J. Physiol. (1958) 144, 525–531

THE RELATIONSHIP OF HISTAMINE AND 5-HYDROXY-TRYPTAMINE TO ANAPHYLACTIC SHOCK IN DIFFERENT SPECIES

By R. K. SANYAL and G. B. WEST

From the Department of Pharmacology, School of Pharmacy, Brunswick Square, London, W.C. 1

(*Received* 27 *June* 1958)

Histamine is released during anaphylactic shock and accounts for many of the observed symptoms. In recent years, interest has been focused on 5-hydroxytryptamine (5-HT) as another amine involved in the anaphylactic reaction. The present study was undertaken to assess the relative importance of histamine and 5-HT in anaphylactic shock in different species. The rat is particularly suitable for this work as its tissues can be preferentially depleted of either of these amines (Parratt & West, 1957*b*) and severe shock can be consistently produced by using *Haemophilus pertussis* vaccine with the sensitizing dose of antigen (Sanyal & West, 1958). In this species the effect on the anaphylactic reaction of depletion of tissue histamine and 5-HT and of specific antagonists of these substances was therefore determined. In addition, the amine release from tissues was studied in the rat as well as in the guinea-pig, rabbit and dog.

METHODS

Female albino rats (100–150 g), adult guinea-pigs, adult rabbits and young dogs were used.

Sensitization. Fresh egg white, which had been mixed with an equal volume of normal saline (aqueous NaCl 0·9 % (w/v)) and strained, or horse serum was used as antigen. Rats were sensitized by an intraperitoneal injection of either 1 ml. of egg white or a mixture of 1 ml. of horse serum and 1 ml. of *H. pertussis* vaccine, phase I, containing $20{,}000 \times 10^6$ organisms (Sanyal & West, 1958). Guinea-pigs were sensitized by an intraperitoneal injection of 0·5 ml. of horse serum. Rabbits received 1 ml. of horse serum intraperitoneally on each of 6 consecutive days. Dogs were sensitized by a subcutaneous injection of 2 ml. of horse serum, followed 2 days later by an intravenous injection of 3 ml. of horse serum.

Challenge. All animals were challenged by an intravenous dose of 1 ml. of antigen. This was given 12–18 days after the last sensitizing dose in rats, 21–28 days in guinea-pigs, 14–20 days in rabbits, and 20–30 days in dogs.

Assessment of shock. The symptoms of shock in the rat were recorded as previously described (Sanyal & West, 1958). Development of asphyxia was taken to indicate the presence of anaphylactic shock in the guinea-pig. A marked fall in blood pressure indicated shock in the rabbit and dog.

Depletion of tissue amines in the rat. To deplete the skin of its histamine before challenge with the antigen, five doses of polymyxin B were given intraperitoneally over 3 days (Parratt & West, 1957*b*), commencing 9 days after sensitization. To deplete the skin and intestine of their 5-HT, one dose of reserpine (10 mg/kg) was given intraperitoneally 24 hr before the challenge. To deplete the stomach and jejunum of their histamine, a dose of 900 r total body irradiation (Eisen, Ellis & Wilson, 1956) was given 6 days after sensitization. To lower the skin histamine of several of the irradiated rats, repeated doses of polymyxin B were given during the 3 days before the challenge.

Use of specific antagonists in the rat. Thirty minutes before the challenge, rats received intraperitoneal injections of either mepyramine, a specific antagonist of histamine, or 2-bromo-lysergic acid diethylamide (BOL 148), a specific antagonist of 5-HT.

Amine release by antigen from the whole animal. Amine release by antigen was studied before and after anaphylactic shock. On the day of challenge with antigen the animals were anaesthetized with urethane (1·5 gm/kg intraperitoneally) and blood pressure recordings were taken from the carotid artery. Artificial respiration was necessary for guinea-pigs and rabbits, in order to remove a piece of right lung for extraction and assay. In dogs pieces of liver and of spleen were similarly removed for assay. Twenty to thirty minutes after the challenging dose of antigen had been given, similar pieces of tissue were removed from each animal for assay. The tissues were extracted either with trichloroacetic acid and assayed for histamine on the isolated guinea-pig ileum or with acetone and assayed for 5-HT on the isolated rat uterus, as described in detail by Parratt & West (1957*a*). All values for histamine and 5-HT in this paper refer to the bases.

