HISTAMINE AND 5-HYDROXYTRYPTAMINE RELEASE IN ANAPHYLAXIS OF GUINEA-PIG TISSUES

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Abstract—Histamine and 5-HT releases in in vitro anaphylaxis were measured spectrophotofluorometrically by immersing passively and actively sensitized guinea-pig small intestine, lung and aorta in Tyrode solution containing antigen. Histamine was released from sensitized tissues by an antigen. Values from three tissues were almost the same in both passive and active sensitizations. In passive sensitization, histamine and 5-HT were released spontaneously from tissues during and after sensitization. In active sensitization, histamine release from tissues increased along with an increase in concentration of antigen, while no increase was seen with 5-HT. 5-HT was released only spontaneously from both passively and actively sensitized tissues.

A previous work using the fluorometrical method showed that a definite amount of histamine was released from sensitized guinea-pig small intestine in anaphylaxis but that 5-hydroxytryptamine (5-HT) was undetectable owing to a disturbance by an interfering substance and/or to low sensitivity of the method (1).

The objective of the present experiments was to determine the anaphylactic release of histamine and 5-HT from the lung and aorta as well as from the small intestine of guinea-pig. Histamine release was demonstrated from all these tissues, and 5-HT release was also successfully determined by a different method from that used previously.

MATERIALS AND METHODS
Male guinea-pigs weighing about 600 g at the time of sacrifice were used. In experiment I, animals were divided into 2 groups: a) nonsensitized animals, from which the small intestine, lung and aorta were to be passively sensitized with serum of immunized rabbit and b) nonsensitized, control for group a), from which the three tissues were to be incubated with serum of non-immunized intact rabbit. In experiment II, 3 groups (c, d and e) of nonsensitized animals were used and the three tissues were incubated in Tyrode solution for 5 min (c), 15 min (d) and 60 min (e). In experiment III [1) and 2)], animals were divided into 4 groups: f), g) and h), actively sensitized animals, from which tissues were immersed in Tyrode solution for 5 min and the antigen was added in amounts of $2.5 \times 10^{-5}$ g/ml (f), $10^{-4}$ g/ml (g) and $10^{-3}$ g/ml (h), and i) nonsensitized control for group f).

Each group included 5 guinea-pigs. These were adapted to the laboratory conditions for at least 7 weeks prior to sacrifice. Animals were fed on a commercial solid diet.
(Oriental Co., Japan), and water was provided ad libitum.

Methods used for sensitization, precipitin test, in vitro anaphylaxis with crystalline ovalbumin (Sigma Chemical Co., U.S.A.) and chemical assay of histamine were similar to those previously employed by the authors (1). Actively sensitized guinea-pigs were obtained by injecting s.c. 5 mg of ovalbumin together with 0.5 ml of Freund's complete adjuvant (Difco Laboratories, U.S.A.) and after a month, a single booster with 20 mg of the same antigen without the adjuvant was given. Seven days after the last injection, the animals were fasted overnight, sacrificed by a blow on the head and exsanguinated by decapitation. The small intestines weighing about 10 g were excised by cutting both ends after removal of the mesenteries. Immediately after, these were washed in Tyrode solution saturated with oxygen at 37°C for a few min. After removing almost the entire contents of each, both ends were bound. These were used for the anaphylaxis test without cutting them into segments. The lungs and aorta were excised and washed in Tyrode solution saturated with oxygen at 37°C for a few min. Weight was about 3.5 g for the lungs and about 140 mg for the aorta.

In passive sensitization, the serum was obtained from a highly sensitized rabbit, lyophilized and stored in a deep freezer, then used for passive sensitization in a dilution of 1:200 with Tyrode solution.

The tissues of actively sensitized guinea-pigs were incubated in 20 ml Tyrode solution at 37°C in 50 ml conic flasks and stirred by bubbling oxygen. The antigen was then added and the whole was shaken at a low speed for 5 min. Tyrode solution was then filtered and divided into 2 ampoules. Immediately after, each was lyophilized and stored in a deep freezer until the assay of histamine and 5-HT, respectively.

Passively sensitized and nonsensitized tissues were similarly incubated in 20 ml Tyrode solution at 37°C, well stirred by bubbling oxygen. The antigen was added and the whole was shaken for 5 min. Tyrode solution was then similarly treated as described above for active sensitization.

Histamine was measured spectrophotofluorometrically by the method of Shore et al. (2). Determination of 5-HT was carried out by the method of Udenfriend et al. (3) replacing Saikawa's modification (4) for the method of Weissbach et al. (5). The values of determination are expressed in µg bases per g of dry tissue wt.

