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Abstract

1. Quantitative examinations were made on the effect of benadryl and neoantergan on the histamine release in vitro from chopped skin of dogs and in vivo from rat skin. For estimation of the in vitro histamine release by biological method, a chemical procedure for separating the diffused-out histamine from mixed antihistamines was carried out. 2. Both antihistamines caused a fairly marked release of histamine from chopped skin tissues in comparatively higher concentrations. This action was synergistic with histamine-releasing effect of sinomenine and anaphylatoxin. 3. In lower concentrations, however, both antihistamines inhibited the in vitro histamine-releasing effect of sinomenine and anaphylatoxin. 4. Administration of a comparatively large amount of benadryl markedly depleted the skin histamine of a rat in vivo but smaller amount clearly suppressed the histamine depletion by subsequently administered sinomenine. 5. Based on the evidence of such dual action of antihistamines, some considerations were made on the site of action of these agents.

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DUAL ACTION OF ANTIHISTAMINES ON HISTAMINE RELEASE*

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It is well known that various antihistamines possess several actions other than specific antagonism against pharmacological actions of histamine[1]. Recent suggestion that these actions of antihistamines may include some effects related to histamine release from tissue cells is of much interest, because this is an important problem to be considered in evaluating antagonistic effect of these agents against histamine, either administered or released under various pathological conditions.

PELLERAT and MURAT[2] reported an increase of histamine in the human blood after parenteral administration of 2339 RP or 2789 RP. Later, OHKURA[3] observed similar phenomenon in the dog plasma after an intravenous administration of benadryl. In his *in vitro* experiment ARUNLAKSHANA[4] observed that histamine was released from human lung tissue by benadryl equally srtong as that by compound 48/80. According to more recent studies of SANUKI and others[5], hypothermia in mice and cutaneous reaction in rabbits caused by histamine or a certain histamine liberator were not antagonized by a large amount of benadryl or neoantergan but were rather strengthened.

On the other hand, there are some indications of the possibility that release of tissue histamine is inhibited by antihistamines. RILEY[6] showed that premedication of antihistamine prevented disruption of tissue mast cells, which are considered to be the chief location of tissue histamine, by chemical histamine liberators or anaphylatoxin. KAWAMOTO[7] confirmed that this is also true in the case of *in vitro* experiments.

The evidences presented by these workers point to the neccessity of systematic studies on the mode of action of these synthetic antihistamines on histamine release in order to understand more correctly the effect of

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these agents against the action of histamine displayed in a biological subject. In the present experiments in accordance with this demand, quantitative examinations were made on the effects of antihistamines in relation to their dosage on the *in vitro* histamine release from dog skin by sinomenine[8,9], a chemical histamine liberator, and anaphylatoxin, and on the *in vivo* histamine release from rat skin by sinomenine.

METHODS

Histamine liberator substances and antihistamines. — As standard histamine liberator substances, sinomenine hydrochloride (Shionogi Research Laboratories) and anaphylatoxin were used. Latter was prepared from rat serum according to the method of BORDET[10]. Benadryl and neoantergan maleate were chosen for antihistamines to be tested. Their concentrations were all expressed in per cent, though the molar ratio of benadryl : neoantergan is approximately 7 : 5.

Determination of in vitro histamine release from dog skin. — Dogs weighing 4—5 kg. were sacrificed by bleeding, the skin on the flanks and inner side of the thigh was shaved with a hair clipper, washed, and

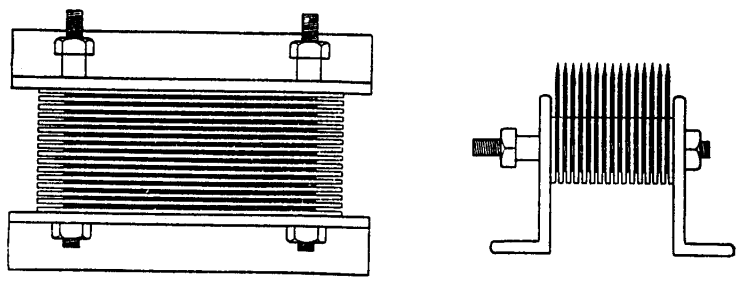

Fig. 1. The chopper for skin. Left: plan; right: end elevation. About 5/6 original size. Somewhat diagrammatic. *See* Text.

