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EFFECT OF HISTAMINASE TREATMENT ON HISTAMINE AND ANAPHYLACTIC SHOCK IN GUINEA PIGS

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In previous reports (1—3) it was shown that the histaminesensitivity of animals and man may be decreased by treatment with histamine over a period of 7 to 10 days. Later (4—6) it was shown that histamine- and anaphylactic shock in guinea pigs could be prevented by pretreatment with histamine. The question of how to account for the increased resistance to histamine demonstrated by these experiments naturally arises. It seemed very probable that the beneficial effects of the pretreatment with histamine might be attributed to the increased activity of the enzyme histaminase. This enzyme, discovered by Best and McHenry (7, 8), has the specific property of destroying histamine and is constantly present in the different organs of the living animal. It seemed possible that treatment with histamine might increase the histaminase-content of the organs.

The following experiments were carried out in order to determine what effect, if any, pretreatment with histaminase might have on histamine- and anaphylactic shock in guinea pigs.

EXPERIMENTS

1. Thirty male guinea pigs weighing 200 to 250 grams were used in this experiment. In 20 animals the jugular vein was exposed under slight ether-narcosis and injected with 3 ampules of Torantil (T 360)[1] dissolved in 2 ml. of physiolo-

[1] Torantil (T 360) is a histaminase preparation of the Winthrop Chemical Co. A unit of histaminase is the amount necessary to detoxify

gical saline. After 15 minutes when the guinea pigs had entirely recovered from the narcosis and behaved in every respect as the 10 normal controls, all 30 animals (the pretreated as well as the non-pretreated controls) were given 4 mgm. of histamine base as histamine dihydrochloride intraäbdominally.

The results may be summarized as follows: Within 3 to 5 minutes all of the 10 non-pretreated control animals showed symptoms of histamine-shock which reached a maximum in 5 to 7 minutes; 7 of these animals died. In the 20 guinea pigs pretreated with histaminase there were no signs of shock during the period when the control animals exhibited symptoms. After 12 to 15 minutes 6 of the 20 animals showed slight dyspnea which in another 5 to 10 minutes decreased in all but 2 of the 6 animals. These 2 animals later developed a real histamine-shock and died 35 minutes after the injection. These results show that histaminase injected intravenously has a very definite effect *in vivo* on histamine-shock. Of the 10 controls all developed severe shock, 7 died; of the 20 pretreated animals, 4 developed slight shock much later than the controls and only 2 died.

2. Thirty 200 to 250 gram male guinea pigs were sensitized with 3 ml. of 50 per cent egg-white subcutaneously. Two to three weeks later 20 of the 30 animals were injected with histaminase intrajugularly under the same conditions and using the same amount as in the first experiment. The 10 control animals received no treatment. Fifteen minutes following the injection of histaminase, all animals received 2 ml. of 50 per cent eggwhite intraäbdominally.

The result were similar to those of histamine-shock. In the control group symptoms of anaphylactic shock began within 4 to 5 minutes in all of the animals. The shock became very severe, with a maximum 10 minutes after the shocking dose of egg-white was administered, and continued for ½ to 1 hour. Four animals died after 10 to 14 minutes. In the pretreated group of 20 animals, no symptoms were apparent

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one mg of histamine dihydrochloride. One ampule of T—360 contains one H. D. (Histamine—destroying) unit. This material was supplied through the courtesy of Dr. H. A. Cave, the Department of Medical Research, Winthrop Chemical Company.

during the first 20 minutes; after 20 minutes, 5 of the animals showed slight symptoms such as scratching of nose, excitement, *etc.*, which lasted for only a few minutes. No other signs of anaphylaxis were observed and none of the animals died.

The objective symptoms observed in both experiments on histamine and anaphylactic shock may be compared as follows: In the control animals of both experiments the temperature fell from the average normal of 37.5 to 38°C. to 30 to 31°C. during the first 6 to 8 minutes. In the animals pretreated with histaminase in both experiments there was no such marked fall; however six of the animals showed a slight drop of ½ to 1°C., 13 to 15 minutes after the histamine-injection, with a continued drop to 31 to 31.5°C. in the two animals that finally died in the experiment on injection of histamine. At necropsy, pulmonary emphysema was noted in all controls and in the pretreated group only in the two animals which died of histamine-shock.

3. Six male guinea pigs weighing 200 to 250 grams were used in this experiment. Three animals previously sensitized to eggwhite in the same manner as described in the second experiment together with three non-sensitized animals were prepared for intrajugular injection of histaminase as previously described. Inactivation of the histaminase was accomplished by heating in a waterbath at 65°C. for 10 minutes (8). Each animal received 3 ampules of the histaminase as in the other experiments. After 15 minutes the shocking dose of 2 ml. 50 per cent egg-white solution was given intraäbdominally to 3 sensitized animals and 4 mgm. of the histamine base were given intraäbdominally to each of a second group of 3 animals.

