Brit. J. Pharmacol. (1957), **12**, 39.

HISTAMINE RELEASE INHIBITION *IN VITRO* AND ANTIANAPHYLACTIC EFFECTS *IN VIVO* OF SOME CHEMICAL COMPOUNDS

BY

F. C. McINTIRE, R. K. RICHARDS AND L. W. ROTH

From the Research Division, Abbott Laboratories, North Chicago, Illinois

(RECEIVED SEPTEMBER 4, 1956)

Several different types of compounds inhibited the release of histamine from the " platelet fraction " of rabbit blood when antigen was added to the blood of a sensitized rabbit *in vitro*. These compounds were all very toxic *in vivo* and could be tested only at low levels for their ability to protect animals against anaphylaxis. None of the compounds gave significant protection to sensitized animals when antigen was given intravenously. Three compounds protected animals against antigen administered as an aerosol. None of these showed any promise of clinical usefulness.

Of a number of compounds which have been reported to alleviate allergic symptoms, or to protect against anaphylaxis, none prevented the release of histamine *in vitro* to any considerable extent.

The efficacy of the antihistamines has given considerable credence to the concept that the release of histamine from sensitized tissue cells is a major event in anaphylactic and allergic reactions. There is now wide acceptance of the view that the antigen–antibody reaction, by an unknown mechanism, causes severe injury to certain tissue cells; histamine and perhaps other highly active compounds which are released from the injured cells produce most of the acute symptoms of many anaphylactic and allergic reactions.

The shortcomings of the antihistamines have encouraged workers in this field to think in terms of inhibiting the release of histamine (Spain and Strauss, 1951). Trethewie (1951, 1952) has reported that aspirin and *p*-aminosalicylic acid, which inhibit anaphylaxis but have no antihistaminic action, inhibit the release of histamine by antigen in the perfused lung from a sensitized guinea-pig. Trethewie and others (Coburn and Kapp, 1943; Campbell, 1948; Lepper, Caldwell, Smith, and Miller, 1950) suggested that the salicylates interfere with the reaction by preventing the union between antigen and antibody. Moss, Beiler, and Martin (1950) attempted to block the release of histamine by inhibiting histidine decarboxylase. Because of the abundance of preformed, loosely bound histamine in the tissues (McIntire, 1955a, 1955b), and because of the long " half-life " of tissue histamine (Schayer, 1952), this approach does not seem reasonable, unless a dearth of histamine could be effected by the long-term treatment with histidine decarboxylase inhibitors. Other workers (Malkiel and Werle, 1951) have not been able to confirm the findings of Moss and his colleagues on the inhibition of anaphylaxis by (+)-catechin.

In our laboratories a screening programme was begun in 1946 for chemicals which would inhibit the release of histamine from the " platelet fraction " when antigen was added to the blood from a sensitized rabbit *in vitro*. We were searching for compounds which might inhibit the reaction at any point before the release of histamine. It was hoped that some of the inhibitors found in this screening programme would inhibit anaphylaxis *in vivo* and be of some value for the treatment of allergic disorders. Approximately 2,500 compounds have been tested. This paper describes the results obtained with the most interesting compounds of this group.

METHODS

Details of rabbit sensitization and bleeding were presented in a previous publication (McIntire, Roth, and Richards, 1949). The blood was kept in an ice-bath until used. All glassware in which blood was handled was coated with silicone, either General Electric Dri Film 9987 or Dow Corning Pan Glaze. *In vitro* histamine release reactions were carried out

as follows: The chemical to be tested, in isotonic solution, was pipetted into a heavy duty 12 ml. conical centrifuge tube in an ice-bath. The volume was diluted to 2.6 ml. with isotonic sodium chloride. To this solution 2 ml. of blood was added, followed by thorough mixing, and then was added 1 ml. of a 1:100 dilution of egg white in saline. The contents of each tube were mixed thoroughly; the tubes were placed in a 37° C. bath for 20 min, and then returned to the ice-bath. Blood cells were removed by centrifugation at approximately 4° C. A 4.5 ml. aliquot of the supernatant fluid was taken for histamine determination (Code and McIntire, 1956). The final volume of histamine-release reaction-mixture was 5.6 ml., but, since 0.6 ml. of this was blood cells, drug concentrations and histamine assays were calculated for the 5 ml. of extracellular solution.

The inhibition of histamine release was calculated as follows:

$$\% \text{ inhibition} = \frac{H_c - H_i}{H_c - H_b} \times 100, \text{ where}$$

H_b histamine in plasma from "blank" reaction, without egg white

H_c histamine in plasma from reaction with egg white

H_i histamine in plasma from reaction with egg white in the presence of the chemical being tested for inhibition.