excised. The piece of skin from which subcutaneous tissues had been removed was pinned to the original size on a cork board and cut into many fine strips with a tissue chopper having razor blades fixed in parallel at 0.7-mm. intervals (Fig. 1). A series of flat-bottomed test tubes, each containing 150 mg. of the chopped skin tissue, 1 cc. of 0. 9 per cent sodium chloride solution, and one glass bead (diam., 3 mm.), were suspended in a constant-temperature bath of 37°C and shaken horizontally

at 100 rounds per minute. The diffusion rate of histamine liberated spontaneously from tissue pieces by this procedure was larger at the beginning, approximately 60 per cent of the total histamine liberated during 60 minutes having been released in the first 15 minutes. After 15 minutes of this procedure, the saline solution in the tubes was exchanged with new saline solution containing a histamine liberator substance and/or an antihistamine to be tested, for the latter in graded concentration, and that of remaining one tube with a simple saline solution (for spontaneous release), in order to determine later release of histamine. After the rocking for further 60 minutes, the saline media containing diffused-out histamine was taken out with the pipette described by MONGAR and SCHILD[11], filtered, and boiled for 1 minute. For the determination of residual histamine, the tissue pieces were ground up and boiled for 3 minutes with 2 cc. of saline solution. The amount of water lost by evaporation was supplemented and these solutions were submitted to the histamine assay. For solutions containing antihistamine the following procedure for separation of histamine were needed before the assay.

Separation of histamien from the test solution containing antihistamine. — It was ascertained that the method used by WARTHERT[12] was suitable for the purpose of removing benadryl or neoantergan from its mixture with histamine. By the use of this method, histamine can be recovered from its mixture in a good yield of 94—97 per cent. The procedure used was as follows :

The test solution was diluted to 10 cc. with distilled water and 2 cc. of saturated barium hydroxide solution was added. This solution colored phenolphthalein paper rose pink. The solution was shaken with three 10 cc. portions of ether for 10 minutes each. To the separated aqueous layer, 0.6 cc. of N-sulfuric acid was added and the barium sulfate precipitate thereby formed was centrifuged off. The supernatant was filtered, evaporated to dryness on a boiling water bath, neutralized with N-sodium hydroxide, and diluted to a definite quantity with distilled water. This solution was submitted to the histamine assay.

In the same series of experiment, similar treatment was made with the saline media when only histamine liberator substance other than antihistamine was used.

Method of histamine determination and calculation of the rate of histamine release. — The amount of histamine in the neutralized test solution was determined by terminal ileum preparation of a guinea pig, suspended in a bath of 10 cc. of Tyrode solution containing 0.05 μg./cc. of atropine sulfate. It was ascertained that the height of ileum contrac-

tion by the test solution was suppressed by neoantergan to the same extent as that by equiactive dose of histamine. The percentage rate of histamine release *in vitro* by test agents was calculated from the following equation:

$$\left(\frac{Ct}{Ct + Cr_1} - \frac{Cs}{Cs + Cr_2}\right) \times 100\ \%$$

where Ct is the total release of histamine in the drug saline media, Cs is the amount of histamine by spontneous releaset, and Cr_1 and Cr_2 are the residual amount of hisamine in the tissue. The histamine content in the skin of afore-mentiond locations from 24 dogs was 4.9 — 18 μg./g., an average of 8.9 μg./g., and spontaneous release during the period exposed to the agents (60 minutes) was 4.8 — 21 per cent, an average of 7.5 per cent.

