SEROTONIN AND HISTAMINE RELEASE DURING ANAPHYLAXIS IN THE RABBIT

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The release of physiologically active substances in animal tissues during anaphylaxis has been a subject of considerable interest for many years. Most of this interest has centered on the release of histamine from a bound to a free form, the latter being capable of producing marked physiological changes (1). However, despite the toxicity which can be attributed to histamine, attempts to explain all the alterations seen in anaphylaxis on the basis of histamine release alone have been unsatisfactory (2).

Many of the effects produced by serotonin (5-hydroxytryptamine) in animals (3) resemble the manifestations of hypersensitivity reactions. In addition, patients with malignant carcinoid, a syndrome (4) in which there is present an excessive amount of serotonin, frequently show symptoms resembling those of an allergic process. These findings have suggested that this amine may play a role in anaphylaxis and other allergic phenomena.

Serotonin is found in the blood platelets of animals and man (5). Anaphylaxis is known to produce profound changes in these blood cells (6). Recently, Humphrey and Jaques (7) showed that both serotonin and histamine were released from platelets upon addition of purified antigen and antibody to normal rabbit platelets suspended in plasma. Also, Herxheimer (8) reported that in guinea pigs an interrelationship exists between desensitization and tolerance to serotonin.

The present study was designed to determine whether serotonin is released during anaphylaxis. The experiments outlined below for serotonin are similar to those that were previously used to demonstrate the changes in blood histamine which occur during anaphylactic shock (9–12). Simultaneous determinations for both serotonin and histamine were carried out for comparative purposes. In this work, it has been shown that both amines appear in a free form in the plasma when a specific antigen is injected into sensitized rabbits.

MATERIALS AND METHODS

The rabbit was chosen for these experiments for several reasons: (a) the large amounts of both serotonin and histamine carried in the blood platelets, (b) the ease of obtaining serial blood samples, and (c) the ability of the rabbit to produce adequate amounts of antibody.

Male rabbits (1.5 Kg.) were sensitized to either egg albumin or horse serum. Those sensitized to egg albumin were given 10 ml. of a 10 per cent solution in saline intraperitoneally every other day for four injections. Those sensitized to horse serum were given 1 ml. of the undiluted serum intraperitoneally every day for six days. After three to five weeks, the rabbits were used as indicated. At this time, complement fixation antibody titers were high.

Siliconized glassware was employed wherever whole blood had to be handled. Blood for the *in vitro* studies was obtained by intracardiac puncture. Blood samples for the *in vivo* studies were obtained by means of a plastic tube inserted into the carotid artery.

Both serotonin and histamine were determined in all samples of plasma and whole blood. For plasma, 2 ml. were treated according to the method of McIntire, Roth, and Shaw (13) for the isolation of histamine. The method utilizes cotton acid succinate as a cation exchange medium after extraction of amines into n-butanol. It was found that histamine is removed from the butanol solution by the cotton acid succinate whereas serotonin is not. This procedure gives a convenient method for separating serotonin and histamine. Serotonin was then determined spectrophotofluorometrically (14), and histamine by modification of the methods of Lowry, Graham, Harris, Priebat, Marks, and Bregman (15) and McIntire, White, and Sproull (16). For assay of these amines in whole blood, 1 ml. was diluted with 7 ml. of water. After hemolysis, 1 ml. of 10 per cent zinc sulfate solution, and 0.5 ml. of 1 N sodium hydroxide were added to deproteinize the blood. After centrifugation, 1 ml. of the supernatant was taken for spectrophotofluorometric assay of serotonin; the remainder of the supernatant was assayed for histamine as described for plasma. The procedures employed in this study were convenient because both serotonin and histamine could be determined on the same deproteinized blood sample. The details of these methods will be published in a separate communication.

RESULTS

In vitro studies

Table I shows that upon addition of a specific antigen to the whole blood of sensitized rabbits, serotonin, as well as histamine, were liberated into the plasma. The values for serotonin and histamine in the experimental plasma samples were five to eight times greater than the values for the control plasmas, handled in the same manner.