Amine release by antigen from isolated tissues. This was carried out by a modification of the method of Campbell & Nicoll (1940). Pieces of tissue of similar weight from non-sensitized or sensitized rats, guinea-pigs or dogs were suspended in the organ bath (vol. 20 ml.) in which was set up an assay preparation of either guinea-pig ileum, rat uterus or rat colon. The bath fluid contained atropine (10^{-7}). The specific antigen (0·2 ml.) was then added and the contraction produced was compared with those resulting from the addition of doses either of histamine on the guinea-pig ileum or of 5-HT on the rat preparations. The experiments were then repeated with other pieces of tissue on the guinea-pig ileum in the presence of mepyramine (10^{-7}) or on the rat preparations in the presence of BOL 148 (10^{-7}).

Histological examination. Fresh tissue spreads of rat subcutaneous connective tissue and of guinea-pig phrenic nerve with its attached pleura were made before and after challenge with the antigen. They were fixed in alcohol, stained with toluidine blue and mounted (Parratt & West, 1957*a*).

RESULTS

Anaphylaxis in the rat. The results were the same whether horse serum or egg white was used as the antigen. Depletion of tissue histamine by treatment with polymyxin B only slightly lowered the average shock value of rats undergoing anaphylaxis and deaths were recorded in each group. Depletion of tissue 5-HT by treatment with reserpine likewise had little or no effect on the shock values. In sensitized rats receiving X-irradiation there was an indication that the shock produced was more intense than in control sensitized rats given the challenge, as fatal reactions were obtained much earlier. X-irradiation did not alter the shock value of rats whose histamine had been depleted by treatment with polymyxin B. The results obtained with horse serum are summarized in Table 1.

Pre-treatment with mepyramine (10 mg/kg) or with BOL 148 (4 mg/kg) or with both antagonists did not significantly reduce the severity of anaphylactic

shock. The results using horse serum as antigen are shown in Table 2 and similar values were obtained for egg white.

When the amine release from isolated rat intestine by antigen was examined, it was found that both horse serum and egg white were effective in releasing substances which stimulated the guinea-pig ileum, rat uterus and rat colon, and there was no difference whether the intestine was from sensitized or non-sensitized rats.

TABLE 1. The effect of different treatments on the average shock value of rats undergoing anaphylactic shock. Sensitization with horse serum and *H. pertussis* vaccine; challenge with horse serum 12 days later

Treatment	Number of rats used	Average shock value (%)
None	18	100
Polymyxin B	12	81
Reserpine	9	90
X-irradiation	5	100
X-irradiation + polymyxin B	5	80

TABLE 2. The effect of specific antagonists of histamine and 5-hydroxytryptamine on the average shock value of rats undergoing anaphylactic shock. Sensitization and challenge as described in Table 1

Antagonist	Number of rats used	Average shock value (%)
None	18	100
Mepyramine	6	92
BOL 148	6	92
Mepyramine + BOL 148	4	100

The appearance of the mast cells of the subcutaneous connective tissue was not altered by anaphylactic shock, and even when the mast cells had been completely disintegrated by repeated injections of polymyxin B severe and often fatal shock was produced.

Anaphylaxis in the guinea-pig. Histamine is known to be released from guinea-pig lungs during anaphylaxis. In the present experiments, horse serum reduced the histamine content of the lungs of four non-sensitized animals by about 16% (range 14–25%) whereas in eight sensitized animals the average reduction was 62% (range 31–88%). The histamine release from isolated guinea-pig lungs during anaphylaxis was not studied because this fact is well established. Anaphylactic shock also degranulated and distorted the pleural mast cells, but did not alter the 5-HT content of the lungs.

In contrast to the rat experiments, the isolated rat uterus and colon were not stimulated when pieces of lungs of non-sensitized guinea-pigs were suspended in the organ bath and the antigen was added. A smooth-muscle-stimulating substance was released, however, when a piece of lung of a sensitized animal was present, and estimates of its activity, calculated as 5-HT, gave similar values on both rat preparations (0·08 μg/g tissue). The stimulant action was not observed in the presence of BOL 148 (10^{-7}), and this result

further suggested that the stimulation was due to the release of 5-HT from the tissues. Release of a similar smooth-muscle-stimulating substance was also obtained from pieces of spleen of sensitized animals on the addition of the antigen but no such release occurred from aorta or oesophagus.

Anaphylaxis in the rabbit. Intravenous doses of horse serum produced no alteration in the histamine or 5-HT contents of the lungs of non-sensitized rabbits but considerably raised the levels of both amines in the lungs of sensitized animals (see Table 3).