Determination of histamine release in vivo from rat skin. — From male albino rats weighing 100—150 g., 150—200 mg. of skin each was excised from one side of the abdomen under ether anesthesia and the incision wound was sutured aseptically. After three days (this interval was provided for the recovery of any change in skin histamine which may be caused by remote injuries[13]), sinomenine (intraperitoneally) was administered and a skin specimen from the other side of the abdomen was similarly obtained after an hour. When both benadryl and sinomenine were administered to the same rat, they were given in this order with 30 minutes' interval. The hair and subcutaneous tissues were removed from the excised skin, placed in a boiling water bath with 5 cc. of N-hydrochloric acid, and ground with quartz sand treated with hydrochloric acid. This was centrifuged, an aliquot of the supernatant was neutralized, and submitted to histamine assay. Percentage of histamine release was calculated from the contents of skin histamine before and after the injection of drugs.

RESULTS

1. Effect of antihistamines on the in vitro histamine release from dog skin

Effect of antihistamines on histamine release by sinomenine.—Liberation of histamine from the chopped skin of a dog by 0.2 — 1.0 per cent of sinomenine was clearly increased by the presence of 0.2 per cent of benadryl, but the action of sinomenine was inhibited by benadryl in concentrations below 0.05 per cent. This inhibitory action of benadryl was observed in a highly diluted solution such as 0.0001 per cent, but was

most marked in the range of 0.01 — 0.005 per cent (Table 1). The effect of neoantergan in increasing the histamine-releasing action of sinomenine seemed to be somewhat weaker than that of benadryl in the same per cent concentration. Neoantergan also inhibited this action of sinomenine in

Table 1. *In vitro* histamine release from chopped skin of dogs. Combined effect of sinomenine and benadryl.

Agents (%)	Percentage histamine release			
	I	II	III	Average
Sinomenine 1.0	8.5	6.8	30.2	*15.2*
„ + Benadryl 0.2	9.0	9.2	32.0	*16.7*
„ + „ 0.01	5.6	3.0	21.6	*10.1*
„ + „ 0.001	6.2	2.0	20.7	*9.6*
	IV	V	VI	Average
Sinomenine 0.2	13.9	13.2	12.4	*13.2*
„ + Benadryl 0.2	22.1	16.5	26.8	*21.8*
„ + „ 0.05	11.5	7.6	6.8	*8.6*
„ + „ 0.01	7.9	3.8	2.5	*4.7*
„ + „ 0.005	6.9	4.5	3.0	*4.8*
	VII	VIII	IX	Average
Sinomenine 0.2	14.0	12.2	12.8	*13.0*
„ + Benadryl 0.005	4.0	5.1	5.0	*4.7*
„ + „ 0.0001	10.0	9.3	8.9	*9.4*
„ + „ 0.00001	13.5	12.2	12.7	*12.8*
„ + „ 0.000001	13.8	12.1	12.7	*12.9*

Note. — Roman figures denote serial number of dogs used. This number is provided separately for each table.

concentrations below 0.01 per cent. When neoantergan was added to the medium 15 minutes prior to the application of sinomenine, the inhibitory effect of neoantergan did not practically differ from when both were added at the same time though the synergistic effect of 0.2 per cent of it could not be observed (Table 2).

Effect of antihistamines on the histamine release by anaphylatoxin.— The effect of benadryl on the release of histamine from chopped skin of a dog by rat serum anaphylatoxin was also dual according to the concentration as observed for histamine release by sinomenine. The synergistic effect was more marked in this case (Table 3).

Histamine releasing action of antihistamines. — In concentrations

Table 2. *In vitro* histamine release from chopped skin of dogs. Combined effect of sinomenine and neoantergan.

Agents (%)	Percentage histamine release			
	I	II	III	Average
Sinomenine 0.2	10.6	11.4	19.0	*13.8*
„ + Neoantergan 0.2	10.8	12.2	19.5	*14.2*
„ + „ 0.01	1.1	5.8	12.4	*6.4*
„ + „ 0.001	1.4	5.0	11.7	*6.0*
	IV	VI	VII	Average
Sinomenine 0.2	26.3	1.8	17.6	*18.6*
„ + Neoantergan 0.2*	14.4	7.9	16.8	*13.0*
„ + „ 0.01*	5.9	5.4	6.0	*5.8*
„ + „ 0.001*	6.5	5.8	6.2	*6.2*

* Sinomenine was added after 15 minutes' exposure to neoantergan.