Release into plasma in vivo

Results obtained with horse serum and egg albumin in intact rabbits are shown in Figures 1 and 2. In every case, serotonin and histamine were found in the plasma following the injection of the specific antigen. Both amines appeared almost immediately, the maximum values being reached within one or two minutes. The serotonin levels were consistently lower and persisted for a shorter period of time than the histamine levels.

In an attempt to estimate the possible quantitative significance of the increased amounts of these plasma amines appearing during anaphylaxis, solutions containing both serotonin and histamine were injected rapidly into the marginal ear vein of normal rabbits, and the plasma levels of serotonin and histamine determined. The results in Figure 3 show that under these conditions, too, serotonin and histamine disappear rapidly from plasma. In order to produce levels as high as those found dur-

TABLE I

Release of serotonin and histamine in rabbit blood in vitro *

Rabbits	Number	Serotonin (γ/ml.)		Histamine (γ/ml.)	
		1:100 Horse serum	Isotonic saline	1:100 Horse serum	Isotonic saline
Sensitized to horse serum	1	3.0	0.4	2.5	0.4
	2	2.6	0.2	3.9	1.0
	3	1.4	0.2	2.0	0.7
	4	5.0	0.9	3.2	0.9
	5	2.7	0.3	3.2	0.4
	6	2.6	0.1	3.5	0.4
	Average	2.9	0.4	3.1	0.6
Normal† controls	1	0.1	0.3	0.8	1.1
	2	0.1	0.1	0.6	0.6
	3	0.3	0.3	0.8	0.3
	4	0.8	0.5	0.8	0.4
	Average	0.3	0.3	0.8	0.6
		1:100,000‡ Egg albumin	Isotonic saline	1:100,000‡ Egg albumin	Isotonic saline
Sensitized to egg albumin	1	5.6	0.7	3.0	0.9
	2	4.5	0.3	1.9	0.5
	3	5.7	0.7	3.1	0.4
	4	5.1	0.8	4.0	0.5
	Average	5.2	0.6	3.0	0.6
Normal† controls	1	0.4	0.3	0.6	0.5
	2	0.4	0.8	0.4	0.3
	3	0.5	0.7	0.5	0.8
	Average	0.4	0.6	0.5	0.5

* Whole blood was incubated at 37°C. for 30 minutes with 50 per cent by volume of saline solution (1:100 horse serum or 1:100,000 egg albumin) of the antigen. The tubes were swirled every five minutes. After centrifugation, the diluted plasma was analyzed for serotonin and histamine. The values were calculated on the basis of the original plasma.

† Some of the control levels for serotonin and histamine in these experiments are higher than usually found. This is presumed to be due to the excessive handling of the blood in setting up and carrying out the incubations with subsequent breakage of some of the platelets to release these amines into the plasma.

‡ This dilution of egg albumin was used because more concentrated solutions caused release of serotonin and histamine in normal blood.

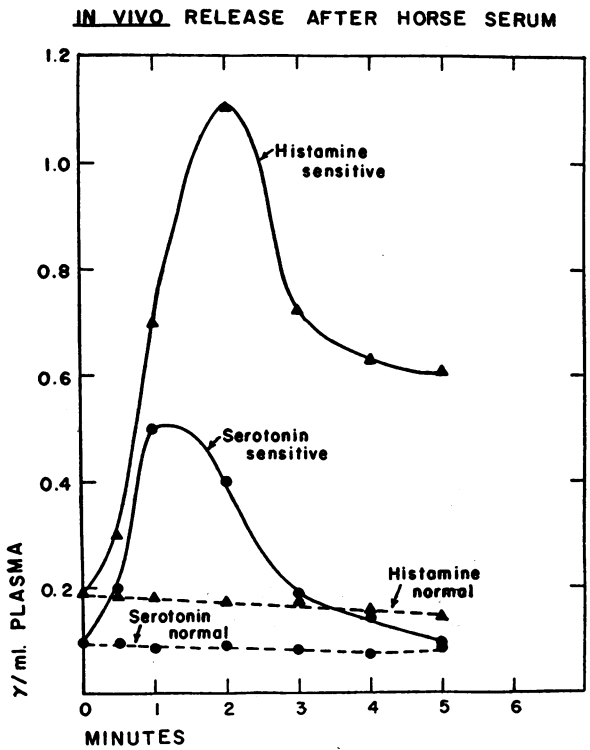

FIG. 1. *IN VIVO* RELEASE AFTER HORSE SERUM

Undiluted horse serum (1 ml. per Kg.) was injected intravenously into both sensitized and normal control rabbits, anesthetized with Nembutal® and ether. Plasma serotonin and histamine values were determined at the times indicated. The values are the averages of four sensitized animals and six normal controls.