TABLE 3. The influence of anaphylactic shock on the histamine and 5-hydroxytryptamine contents (μg/g) of rabbit lung

	Histamine			5-HT		
Rabbit no.	Pre-shock value	Post-shock value	Change (%)	Pre-shock value	Post-shock value	Change (%)
1	4·2	8·0	+ 90	1·7	4·3	+153
2	7·5	16·4	+118	2·1	6·8	+228
3	9·9	25·6	+158	7·1	20·3	+185
4	3·6	11·3	+213	1·5	2·5	+ 66

TABLE 4. The influence of anaphylactic shock on the histamine and 5-hydroxytryptamine contents (μg/g) of dog liver

	Histamine			5-HT		
Dog no.	Pre-shock value	Post-shock value	Change (%)	Pre-shock value	Post-shock value	Change (%)
1	6·8	4·4	−35	1·55	1·60	+ 3
2	14·0	2·0	−86	0·50	0·45	−10
3	13·0	5·0	−62	0·35	0·35	0
4	16·0	2·0	−82	0·45	0·45	0

Anaphylaxis in the dog. The injection of horse serum into control dogs did not produce a fall in arterial blood pressure nor did it alter the histamine or 5-HT contents of the liver. In sensitized dogs, however, the injection produced an abrupt fall in blood pressure accompanied by a considerable loss of histamine, but not of 5-HT, from the liver (Table 4). The loss of histamine was not confined to the liver, as the spleen also showed a loss of histamine, though not of 5-HT.

The isolated rat uterus was not stimulated when a piece of liver of a sensitized dog was suspended in the bath. There was also no stimulation when horse serum was added as well. Nevertheless, the dogs were sensitized since the intravenous injection of horse serum produced an abrupt lethal fall in arterial blood pressure.

DISCUSSION

The present results show that neither histamine nor 5-HT plays a role in the anaphylactic shock of the rat. This conclusion is based on the finding that the shock is little altered when the skin and intestinal histamine and 5-HT are depleted and when the animals have been pre-treated with specific antagonists

of both amines. Further, the release of histamine and 5-HT from sensitized tissues on addition of antigen is no more than that from non-sensitized tissues. Tissue mast cells which contain histamine and some of the 5-HT also fail to exhibit major changes following anaphylaxis, and their complete disruption does not reduce the severity of the reaction. This conclusion is in agreement with our earlier findings (Sanyal & West, 1958). It is thus possible that anaphylaxis in the rat is mediated by substances which have not so far been identified or that the antigen-antibody reaction directly damages the susceptible cells.

A different conclusion concerning the role of histamine in rat anaphylaxis has been reached by Mota (1957). He based his conclusions on the following three points: (1) Tissue mast cells which contain histamine are disrupted during anaphylaxis. (2) A rise in plasma histamine occurs in the anaphylactic reaction. And (3), rats can be protected against anaphylactic shock by pretreatment either with chlorprophenpyridamine maleate (Alergon), an antihistamine drug, or with compound 48/80, a potent histamine liberator. Examination of his published data, however, reveals that only a proportion of the tissue mast cells showed granule extrusion with little disruption; however, such changes are frequently encountered in tissue spreads from control rats. Moreover, as shown in the present experiments, complete degranulation and disruption of mast cells as, for example, by treatment with polymyxin B, is followed by severe and often fatal anaphylactic shock. Mota did not report the plasma histamine levels of non-sensitized rats receiving the antigen (horse serum) and it is difficult to assess whether the slightly raised values of plasma histamine in some of his rats are related to anaphylaxis, as horse serum releases histamine from tissues of non-sensitized animals (Sanyal & West, 1958). Further, the protective effect of compound 48/80 (as reported by Mota) loses much of its significance as the method of sensitization used produced mild, and at no time fatal, anaphylactic shock.

Histamine release from the lungs of guinea-pigs during anaphylaxis has been confirmed (for references, see Rocha e Silva, 1955). Although horse serum releases histamine from the lungs of control guinea-pigs, the release from the lungs of sensitized animals is considerably greater. Moreover, pleural mast cells show changes during anaphylaxis which are suggestive of histamine release. There is a release of a minute amount of a smooth-muscle-stimulating substance other than histamine in the tests of anaphylaxis *in vitro* and its action is similar to that of 5-HT. As the injection or inhalation either of histamine or of 5-HT produces in guinea-pigs symptoms which resemble those found in anaphylactic shock, it is apparent that both substances may play a part in anaphylaxis in this species. Histamine is likely to be the major component responsible for asphyxial death but 5-HT may play an accessory role. The complete obstruction of the respiratory passages, which is a characteristic

feature of anaphylactic shock in the guinea-pig, results from the special arrangement of the smooth-muscle fibres in the trachea and bronchi which, in the guinea-pig, in contrast to the arrangement in other species, are attached to the inner aspect of the cartilage. Both histamine and 5-HT stimulate these smooth muscle fibres *in vitro* (Brocklehurst, 1958) and their contractions in anaphylaxis may produce dove-tailing and folding of the bronchial mucosa, as reported by Schultz & Jordan (1911), resulting in respiratory obstruction and death.