Frequently H_c would account for as much as 75% of the total histamine in the blood. H_b was rarely more than 20%, and usually less than 10%, of the total histamine. In most experiments H_b was not more than 20% of H_c.

Compounds were tested *in vivo* for antihistaminic activity in guinea-pigs by exposing the animals to a histamine aerosol 30 min. after intraperitoneal injection of the test drug. Antianaphylactic activity was tested in guinea-pigs which had been actively or passively sensitized to whole egg white. These were exposed to aerosolized antigen according to the method of Herxheimer (1952). In both tests, protection was evaluated by the prolongation of exposure before development of critical signs.

Irritation was tested by subcutaneous injection in rabbits, instillation of solutions into the rabbit eye, and, ultimately, testing solutions in the eyes of human volunteers. Administration of aerosols of the drugs to guinea-pigs provided another assessment of irritation to mucous membranes.

RESULTS

Inhibitors of Histamine Release

Early in our screening programme it became evident that many types of compounds, if present at a concentration of 0.5 to 1 mg./ml., would inhibit histamine release in rabbit blood. Most of these compounds had no effect at a concentration of 0.1 mg./ml. or less. Subsequently, all compounds were screened at a concentration of 0.04 mg./ml. in the diluted blood. Only compounds producing an inhibition of at least 40% were considered for *in vivo* studies.

Table I lists those compounds of various structure which met the criteria regarding potency set forth above. It will be noted that most of them are of strongly cationic character; many are quaternary ammonium bases. Only one (compound B) is nitrogen free, but it is strongly cationic. Compounds A and C proved the most potent *in vitro*; they exerted a definite effect at a very low concentration, 0.00002 M. The maximal inhibition usually occurred at 0.0002 M. In one instance at the highest concentration (compound C), there was a slight to moderate increase, rather than a decrease of histamine release, which is designated by −20 in Table I.

Several compounds which had shown activity in the foregoing *in vitro* tests were selected for further evaluation. Each compound was first administered to groups of normal guinea-pigs. Thirty minutes later they were exposed to a histamine aerosol, of a concentration in which unprotected animals developed marked dyspnoeic symptoms within 2 min. Any compound which afforded significant protection to the animals was rejected, since antihistaminic activity would mask any evaluation of the ability of the compound to inhibit histamine release.

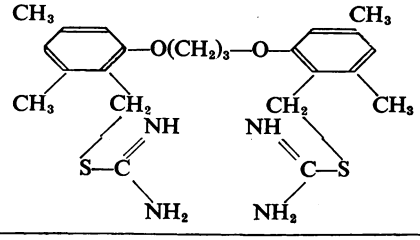
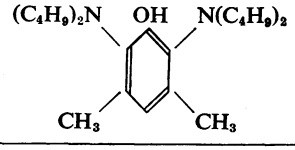
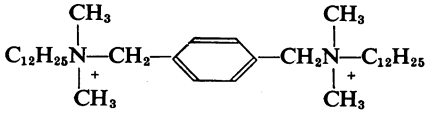
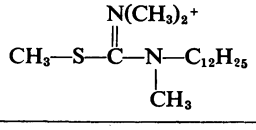
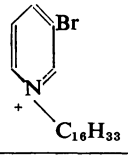
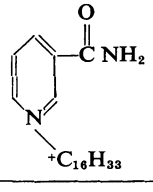
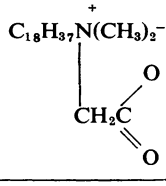
If little or no protection was evident, such compounds were then tested for antianaphylactic activity. Earlier tests involved intravenous challenge in actively or passively sensitized animals, but this was found to be too severe a test. Subsequent testing was by the Herxheimer method, in which sensitized animals were exposed to aerosol of egg white. Sixty-two control animals developed shock symptoms within an average of 2.2 min.

Of the compounds shown in Table I, A, E, and H were found to afford no protection against egg-white aerosol challenges at the maximal tolerated dose of the drug (20 mg./kg. for A and H, 10 mg./kg. for E). Compounds F and I were found to be too toxic to consider for testing.