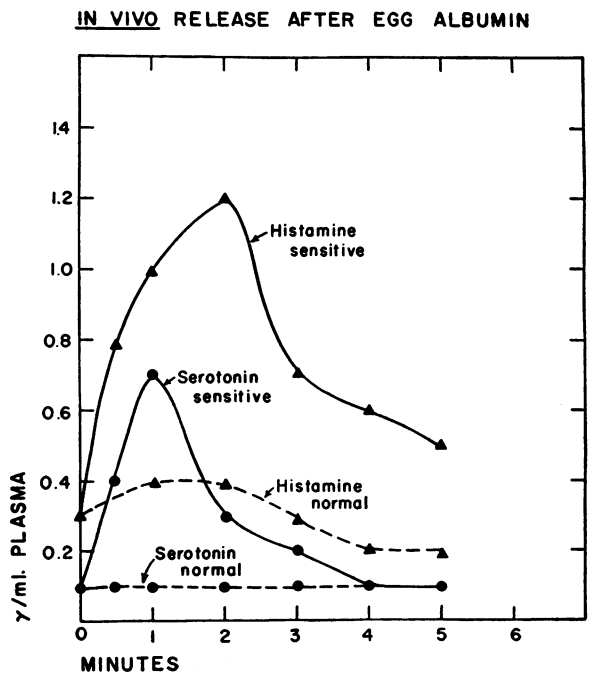

FIG. 2. *IN VIVO* RELEASE AFTER EGG ALBUMIN

Ten per cent egg albumin solution in saline (2 ml. per Kg.) was injected into both sensitized and normal control rabbits anesthetized with Nembutal® and ether. Plasma serotonin and histamine values were determined at the times indicated. The values are the averages of three sensitized animals and three normal controls.

ing anaphylaxis, it was necessary to inject at least 50 γ per Kg. of histamine and 150 γ per Kg. of serotonin. When 50 γ per Kg. of serotonin were injected, no increase in plasma serotonin could be detected.

The rabbit, during anaphylaxis, may not show many objective signs of toxicity despite the fact that chemical changes do occur. However, the most prominent toxic manifestation produced both by anaphylaxis and by injection of serotonin and histamine was respiratory in nature as shown by rapid, labored breathing. The effects following anaphylaxis were prolonged, whereas those following injection of the amines were transient.

Effect of anaphylaxis on serotonin and histamine in whole blood

Although the plasma levels of both amines were found to be increased as a result of anaphylactic shock, the total amounts of circulating serotonin and histamine were markedly diminished. This drop in total circulating amines resulted from a disappearance of blood platelets from the circulation. The experiments, shown in Figure 4 and Table II, indicate that following the injection of the antigen [1] into sensitized animals, the whole blood histamine and serotonin levels fell rapidly, as did the number of circulating platelets and leucocytes. Minimum values were reached in four minutes. The reduction in platelets persisted for one to two hours. At the end of this time the platelet counts had returned to values equal to those of the control blood samples, but the diminution of the amines persisted for at least four hours,

[1] For these experiments horse serum diluted 1:100 or 1:1,000 with saline was used since the animals often died too soon following injection of the undiluted horse serum. The diluted antigen, although very active in lowering the platelet count, increased plasma amine levels only slightly.