Anaphylaxis in the rabbit results in a decrease in blood histamine, blood 5-HT and the number of circulating platelets (Waalkes, Weissbach, Bozicevich & Udenfriend, 1957). As rabbit platelets are rich in both histamine and 5-HT and release these two amines during the antigen-antibody reaction (Humphrey & Jaques, 1954), the increase in the histamine and 5-HT contents of the lung noted in the present experiments after anaphylaxis may be accounted for by the trapping of platelets in the pulmonary bed, as suggested by Schachter (1953).

In the dog histamine release from the liver during anaphylactic shock has been confirmed. No evidence however has been obtained to suggest that 5-HT is released either from the liver or from the spleen, although appreciable quantities of 5-HT are normally located in these two tissues in this species.

The present results show that there is considerable species variation in the relative importance of histamine and 5-HT in the production of anaphylactic shock. Both amines are involved in the rabbit but only histamine in the dog. In the guinea-pig 5-HT is involved but it probably plays a minor role. In the rat, however, neither histamine nor 5-HT appear to participate. The characteristic lesions of anaphylaxis in the rat consist of haemorrhage in the small intestine, and similar lesions have also been found in the dog and guinea-pig, especially when death is delayed as after the intraperitoneal or subcutaneous challenge with antigen (Williamson, 1936) or after an antihistamine drug (Green, 1953). Such changes therefore represent a pattern of lesions in anaphylaxis common to several species.

SUMMARY

1. The role of histamine and 5-hydroxytryptamine in the production of anaphylactic shock has been examined in rats, guinea-pigs, rabbits and dogs.

2. In rats no evidence could be found that either amine participates in the symptomatology of anaphylactic shock. Shock occurs in animals whose skin and intestinal histamine and 5-hydroxytryptamine have been depleted. Specific antagonists of both amines similarly fail to modify the severity of the shock.

3. In guinea-pigs and rabbits both histamine and 5-hydroxytryptamine are involved in anaphylactic shock.

4. In dogs the release of histamine, but not of 5-hydroxytryptamine, occurs as a result of the anaphylactic reaction.

REFERENCES

BROCKLEHURST, W. (1958). The action of 5-hydroxytryptamine on smooth muscle. *5-hydroxytryptamine*, pp. 172–176. London: Pergamon Press.

CAMPBELL, D. H. & NICOLL, P. A. (1940). Studies on *in vitro* anaphylaxis and release of an active non-histamine material from sensitized guinea-pig lung. *J. Immunol.* **39**, 103–112.

EISEN, V. D., ELLIS, R. E. & WILSON, C. W. M. (1956). The effect of X-irradiation on tissue histamine in the rat. *J. Physiol.* **133**, 506–519.

GREEN, A. F. (1953). The antagonism of histamine and the anaphylactic response by phenylpyridylallylamine. *Brit. J. Pharmacol.* **8**, 171–176.

HUMPHREY, J. H. & JAQUES, R. (1954). The histamine and serotonin content of the platelets and polymorphonuclear leucocytes of various species. *J. Physiol.* **124**, 305–310.

MOTA, I. (1957). Action of anaphylactic shock and anaphylotoxin on mast cells and histamine in rats. *Brit. J. Pharmacol.* **12**, 453–456.

PARRATT, J. R. & WEST, G. B. (1957*a*). 5-hydroxytryptamine and tissue mast cells. *J. Physiol.* **137**, 169–178.

PARRATT, J. R. & WEST, G. B. (1957*b*). Release of 5-hydroxytryptamine and histamine from tissues of the rat. *J. Physiol.* **137**, 179–192.

ROCHA E SILVA, M. (1955). *Histamine, its role in anaphylaxis and allergy.* Illinois: C. C. Thomas.

SANYAL, R. K. & WEST, G. B. (1958). Anaphylactic shock in the albino rat. *J. Physiol.* **142**, 571–584.

SCHACHTER, M. (1953). Anaphylaxis and histamine release in the rabbit. *Brit. J. Pharmacol.* **8**, 412–419.

SCHULTZ, W. H. & JORDAN, H. E. (1911). Physiological studies in anaphylaxis—a microscopic study of the anaphylactic lung of the guinea-pig and mouse. *J. Pharmacol.* **2**, 375–389.

WAALKES, T. P., WEISSBACH, H., BOZICEVICH, J. & UDENFRIEND, S. (1957). Serotonin and histamine release during anaphylaxis in the rabbit. *J. clin. Invest.* **36**, 1115–1120.

WILLIAMSON, R. (1936). Late deaths occurring in active anaphylaxis. *J. Hyg., Camb.*, **36**, 588–593.