Additional groups of guinea-pigs, or the same groups tested in cross-over experiments, were pretreated with one of the three remaining compounds, B, C, or D. These were the only compounds which had not been eliminated by previous *in vivo* screening tests on the basis of the limitations described. The protective dose was administered intraperitoneally 30 to 180 min. before exposure to the antigen aerosol. In doses of 10 mg./kg. compound C lengthened the shock time to an

TABLE I

INHIBITION OF HISTAMINE RELEASE BY DIFFERENT TYPES OF COMPOUNDS

Ref. Letter	Inhibitor	% Inhibition* at Various Concentrations			
		4×10^{-4}M	2×10^{-4}M	7×10^{-5}M	2×10^{-5}M
A		78	78	39	59
B	$C_{12}H_{25}S^{+}(CH_3)_2$	31	48	42	8
C		−20	61	70	41
D		79	88	45	16
E		2	47	24	0
F		42	45	20	0
G			51 ($1\cdot1\times10^{-4}$M)		
H		57	67	24	10
I	$C_{18}H_{37}N^{+}(CH_3)_3$		69 (9×10^{-5}M)		

* The values given for % inhibition are the means of experiments on four rabbit bloods. As the standard deviation was independent of the % inhibition, a pooled standard deviation was determined. The standard error of the mean of four rabbit bloods is 10.

average of more than 10 min., compound B to 8 min., and compound D to 9.4 min.

It should be emphasized that, after the control animals had been removed from the chamber upon signs of shock appearing, these signs usually progressed further, sometimes terminating in anaphylactic death. Likewise, if they were allowed to remain in the chamber, the same factors would inevitably cause anaphylactic death. After injection of compounds B and D, no progression of signs was noted when the animals were removed from the chamber. Therefore, two other groups were deliberately left in for the arbitrary period of 10 min. In these animals there was no progressive increase in the signs of shock, and all the animals survived the 10 min. exposure. The results are summarized in Table II.

TABLE II

DRUG PROTECTION AGAINST MICROSHOCK ANAPHYLAXIS

Drug*	No. of Animals	Dose		Shock Time, Minutes (Mean and Standard Error)†
		mg./kg.	% of LD50	
Controls	62	10	20	2·15±0·23
C	16	10	20	>10
B	41	10	20	8·07±0·74
D	27	5	20	7·31±0·92
	9	10	40	9·38±0·98
B	5	10	20	10‡
D	5	10	40	10‡

* See Table I for formulae of compounds tested.

† The means and their standard errors were calculated using the one-sided censored normal distribution method given by A. Hald, *Statistical Methods with Engineering Applications*, and A. Hald, *Statistical Tables and Formulas* (London: Chapman & Hall, 1952).

‡ Deliberately left in 10 min. Signs did not progress further, and all animals survived.

The limiting factor in the above experiments was that the protective dose in these animals was also a toxic dose. In a few experiments where the dose was increased, the toxic effects of the drug were great enough to cause a higher mortality rate in the treated animals than in the controls.

Because of this limitation, topical application appeared to be the remaining possibility for clinical application. Tests in rabbit corneas showed compound C to be irritating upon local instillation. Compounds B and D, similarly tested, showed no corneal irritation or damage. However, when these were dropped into the eyes of human volunteers, both were judged to cause sufficient discomfort to preclude their acceptability for this use. Aerosols of compounds B and D caused marked sneezing, retching, and coughing in guinea-pigs exposed to mists for only a few minutes. This, and the observations on the human eye, were evidence against their suitability for use on mucous membranes. A limited trial in patients of compounds B and D was attempted by local application to the scarified area of scratch tests for specific antigens. Pretreatment with these compounds gave no evidence for a reduction of the local reaction produced by the antigen.

Other Compounds Giving Protection against Anaphylaxis

Several drugs have been reported to alleviate allergic symptoms or to protect experimental animals against anaphylaxis (Goth and Holman, 1947; Wolfsohn, 1944; Wilson and DeEds, 1948; Raiman, Later, and Necheles, 1947; Hiller, 1950). An attempt was made to test these preparations, including several of the antihistamines, for inhibition of histamine release. All were tested at 0.04 mg./ml., except aspirin and sodium salicylate, which were tested at concentrations of 0.2 mg./ml. The results are presented in Table III.

