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A SEA ANEMONE EXTRACT (THALASSINE) WHICH LIBERATES HISTAMINE AND A SLOW CONTRACTING SUBSTANCE

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The discovery of anaphylaxis was made during a study of the toxicity of extracts of the stinging tentacles of various coelenterates such as *Physalia* and *Actinia* (Portier and Richet, 1902; Richet, Perret, and Portier, 1902; Richet, 1905). From a crude extract of *Actinia* tentacles these workers separated three pharmacologically distinct fractions which on intravenous injection into dogs produced severe generalized pruritus, pulmonary oedema, or sedation. They termed these fractions thalassine, congestine, and hypnotoxin respectively. Thalassine, the pruritogenic fraction, is obtained as a stable, dry powder by repeated alcohol extraction.

Since generalized pruritus occurs in dogs after the systemic administration of various drugs known to release histamine (Paton and Schachter, 1951), and since cutaneous reactions may be produced in man by the local application of anemone tentacles (Gosse, 1860; Stephenson, 1935), it seemed to us that thalassine might cause the release of histamine.

The present experiments demonstrate that thalassine does in fact release histamine from the isolated, perfused cat skin. On intravenous injection into cats it produces a prolonged increase in the plasma histamine concentration, and also liberates a substance producing a delayed, slow contraction of the guinea-pig ileum.

METHODS

Cats were anaesthetized with chloralose (80 mg./kg.) intravenously, preceded by ethyl chloride–ether induction. Blood samples taken from the femoral artery were heparinized, centrifuged, and the plasma tested for histamine on the isolated guinea-pig ileum. Blood pressure was recorded from the carotid artery by a mercury manometer.

Cat skin preparations were prepared by the method of Feldberg and Paton (1951) as modified by Feldberg and Schachter (1952). Injections were made into the saphenous artery in 0.5-ml. volumes, and the perfusate collected from the corresponding vein. The infusion was arrested for 10–20 sec. during the injection, thus permitting the injected material to remain in contact with the tissues for this period.

Histamine assay of perfusates and plasmas was carried out on the isolated guinea-pig ileum suspended in magnesium-free Tyrode solution in an 18-ml. bath. Atropine (0.2 μg.) was added to the bath after each test to abolish the spontaneous activity of the preparation. Mepyramine (0.5 μg.) was added when required to antagonize the histamine activity of the test samples.

Preparation of Thalassine from Anemone Tentacles.—Thalassine was prepared from anemone tentacles by repeated alcohol extractions, as described by Richet (1905). Sea anemones (mostly *A. equina*, but including a small proportion of *A. sulcata*) were obtained from the south coast of England and kept alive in sea water until the chemical extraction was begun. From approximately 700 g. of these tentacles, 2.85 g. of dry material was obtained. The powder was readily soluble in distilled water in amounts up to 100 mg./ml. A 1.0% (w/v) solution in saline was slightly acidic (*p*H 6.5) because acetic acid was used at one stage of the extraction to facilitate precipitation of the active material.

Two thalassine preparations were made from separate batches of sea anemones. One was more effective as a histamine liberator and was used in most experiments. Neither preparation had any effect on the guinea-pig ileum, even in high concentrations, and contained less than 0.5 μg. histamine per gram of dried powder. Fresh tentacle itself also contained only small amounts (2.0–6.5 μg./g.) of histamine on extraction with hydrochloric acid.

RESULTS

Release of Histamine From Perfused Cat Skin by Thalassine

Thalassine (0.05–5.0 mg.) was injected intra-arterially into perfused cat skin preparations in six

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TABLE I

RELEASE OF HISTAMINE FROM PERFUSED CAT SKIN FOLLOWING THE INTRA-ARTERIAL INJECTION OF SEA ANEMONE TENTACLE EXTRACT (THALASSINE)

	Amount of Thalassine Injected (mg.)	Total Histamine Collected in Perfusate (μg.)
Less potent preparation	5·0	35·0
	2·0	37·3
	0·25	0·9
More potent preparation	0·5	31·7
	0·1	16·8
	0·05	3·1

experiments. Histamine was released in every instance. The total amount collected in the perfusate in each experiment is shown in Table I. The minimal dose of thalassine required to release histamine from the isolated skin was 0.05 mg. The corresponding threshold dose of compound 48/80 was 0.001 mg. After the intra-arterial injection of thalassine, as of 48/80, the histamine concentration in the effluent was greatest in the first 10-minute sample in five of the six experiments. In one experiment, however, the release was more protracted and the histamine concentration was highest in the second 10-minute sample. The perfusates remained clear throughout, and their activity on the guinea-pig ileum was completely abolished by mepyramine.