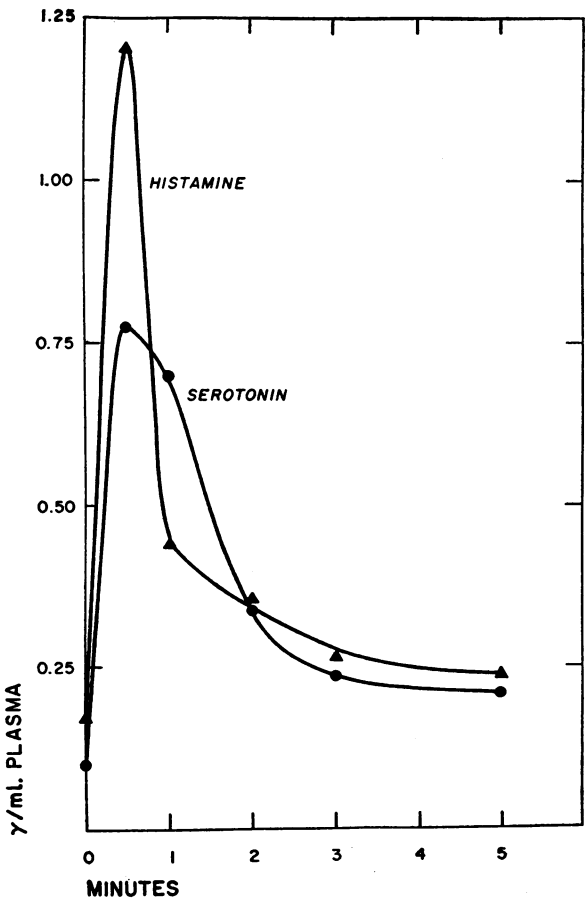

FIG. 3. PLASMA SEROTONIN AND HISTAMINE FOLLOWING INJECTION OF THE AMINES

Histamine 50 γ per Kg. and serotonin 150 γ per Kg. were injected into a marginal ear vein of a normal rabbit. Plasma was taken, after centrifugation, from blood obtained by means of a plastic catheter inserted into a carotid artery.

suggesting that there had been a release of serotonin and histamine from the platelets.

DISCUSSION

The finding that serotonin is released from platelets when antigen is added to blood from sensitized animals is in agreement with the results of Humphrey and Jaques (7), although the two experiments were not carried out in the same manner. The demonstration that histamine is released during antigen-antibody interaction is in agreement with the findings of many previous investigators (9, 10, 17).

The *in vivo* results show that both serotonin and histamine are released into plasma following the intravenous injection of a specific antigen into sensitized rabbits. Although the increase in plasma serotonin is transient, the fact that it does appear in a physiologically active form implicates serotonin along with histamine in the manifestations of anaphylaxis.

The problem of the exact site or sites in the body from which serotonin is liberated during anaphylaxis has not been solved. The *in vitro* results suggest that much of it is released from platelets. However, serotonin is found in many tissues including brain and intestinal tract, and it is important to determine whether these tissues also release serotonin during anaphylaxis. Of particular interest in this respect is the recent finding of serotonin in lung (18). A release of serotonin within this organ could be a factor in the pulmonary aspects of anaphylaxis.

The marked and prolonged diminution in circulating platelets during anaphylaxis may be an important phenomenon. First, it produces a corresponding drop in total circulating serotonin and histamine in the rabbit, although as has been pointed out above, the amounts of these free amines in the plasma are increased. Secondly, platelets are thought to play an important role in inactivating free serotonin and other amines (19) found in blood. Consequently, the paucity of circulating platelets, at a time when large amounts of amines are being released into the blood, may be an important factor in anaphylaxis.

TABLE II

Whole blood serotonin, histamine, platelets, and leucocytes during anaphylaxis *

Time	Serotonin (γ/ml. of whole blood)	Histamine (γ/ml. of whole blood)	Platelets (No./cu. mm.)	Leucocytes (No./cu. mm.)
Control	4.5	2.3	350,000	5,000
4 min.	0.8	0.6	<10,000	700
30 min.	1.2	0.9	75,000	3,200
1 hour	2.1	0.9	185,000	7,800
2 hours	3.0	1.2	385,000	10,200
3 hours	3.0	1.1	330,000	13,500
4 hours	2.9	1.1	265,000	15,000

* Whole blood serotonin and histamine levels, and platelet and leucocyte counts were determined at specific times following the injection of diluted horse serum (1:100 in saline; 1 ml. per Kg.) into sensitized rabbits. The values given represent the average of two experiments.