Symtoms of anaphylactic and histamine-shock appeared within 4 to 5 minutes as in the controls of the first two experiments. The symptoms were very marked and severe, more so than in the control group, and the shock rapidly progressed and culminated in all cases in death of the animals within 10 minutes. Apparently the injection of the inactivated histaminase served to aggravate and increase the symptoms of shock.

DISCUSSION

The purpose of the experiments just described was to provide an experimental basis for the recent successful administration of histaminase (T 360) in the treatment of disorders based on release of histamine: physical allergy (9, 10) serum-sickness (11) hay-fever (12) bronchial asthma (13), inasmuch as such successful results are contrary to what might be expected from the studies of Best and McHenry on histaminase.

According to these authors the inactivation of histamine by histaminase even under optimal conditions (37°C., pH 7.2) requires several hours and is complete only after 24 hours. If so, the administration of histaminase as a therapeutic measure is not justified. How could one, for instance, explain the results of Roth and Horton (9, 10) who were able to prevent the symptoms of a physical allergy (cold-supersensitiveness) which would ordinarily occur on the immersion of the hands and arms of the patient in ice-cold water, by a previous administration of histaminase? According to the experiments of Best and McHenry, it would take hours for the histaminase present in the blood to destroy the released histamine and before it could be destroyed local and systemic reactions could occur.

The same objection may be raised in the case of treatment with histamine even if the histaminase-content of the organism is increased by pretreatment with histamine, such an increase could have no influence in the appearance of allergic symptoms since the histamine suddenly released, which produces the symptoms, could be destroyed by the histaminase only after hours.

On the basis of the results of clinical investigations then, one must suppose that the histaminase *in vivo* acts differently than *in vitro, i. e.*, without the necessary *in vitro* incubationary period. If this is so the histaminase-treatment of allergy is rational, but if such is not the case, then the successful results reported must be ascribed merely to accident or to some unknown factors and until such factors are discovered the administration of histaminase would on a theoretical basis be unwarranted.

We believe that our experiments afford evidence sup-

porting the therapeutic use of histaminase and indicate that it acts apparently more rapidly *in vivo* than *in vitro*. This is in accordance with the recent results of Takebayashi (14).

Experiments on the guinea pig (15) have shown that a mild alarm-reaction elicited by exposure to some damaging agent decreased the anaphylactic response of sensitized guinea pigs. One might suppose that the intravenous injection of 3 ampules of histaminase might be sufficient to act as a damaging agent and thus increase the nonspecific resistance of the organism. That this objection is not valid is shown by the third experiment in which inactivated histamine was injected with no beneficial effect on anaphylactic or histamine-shock.

At the beginning of our experiments we believed that the effect of the histamine-treatment was due to an increase in the histaminase-content of the blood and tissues. However, recent investigations by Rose *et al.* (16) have shown that there is no increase in the histaminase-content of rat tissues after pretreatment with histamine. Explanation of the good effects of histamine-pretreatment must be found in some other mechanism. It has been shown (2, 17) that an important feature of histamine shock, the decrease in blood-volume, not only does not occur after histamine-pretreatment, but the organism actually responds with a marked increase of blood volume. This reverse response cannot merely be due to an increased rate of destruction of histamine, such as might be caused by an increased histaminase-content of the tissues, but represents, rather a difference in response to histamine itself. Such an altered response of the blood vessels for example has been shown (3) also in experiments involving acute reactions (tachyphylaxis).

SUMMARY

1. In guinea pigs, the intravenous injection of histaminase 15 minutes before the intraäbdominal injection of 4 mgm. of histamine prevented in most instances the symptoms of histamine shock. Of 10 control animals, all showed marked symptoms within 5 to 7 minutes and 7 animals died shortly after. Ofthe 20 animals pretreated with histaminase, 6 showed

slight symptoms after 15 minutes, which disappeared in a few minutes in all but 2 animals that died 35 minutes after the injection of the histamine.

2. Histaminase given intravenously 15 minutes before the administration of egg-white intraäbdominally to previously sensitized guinea pigs prevented an anaphylactic shock completely in 15 out of 20 animals. The other 5 guinea pigs showed only very slight symptoms beginning 20 minutes after the injection of egg-white and lasting for a few minutes. Of the 10 control animals all showed severe anaphylactic shock developing within 5 to 10 minutes and 4 animals died 10 to 14 minutes after the injection of the egg-white.

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