Release of Histamine by Intravenous Injection of Thalassine in the Intact Cat

Thalassine (10 mg./kg.) was injected intravenously in six cats after cervical section of the vagus nerves. After the injection all animals showed a prolonged fall of the arterial blood pressure, with only partial recovery in one hour. The fall of blood pressure was regularly delayed for approximately 30 seconds following the injection. This delay is characteristic of substances whose main circulatory depressor action is due to the release of histamine (MacIntosh and Paton, 1949). In two experiments, thalassine also caused a slight, short-lasting, immediate fall of the blood pressure which recovered before the onset of the severe prolonged depression. Fig. 1 shows the prolonged circulatory response to thalassine, together with the short-lasting immediate fall that sometimes precedes it.

Blood samples for plasma histamine assay were taken from the femoral artery before, and at intervals of 4 to 90 minutes after, the intravenous injection of thalassine. Injection of thalassine always produced a considerable increase in plasma histamine concentration which was still detectable one hour later. The results of these experiments are shown in Table II. The histamine releasing effect of thalassine solutions was unaffected by heating for five minutes in a boiling water bath.

TABLE II

PLASMA HISTAMINE CONCENTRATIONS (μG./ML.) BEFORE AND AT VARIOUS INTERVALS AFTER THE INTRAVENOUS INJECTION OF THALASSINE (10 MG./KG.) INTO CATS

Exp. No.	Before Injection	Minutes after Injection 4	10	40	60	90
1	0·02	0·10	0·14	—	0·05	—
2	0·03	0·06	0·20	0·18	0·17	0·10
3	0·04	0·10	0·15	0·18	0·17	0·06
4	0·03	—	0·18	0·30	0·25	—
5	0·01	0·08	0·14	0·12	—	—
*6	0·02	0·11	0·21	0·28	0·17	0·12

* Solution heated in boiling water bath for 5 minutes before injection

In assaying the post-injection plasma samples for histamine, it became apparent that the later samples produced a delayed contraction of the guinea-pig ileum. However, by using small amounts of plasma for histamine assay, 0.2 ml. or less, it was possible to eliminate this effect. The quantitative nature of the plasma histamine assay was subsequently confirmed by the complete abolition of the effect of 0.2 ml. of plasma on the

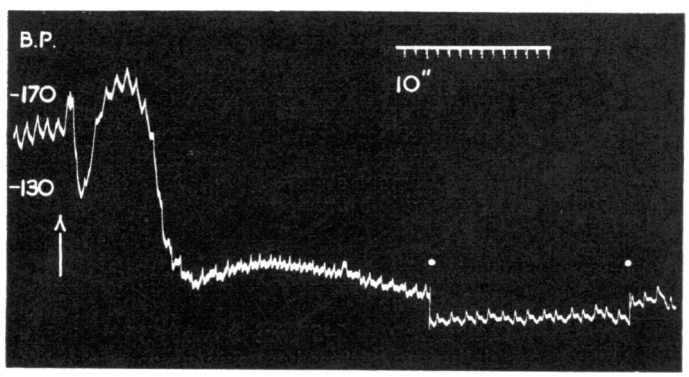

FIG. 1.—Prolonged depression of arterial blood pressure of a cat following intravenous injection of thalassine. Cat 3.0 kg., vagi cut in neck. Thalassine, 10 mg./kg., injected at arrow. Recording stopped for 15 minute intervals at dots.

guinea-pig ileum by mepyramine. The delayed contraction was itself subsequently studied.

