
		2·0	9·2±0·7	0
		0·2	6·4±1·3	0
3	Three Bagg mice injected i.v. with mepyramine 5 mg/kg	Bradykinin 1·0	9·2±1·6	4·6±2·8
		0·2	6·9±1·3	0·9±1·3
		0·04	4·9±3·0	0·9±1·3
4	Five Bagg mice injected i.v. with mepyramine 0·5 mg/kg	Bradykinin 1·0	7·5±1·1	5·0±1·9
		0·2	5·5±0·4	3·4±1·1
		0·04	2·6±1·7	0·8±1·1
5	Five ICR mice injected i.v. with triprolidine 0·5 mg/kg	Bradykinin 1·0	8·8±2·3	3·8±2·6
		0·2	6·3±1·1	2·7±1·4
		0·04	3·7±1·3	1·5±1·0
6	Four Bagg mice injected i.v. with mepyramine 0·5 mg/kg	Histamine 20.0	11.6±1.2	7.2±1.2
		2·0	8·3±1·0	4·0±1·0
		0·2	4·3±2·1	2·0±0

*Mean and standard deviation.

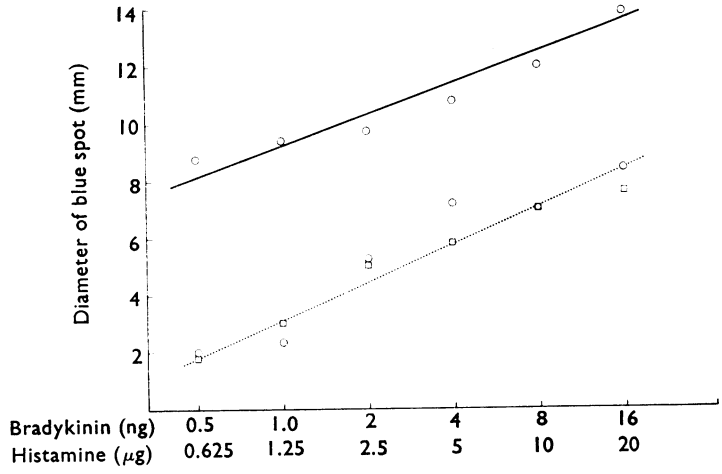

FIG. 3. Inhibition by triprolidine (1 mg/kg) of permeability increasing effect of histamine but not bradykinin in the skin of the guinea-pig. Each point is an average from six guinea-pigs. Histamine before injection ○——○; points after triprolidine are not shown because there was complete inhibition. Bradykinin before triprolidine ○- - -○; after triprolidine □- - -□.

Guinea-pigs. Figure 3 demonstrates that in this species triprolidine 1 mg/kg had no detectable effect on bradykinin even though it completely suppressed the effect of histamine. The points representing the effect of histamine after the injection of triprolidine are not shown because they all lay on the abscissa.

Discussion

The results presented here clearly indicate that the antihistamines principally used here—triprolidine and mepyramine—inhibited the permeability increasing effects of bradykinin as well as histamine in the skin of rabbits, rats and mice but not in the skin of guinea-pigs. In rabbits, triprolidine was only approximately one-third and mepyramine only about one-tenth as active in inhibiting bradykinin as they were in inhibiting histamine. The data from rats and mice did not permit a comparison of the relative potency of the inhibitors as antibradykinin and as antihistaminic agents, but they were clearly less potent as antibradykinin agents than as antihistaminics.

Triprolidine and mepyramine are considered among the most specific of the antihistaminics. The fact that they can prevent the permeability increasing effects of bradykinin in at least certain species raises the question of whether bradykinin increases vascular permeability in the skin of these species by releasing histamine. There is evidence that this might occur. Goth (1967) has cited unpublished observations indicating that bradykinin caused significant release of histamine from rat peritoneal mast cells. The concentration of bradykinin required to obtain detectable histamine release was 10 μg/ml.; this is the same as the highest concentration used in rats and one-half the concentration used in mice in the experiments reported here. There is also the less direct evidence presented by Stern, Nikulin & Ferluga (1962) and Zweifach (1966) that pretreatment of rats with 48/80, which depletes the tissues of histamine, presumably by releasing material from mast cells, decreases the permeability effects of bradykinin.

Our findings confirm the report of Holdstock, Mathews & Schachter (1957) that mepyramine does not inhibit the effect of bradykinin on guinea-pig skin. It is probable that humans behave like guinea-pigs in this respective, for Greaves & Shuster (1967) have observed that the wheal and flare induced in human skin by bradykinin is not inhibited by antihistamines. These species differences in the effect of antihistamines on bradykinin are difficult to reconcile with the hypothesis that the permeability increasing activity of bradykinin is exerted through the histamine it releases. Zweifach (1966) considers that although histamine release due to bradykinin may occur the permeability increasing effects of bradykinin are largely independent of histamine. It is possible that in guinea-pigs and humans the direct action of bradykinin is much more important than the release of histamine.

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