Table 3. *In vitro* histamine release from chopped skin of dogs. Combined effect of anaphylatoxin and benadryl.

Agents (%)	Percentage histamine release			
	I	II	III	Average
Anaphylatoxin 1.0	5.5	10.5	6.3	*7.4*
„ + Benardryl 0.2	46.2	28.4	22.8	*32.5*
„ + „ 0.001	1.2	2.5	2.6	*2.1*

above 0.2 per cent, both benadryl and neoantergan caused release of histamine from the chopped skin of a dog. The action of 0.2 per cent of benadryl was comparable to that of sinomenine in the same per cent concentration. In both of these antihistamines, this action disappeared in concentrations below 0.01 per cent. The effect of histamine release was

Table 4. *In vitro* histamine release from chopped skin of dogs. Effect of benadryl.

Agents (%)	Percentage histamine release			
	I	II	III	Average
Benadryl 1.0	48.5	31.5	32.6	*37.5*
„ 0.2	13.4	16.8	15.1	*15.1*
„ 0.01	−1.6	−0.4	0.0	*−0.7*
„ 0.001	−1.7	−0.5	−0.1	*−0.8*

more marked in benadryl than neoantergan in per cent concentration but this difference might not be true in molar concentration (Table 4 and 5).

Table 5. *In vitro* histamine release from chopped skin of dogs. Effect of neoantergan.

Agents (%)		Percentage histamine release			
		I	II	III	Average
Neoantergan	1.0	30.2	17.2	17.7	*21.7*
„	0.2	10.5	1.0	6.9	*6.1*
„	0.01	−0.8	−0.07	−0.6	*−0.5*
„	0.001	−0.7	−0.08	−1.2	*−0.7*

2. Effect of antihistamines on the in vivo histamine release from rat skin

The results are summarized in Table 6. About one third of histamine content in the rat skin was depleted by the intraperitoneal administration of 50 mg./kg. of sinomenine and about the same effect of skin histamine depletion was observed on subcutaneous injection of 25 mg./kg. of benadryl. Concurrent use of these two agents in the foregoing doses indicated the presence of synergy in histamine depletion effect. In a smaller dose of benadryl, however, such as in 5 mg./kg., the agent itself was incapable of causing histamine depletion and evidently inhibited the histamine releasing effect of sinomenine administered 30 minutes later.

Table 6. *In vivo* hiatamine release from the skin of rats. Effects of sinomenine and benadryl, and their combined effect.

Agents	Percentage histamine release	
		Mean ± S.E.
Sinomenine (i.p.) 50 mg./kg.	30.3, 40.7, 36.7, 29.1, 32.8, 29.3	*33.1±1.9*
Benadryl (s.c.) 50 mg./kg.	0, 0, 5.2, 9.0, 1.4, 2.2	*3.0±1.43*
25 mg./kg.	28.6, 40.1, 27.9, 26.8, 30.7, 28.5	*30.3±2.0*
Sinomenine 50 mg./kg. (s.c.) after benadryl 5 mg./kg. (i.p.)	5.3, 6.5, 10.2, 8.5, 12.1, 8.6	*8.5±1.0*
Sinomenine 50 mg./kg. (s.c.) after benadryl 25 mg./kg. (i.p.)	37.3, 45.8, 39.1, 47.0, 41.8, 40.3	*41.9±1.5*

Remark. — Histamine content of the skin of 30 rats fluctuated between 28 and 59 μg./g. with an average of 43 μg./g. tissue.