Effect of Plasma, Obtained After Injection of Thalassine, in Producing a Delayed, Slow Contraction of the Guinea-pig Ileum

The effect of cat plasma taken before and at intervals after the intravenous injection of thalassine (10 mg./kg.) was tested on the guinea-pig ileum in the presence of mepyramine. The addition to the bath of 1.0 ml. of plasma obtained 30 minutes after injection of thalassine produced a marked contraction of the guinea-pig ileum, whereas control plasma was either without effect or produced a much smaller contraction. Such an effect was regularly observed in five experiments and the results of two of these are shown in Fig. 2. This smooth muscle stimulating action of

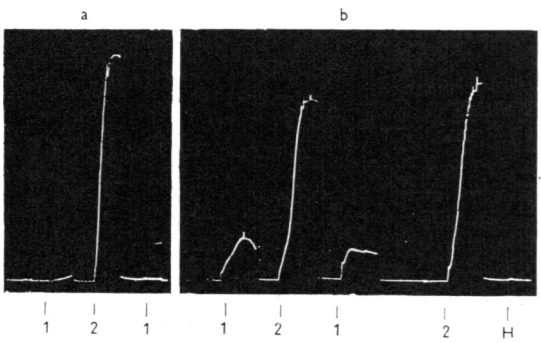

FIG. 2.—Effect of cat blood plasma taken before and 30 minutes after the intravenous injection of thalassine (10 mg./kg.) on the isolated guinea-pig ileum in the presence of atropine (0·2 μg.) and mepyramine (0·5 μg.). a, and b, are results of separate experiments. The following were added to the 18 ml. bath :— 1, 1.0 ml. cat plasma taken before injection of thalassine. 2, 1.0 ml. plasma obtained 30 minutes after injection. H, 0.3 μg. histamine.

plasma differs from that of histamine, acetylcholine, 5-hydroxytryptamine, and other smooth muscle stimulants, in that there is a delay of 10–20 seconds before contraction begins and that a longer interval, usually about 60 seconds, is required for the contraction to reach its maximum. This property of cat plasma was regularly present 30 minutes after the injection of thalassine and gradually disappeared, becoming undetectable in 90 minutes.

In one experiment, plasma obtained 30 minutes after injection of thalassine produced a greater contraction of the rat colon than did control plasma. Since this effect persisted after tryptamine desensitization it could not be caused by 5-hydroxytryptamine ; it would thus appear to be due to the same substance as produces the delayed, slow contraction of the guinea-pig ileum.

DISCUSSION

The demonstration that an alcoholic extract of sea anemone tentacles is an effective histamine releaser suggests that the pruritogenic activity of anemone extracts is due to this property. It is possible that histamine releasing substances may be widespread in nature and may be present in secretions of various organisms, such as jellyfish, lice, mites, cercaria, etc., which provoke local pruritus on contact with human skin. The potency of our thalassine preparation, which is not chemically homogeneous, is approximately 1/50th that of compound 48/80, one of the most potent histamine releasing substances hitherto studied. If the histamine releasing property of thalassine derives from a single substance, this would appear to be of the same order of potency as 48/80. The histamine releasing property, however, represents only one of the pharmacological effects of anemone tentacles. Thus, even large doses of thalassine failed to produce pulmonary oedema, whereas severe pulmonary exudation followed the injection of small amounts of a saline extract of tentacles.

The ability to cause the appearance in plasma of a substance producing a delayed, slow contraction of the guinea-pig ileum is not confined to thalassine. For example, Feldberg, Holden, and Kellaway (1938) demonstrated a " slow reacting substance " in lung perfusates following perfusion with snake venoms ; Beraldo (1950) reported the release of " bradykinin " during anaphylactic shock in dogs ; and Paton (1951) has recently described a similar activity in cat plasma after injection of 48/80. Since thalassine, snake venoms, antigen and 48/80 all release histamine, it seems that the appearance of a slow reacting substance is closely associated with the process of histamine release, as previously suggested by Paton (1951). However, although we regularly detected this slow reacting activity in cat plasma following injection of thalassine into the whole animal, it was never detected in perfusates from cat skin preparations after intra-arterial injection of thalassine—despite the release of histamine. It would thus seem that histamine can be released from tissues without the release or formation of a slow contracting substance. It is also probable that there exists a variety of substances able to provoke a slow contraction of the guinea-pig ileum.

SUMMARY

1. Thalassine, an alcohol extract of sea anemone tentacles, has been shown to possess potent histamine releasing activity.

2. It regularly released histamine from the perfused cat skin, and raised the plasma histamine concentration in this species following its intravenous administration.

3. On intravenous injection in the cat, thalassine also caused the appearance in plasma of a substance producing a delayed, slow contraction of the guinea-pig ileum.

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