It is evident that serotonin release occurs during anaphylaxis in the rabbit. These studies are being extended to other animal species to determine whether serotonin liberation is generally involved in anaphylaxis. Recently, Fink (20) demonstrated the possible importance of serotonin in anaphylaxis in the mouse by the *in vitro* Schultz-Dale technique. Pallotta and Ward (21) have reported that lysergic acid diethylamide, a serotonin antagonist, will protect sensitized guinea pigs from anaphylactic shock. In addition, Rowley and Benditt (22), and West (23) have found that serotonin release is important in the anaphylactoid edema of rats produced by foreign protein. These findings suggest that serotonin release may be a factor in anaphylaxis in several animal species.

In addition to the findings concerning serotonin, this study may help to clarify the role of histamine in rabbit anaphylaxis. Previous investigators have shown that whole blood histamine values increase during anaphylaxis in the dog and guinea pig (24), while in the rabbit, whole blood histamine levels decrease (11). Because of this difference, it has even been suggested that histamine release might not be an important factor in anaphylaxis in the rabbit. It was only recently that Schachter (12) found that histamine was released into plasma during anaphylaxis in the rabbit. The present findings confirm not only the drop in the whole blood histamine, but also its release into plasma. An unusual feature about rabbits is that their platelets contain a much greater amount of histamine than do those of the dog or guinea pig. Consequently, a marked decrease in the number of platelets in rabbits causes a correspondingly marked decrease in the whole blood histamine level. This drop in whole blood histamine is so great that it obscures the transient rise in plasma histamine. In the other laboratory animals the amount of histamine present in their platelets is smaller than the amount released into plasma during anaphylaxis.

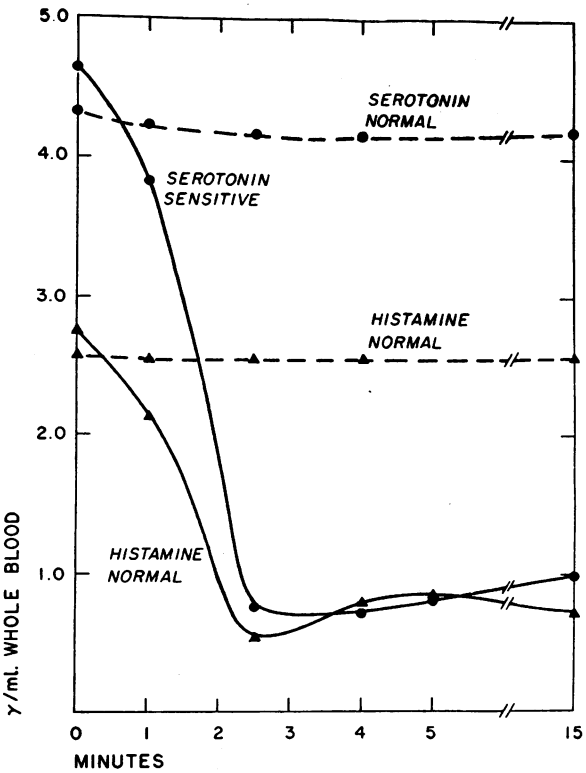

FIG. 4. WHOLE BLOOD SEROTONIN AND HISTAMINE DURING ANAPHYLAXIS

Diluted horse serum (1:100 or 1:1,000 in saline; 1 ml. per Kg.) was injected into sensitized and normal control anesthetized rabbits. Whole blood serotonin and histamine levels were determined at the times indicated. The points represent the average values obtained on seven sensitized and five control animals.

SUMMARY

1. Both serotonin and histamine were released into the plasma when a specific antigen was added to the whole blood from a sensitized rabbit.

2. Following injection of antigen into sensitized rabbits free serotonin and histamine appeared in the plasma. Maximum levels of serotonin and histamine were reached in one minute and declined to control values within two to three minutes. Histamine rose to higher levels than serotonin and declined more slowly.

3. Anaphylaxis was also accompanied by a marked fall in the levels of serotonin and histamine in whole blood due to a decrease in the number of circulating platelets. The diminished values were maintained for over two hours.

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