DISCUSSION

It is generally believed that the beneficial effect of antihistamines on some manifestations of anaphylaxis and allergy is the result of counteraction of these agents against the abominable effect of the liberated histamine, considered to be the culprit of such manifestations. However, the present studies have revealed that antihistamines also possess actions entirely different from those mentioned above and expected to give a considerable effect on the action of histamine under such pathological conditions; viz. dual actions on the release of tissue histamine, inhibiting the release of histamine in small doses and causing histamine release by themselves when used in a large amount. Occurrence of such actions either *in vitro* or *in vivo* suggests that these are direct action of these agents on tissue cells.

In 1950, DALE[14] assumed the possibility of the antihistamine showing its effect against histamine by interfering in the release of histamine from its depots int he tissues, besides blocking tissue receptors for circulating histamine. This possibility became more likely by later observations[6,7] that the disruption of mast cells by chemical histamine liberators and anaphylatoxin can be defended by antihistamines. Our present experiments have actually confirmed this fact in regard to histamine release from tissues.

There are already a few observations[2,3,4] on the histamine-releasing action of antihistamines *in vitro* and *in vivo*. This fact will be able to elucidate the seemingly paradoxical phenomenon that the lethal effect of histamine in mice is not weakened but rather strengthened by antihistamines[15,16,17], because in such observations, a far larger dose of antihistamines per weight is used than in similar experiments with animals more sensitive to histamine, such as guinea pig. SANUKI and others[5] demonstrated that a large dose like 25 mg./kg. of antihistamine will strengthen the histamine action, though a small amount like 5 mg./kg. of antihistamine can inhibit the lethal effect of and fall of body temperature by histamine in mice. The result of our present experiment on histamine release *in vivo* agrees well with the observations of SANUKI and others[5].

The mechanism of competitive antagonism that the sites obtained by histamine on the surface of tissue cells susceptible to its action or in the interior of the cells into which a part of it is accessible are occupied by antihistamines, may be related to some similarity in the configuration of antihistamines to that of histamine[18]. If this view is followed, it seems reasonable to assume the possibility of releasing histamine, when the

amount of the antihistamine is sufficiently large, as a result of replacing histamine with antihistamines from the bonding of histamine molecules with any structure of the cells. However, this does not seem to be quite justifiable because specific histamine releasers, which are basic compounds and have no similarity whatever with the configuration of histamine, also causes similar release of histamine[19].

Recently, MCINTIRE and colleagues[20] reported another example of dual action similar to that of antihistamines. These workers observed that histamine release by tanigen from platelet fraction of sensitized rabbits was inhibited by a comparatively low concentration of a certain compound while the action was accelerated in higher concentrations. They[21] have also found that a slight change in chemical structure, such as the substitution of a carbamide group in the β-position of a certain quaternary pyridinium compound with carbomethoxy group, will change inhibitor of histamine release to histamine liberator. Considering these evidences together with the findings obtained in our present experiments it seems that there is a certain common basis in the mechanism of histamine release when cells are exposed to some histamine-releasing agents or to antigen-antibody reaction and also in the defense mechanism against such release. In the present stage of the studies, nothing definite can be said but we harbor the idea that the site of action of antihistamines on histamine release may be membranes that surround cells and cell-organelles.

SUMMARY

1. Quantitative examinations were made on the effect of benadryl and neoantergan on the histamine release *in vitro* from chopped skin of dogs and *in vivo* from rat skin. For estimation of the *in vitro* histamine release by biological method, a chemical procedure for separating the diffused-out histamine from mixed antihistamines was carried out.

2. Both antihistamines caused a fairly marked release of histamine from chopped skin tissues in comparatively higher concentrations. This action was synergistic with histamine-releasing effect of sinomenine and anaphylatoxin.

3. In lower concentrations, however, both antihistamines inhibited the *in vitro* histamine-releasing effect of sinomenine and anaphylatoxin.

4. Administration of a comparatively large amount of benadryl markedly depleted the skin histamine of a rat *in vivo* but smaller amount clearly suppressed the histamine depletion by subsequently administered sinomenine.

5. Based on the evidence of such dual action of antihistamines, some considerations were made on the site of action of these agents.

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