TABLE III

INHIBITION OF HISTAMINE RELEASE BY ANTIANAPHYLACTIC AND ANTIALLERGIC DRUGS

Compound	% Inhibition	Compound	% Inhibition
Methapyriline hydrochloride	31	Nicotinic acid	22
Tripelennamine ,,	5	Pentobarbitone sodium	0
Diphenhydramine ,,	25	Rutin	0
Pyrilamine maleate	6	Adenosine triphosphate	11
Chlorcyclizine hydrochloride	8	Theophylline	3
Procaine	11	(+)-Catechin	22
Atropine	0	Thorotrast	20
Sodium acetylsalicylate	0	Oleic acid	3
,, salicylate	0	Cortisone	7

DISCUSSION

The failure of aspirin to inhibit histamine release significantly *in vitro* is particularly interesting, inasmuch as we have confirmed (in unpublished work) other reports (Campbell, 1948; Lepper *et al.*, 1950) on the protection of rabbits against anaphylaxis by aspirin. To pursue this point we studied the histamine release in the blood of rabbits which had been treated with sufficient aspirin to protect them against anaphylaxis. Again aspirin failed to inhibit the release of histamine in rabbit blood *in vitro*. These results were somewhat inconsistent with the view that aspirin protected against anaphylaxis by interfering with antigen–antibody interaction.

Our results with oleic acid were not necessarily contradictory to those of Spain and Strauss (1951), who reported that this compound inhibits histamine release. They used approximately 10 times the concentration of oleic acid which we used. Their histamine release system employed human anti-ragweed serum, ragweed antigen, and normal rabbit blood, whereas we used egg white and blood from actively sensitized rabbits. The failure of

the other compounds of Table III to inhibit histamine release significantly does not mean that they might not protect the animal against anaphylaxis. It only suggests that their mode of protection is other than by the prevention of histamine release.

In a previous publication (McIntire, Roth, and Sproull, 1951), it was reported that a relatively slight change of chemical structure, such as the substitution of a carbomethoxy group for a carbamide group in the β-position of certain quaternary pyridinium compounds, changes an inhibitor of histamine release (compound G) into a histamine releaser. Histamine release from cells can be effected as the result of the antigen–antibody reaction, as well as by certain chemicals of relatively small molecular size, such as compounds of the structure referred to above. Thus, a compound only slightly different from a histamine releaser is able to prevent the release of histamine by either procedure. This suggests the possibility of a common basic mechanism of histamine release, and also of a common site of action of the inhibitor in preventing the release when the cells are exposed either to the antigen–antibody reaction or the specific chemical histamine release agent. The cell membrane is likely to be the site of these interactions.

The significant protection afforded by compounds B, C, and D against anaphylactic reactions in guinea-pigs is assumed to be due to some interference with histamine release, since none of them was effective against histamine aerosol shock in the doses employed. In those exposed to antigen aerosol, the interference with the progression of the signs would justify the assumption that the compounds apparently prevented the release of some, but not all, of the histamine resulting from the antigenic challenge.

We are indebted to the Misses M. Sproull, A. Hawthorne, and I. M. Shepperd, and Mr. B. B. Morphis, for technical assistance, and to Dr. S. M. Feinberg for the human scratch testing.

References

Campbell, B. (1948). *Science*, **108**, 478.
Coburn, A. F., and Kapp, E. M. (1943). *J. exp. Med.*, **77**, 173.
Code, C. F., and McIntire, F. C. (1956). *Methods of Biochemical Analysis*, 1st ed., vol. 3, pp. 85–90. New York: Interscience.
Goth, A., and Holman, J. (1947). *J. Pharmacol.*, **89**, 379.
Herxheimer, H. (1952). *J. Physiol.*, **117**, 251.
Hiller, E. (1950). *Münch. med. Wschr.*, **92**, 875.
Lepper, M. H., Caldwell, E. R., Smith, P. K., and Miller, B. F. (1950). *Proc. Soc. exp. Biol., N.Y.*, **74**, 254.
McIntire, F. C. (1955a). *Ciba Foundation, Symposium on Histamine*, p. 170. London: Churchill.
—— (1955b). Ibid., p. 416.
—— Roth, L. W., and Richards, R. K. (1949). *Amer. J. Physiol.*, **159**, 332.
—— —— and Sproull, M. (1951). Ibid., **167**, 233.
Malkiel, S., and Werle, M. D. (1951). *Science*, **114**, 98.
Moss, J. N., Beiler, J. M., and Martin, G. F. (1950). Ibid., **112**, 16.
Raiman, R. J., Later, E. R., and Necheles, H. (1947). Ibid., **106**, 368.
Schayer, R. W. (1952). *J. biol. Chem.*, **199**, 245.
Spain, U. C., and Strauss, M. B. (1951). *J. Allergy*, **22**, 47.
Trethewie, E. R. (1951). *Aust. J. exp. Biol. med. Sci.*, **29**, 443.
—— (1952). *Med. J. Aust.*, **1**, 638.
Wilson, R. H., and DeEds, F. (1948). *Science*, **107**, 369.
Wolfsohn, G. (1944). *Palest. Near East med. J.*, **3**, 11.