RESULTS

I. Release of the mediators from passively sensitized guinea-pig tissues

Table 1 (A and B) summarizes the results of the determination of histamine and 5-HT released in Tyrode solution from passively sensitized guinea-pig tissues in anaphylaxis during 5 min incubation with 2.5×10^-8 g/ml antigen. The values of histamine release from the small intestine, lungs and aorta were 0.277±0.033, 1.205±0.179 and 6.572±0.990 µg/g, respectively. Those of 5-HT were 0.131±0.007, 0.081±0.024 and 2.884 µg/g, respectively.

The histamine release was significantly different in amount from that of the control,
TABLE 1. Histamine and 5-HT release from passively sensitized tissues by antigen.

| Group | Treatment | No. of determin. | Small intestine | Lungs | Aorta |
|-------|-----------|------------------|----------------|-------|-------|
| a     | Sensitization | 5               | 0.277±0.033** | 1.205±0.179** | 6.572±0.990** |
| b     | Control*    | 5               | 0.048±0.023    | 0.086±0.041 (2) | 3.498±1.243 (3) |

B) 5-HT (µg/g±S.E.)

| Group | Treatment | No. of determin. | Small intestine | Lungs | Aorta |
|-------|-----------|------------------|----------------|-------|-------|
| a     | Sensitization | 5               | 0.131±0.007    | 0.081±0.024 (2) | 2.884 (1) |
| b     | Control*    | 5               | 0.122±0.051    | 0.049±0.017 (2) | undetectable |

* Tissues were incubated in Tyrode solution containing non-immunized rabbit serum in dilution of 1:200 for 60 min at 37°C.
** P<0.01
Numbers in parentheses refer to number of detected determinations.
Antigen: 2.5×10⁻⁵ g/ml, for 5 min.

TABLE 2. Spontaneous release of mediators from tissues in Tyrode solution containing rabbit antiserum in dilution of 1:200 during a 60 min-passive sensitization.

| Tissues    | No. of determin. | Histamine (µg/g±S.E.) | 5-HT (µg/g±S.E.) |
|------------|------------------|-----------------------|-----------------|
| Small intestine | 5               | 0.202±0.061           | 0.770±0.063     |
| Lungs      | 5               | 0.475±0.023           | 0.524±0.030     |
| Aorta      | 5               | 3.607±1.576           | 1.533±0.759 (3) |

Number in parenthesis refers to number of detected determinations.
An antigen was not added.

TABLE 3. Effect of incubation time on spontaneous release of histamine and 5-HT from nonsensitized tissues in Tyrode solution.

| Group | Incubation time (min) | No. of determin. | Small intestine | Lungs | Aorta |
|-------|-----------------------|------------------|----------------|-------|-------|
| a     | 5                     | 5                | 0.055±0.014    | 0.215±0.016 | 2.957±0.595 |
| d     | 15                    | 5                | 0.103±0.015*   | 0.290±0.024 | 4.565±1.559 |
| e     | 60                    | 5                | 0.145±0.008*   | 0.259±0.025 | 4.611±1.060 |

B) 5-HT (µg/g±S.E.)

| Group | Incubation time (min) | No. of determin. | Small intestine | Lungs | Aorta |
|-------|-----------------------|------------------|----------------|-------|-------|
| c     | 5                     | 5                | 0.057±0.003    | 0.056±0.005 (3) | 0.532±0.101 (3) |
| d     | 15                    | 5                | 0.123±0.014**  | 0.115±0.017* (3) | 2.545±0.524 (2) |
| e     | 60                    | 5                | 0.434±0.066**  | 0.130±0.013 | 1.154±0.113 (3) |

* P<0.05 comparison between c and d, and d and e.
** P<0.01 comparison between c and d, and d and e.
Numbers in parentheses refer to number of detected determinations.
but 5-HT release, was not always detectable. Moreover, both histamine and 5-HT release were detectable in some controls. It was presumed, that part of the mediators may be released spontaneously from tissues in Tyrode solution during 60 min passive sensitization with a serum dilution (1: 200) of immunized rabbit. As shown in Table 2, considerable amounts of both histamine and 5-HT were released spontaneously from the tissues.

**II. Effect of incubation time on spontaneous release of mediators from nonsensitized tissues in Tyrode solution**

As the mediators were spontaneously released from tissues during passive sensitization, assay was made on histamine and 5-HT release from nonsensitized tissues during incubation in Tyrode solution for 5 min (c), 15 min (d) and 60 min (e). As shown in Table 3 (A and B), release of both histamine and 5-HT from tissues increased with increase in incubation time.

**III. 1) Release of the mediators from actively sensitized guinea-pig tissues**

Table 4 (A and B) summarizes the results of determinations of histamine and 5-HT released in Tyrode solution from actively sensitized guinea-pig tissues in anaphylaxis during 5 min incubation with $2.5 \times 10^{-6}$ g/ml antigen.

### Table 4. Histamine and 5-HT release from actively sensitized tissues.

| Group | Treatment | No. of determin. | Small intestine | Lungs | Aorta |
|-------|-----------|------------------|-----------------|-------|-------|
| f     | Sensitization | 5                | 0.261±0.032*    | 0.909±0.064* | 2.382±0.566 |
| i     | Control     | 5                | 0.029±0.009     | 0.182±0.017 (2) | 3.752±0.140 (2) |

| Group | Treatment | No. of determin. | Small intestine | Lungs | Aorta |
|-------|-----------|------------------|-----------------|-------|-------|
| f     | Sensitization | 5                | 0.128±0.027     | 0.064±0.009 | undetectable |
| i     | Control     | 5                | 0.057±0.010 (4) | 0.053±0.029 (2) | undetectable |

* P<0.01
Numbers in parentheses refer to number of detected determinations.
Tissues were incubated in the presence of an antigen for 5 min at 37°C.
Antigen : $2.5 \times 10^{-6}$ g/ml.

Histamine release from the small intestine, lungs and aorta was 0.261±0.032, 0.909±0.064 and 2.382±0.566 μg/g, respectively. The 5-HT release was 0.128±0.027 and 0.064±0.009 μg/g and was undetectable in any determination in the aorta. The results were quite similar to those in passive sensitization.

2) **Effect of concentration of antigen on the release of mediators from actively sensitized guinea-pig tissues**

Figs. 1 and 2 summarize the results of determinations of histamine and 5-HT released in Tyrode solution from actively sensitized tissues in groups f), g) and h). Histamine release from tissues increased along with the increase in concentration of antigen. The
5-HT release from tissues, however, showed no significant change, with the amount in the aorta particularly insignificant. In none of 5 determinations of group f) (antigen: $2.5 \times 10^{-5}$ g/ml) was it detectable, and in only one case of groups g) (antigen: $10^{-4}$ g/ml) and h) (antigen: $10^{-3}$ g/ml) was it detectable: 1.017 and 1.103 µg/g, respectively.

**DISCUSSION**

There are a few reports on histamine release from thin pieces of guinea-pig small intestine determined by biological assay (6, 7). Using the fluorometrical method, this was...
ascertained utilizing a totally excised small intestine which had not been segmented (1). The present experiments demonstrated this on all three tissues in question.

Attempts to demonstrate 5-HT release from the sensitized guinea-pig ileum have been unsuccessful (8, 9). According to Engelhardt (10), 5-HT was released from the small intestine and spleen but not from the lung in acute anaphylactic shock. In his study, 5-HT release was not directly determined, but was estimated by comparing the content in tissues before and after anaphylactic shock.

In a previous work (1), Saikawa’s modification (4) of Weissbach et al’s fluorometric method (5) failed to demonstrate 5-HT release from sensitized guinea-pig small intestine. In the present experiments, however, 5-HT release from passively sensitized tissues was successfully demonstrated by the method of Udenfriend et al. (3). This may be attributed to difference in wave lengths of excitation and emission, however, as aforementioned, there was no significant difference in 5-HT values between sensitized and nonsensitized tissues. Therefore 5-HT release was presumed to be spontaneous, with no relation to anaphylaxis. In fact 5-HT was demonstrated also in Tyrode solution with antiserum after incubation of the tissue. 5-HT release was then determined from three tissues immersed in Tyrode solution without antiserum for 5, 15 and 60 min, and it was found that 5-HT release increased along with the increase in incubation time.

As it was demonstrated that 5-HT was spontaneously released from all three tissues tested during passive sensitization, 5-HT release was then determined from actively sensitized tissues. It was found to be released from three tissues by an antigen, but no significant difference was observed. Also no significant change occurred when the amount of antigen was increased. Contrastly, histamine release from these three tissues increased along with the increase in amount of antigen, as was shown in a previous work on passively sensitized small intestine.

From the results of the present experiments, it is concluded that histamine was released from tissues in anaphylaxis, while 5-HT was released only spontaneously.

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