Biochemical Pharmacology, Vol. 23, pp. 643–647. Pergamon Press, 1974. Printed in Great Britain.

EFFECT OF ASCORBIC ACID ON DETOXIFICATION OF HISTAMINE UNDER STRESS CONDITIONS

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(*Received* 8 *May* 1973; *accepted* 30 *July* 1973)

Abstract—A variety of stress conditions, namely, administration of vaccines, toxoids and dietary and physical stress in rats and guinea pigs led to an enhanced histamine formation or release in the system. Administration of large doses of ascorbic acid in any of the stressful situations resulted in a marked decrease in the urinary histamine level indicating detoxification of histamine *in vivo*. In the guinea pig, the utilization of ascorbic acid was significantly increased in different histamine forming or histamine releasing stress conditions.

IN A PREVIOUS communication[1] we have shown that autooxidation of L-ascorbic acid in the presence of histamine results in rupture of the imidazole ring, leading to biological inactivation of histamine, and we have indicated a function of ascorbic acid for detoxification of histamine *in vivo*. Recently, we observed[2] that in the rat, administration of a number of drugs led to an increased formation or release of histamine, and ascorbic acid administration along with the drugs resulted in detoxification of histamine in the system. In the guinea pig, the histamine-producing or histamine-releasing drugs resulted in an increased utilization of ascorbic acid. The drugs which did not induce histamine formation or release did not lead to greater utilization of the vitamin in the guinea pig.[2]

It has been reported[3–7] that histamine formation is markedly stimulated in intact animals by a number of different stress conditions, including injection of some chemical compounds, endotoxins, exposure to low temperatures and immunization reaction. We were, therefore, interested to study the effect of ascorbic acid on detoxification of histamine under stress conditions.

MATERIALS AND METHODS

The strains of rats and guinea pigs, the ascorbic acid-free stock diet used (scorbutogenic diet), determination of urinary histamine, histamine-forming capacity (HFC) in the 35,000 *g* supernatant fraction of the gastric mucosa and plasma histaminase, and estimation of ascorbic acid have been described in a previous communication.[2] Unless otherwise mentioned, the temperature of the animal colony was 25 ± 1°.

The HFC and histaminase values were determined approximately 48 hr after administration of the vaccines and toxoids, or exposure of the animals to cold and hot temperatures. These values were maximum at that stage and did not increase on prolongation of the treatment.

To see the effect of ascorbic acid on the urinary excretion of histamine in rats and guinea pigs and under stress conditions, the vitamin was administered on the third day of the treatment when the urinary histamine reached a maximum. In pregnancy,

the maximum effect was during the 17–20th days of gestation in rats. After administration of ascorbic acid, the urinary histamine was determined on the following day. The control group of animals received no ascorbic acid, and instead of vaccine injection received saline injection. In some other control experiments ascorbic acid was given from the start of the treatment. Unless otherwise mentioned, the doses of ascorbic acid used were 100 mg/rat per day and 5 mg/guinea pig per day as described previously.[2] For the production of dietary stress, the rats were fed the different stress diets for one month, following which the HFC, histaminase and the effect of ascorbic acid on histamine excretion were determined. The stress diets consisted of only whole grain cereal flours, e.g. wheat, rice and corn, without supplementation of any other dietary ingredients. The stress diets were given to the guinea pigs for 1 week only.

The vaccines and toxoids were injected intramuscularly as a single dose. The dosages used were: cholera, TAB and TABC vaccines 0·1 ml each, and all other vaccines 0·2 ml each.

Cholera, TAB and TABC vaccines were from Bengal Immunity Co. Ltd. and all other vaccines and toxoids used were from Glaxo Laboratories (India) Private Ltd. L-Ascorbic acid (G.R.) was from S. Merck (India), histidine dihydrochloride and histamine dihydrochloride were from Sigma Chemical Company, U.S.A.

RESULTS AND DISCUSSION

The results (Table 1) indicate that under a variety of stress conditions the urinary histamine level is markedly enhanced in rats. The different stress conditions include (i) injection of different vaccines and toxoids, (ii) putting the animals through dietary stress such as whole cereal diets, or fasting, (iii) pregnancy, and (iv) keeping the rats in a cold room (3–4°) or a hot room (39 ± 1°). The increase in the urinary histamine has been shown to be an index of histamine production or release in the intact animals.[7,8] Table 1 shows that in some cases the increased urinary histamine was due to enhanced HFC in the system. The HFC of the gastric mucosa has been given because it represents about half of the total histamine formed in the body.[7] The increase in the urinary excretion of histamine after administration of some toxoids or putting the rats through chronic dietary stress (Table 1) may be considered as due to histamine release in the body because the HFC was not induced under those conditions. The possibility that the increased urinary histamine level might be due to an inhibited histaminase activity was eliminated because the plasma histaminase value from normal rats (3·1 ± 0·2 ImU/100 ml plasma) was not significantly changed by putting the rats through any of the stress conditions studied except in the case of pregnancy, where the plasma histaminase value was 15·5 ± 0·5 ImU/100 ml plasma.

Table 1 further indicates that irrespective of the stress condition used whenever there was an increase in the urinary level of histamine, administration of ascorbic acid resulted in a significant decrease in the urinary histamine level. This decrease in the urinary histamine level by ascorbic acid under stress condition was not due to its indirect effect on inhibition of HFC or enhancement of histaminase activity. The HFC of rat gastric mucosa (138 ± 5 ng histamine formed/mg protein per 90 min) or the activity of plasma histaminase (3·1 ± 0·2 ImU/100 ml plasma) from normal rats were not significantly changed by administration of ascorbic acid either in the normal condition, or under the different stress conditions used. As indicated

TABLE 1. HFC AND EFFECT OF L-ASCORBIC ACID ADMINISTRATION ON THE URINARY HISTAMINE LEVEL OF RATS UNDER STRESSFUL CONDITIONS

Treatment	HFC of gastric mucosa*	Urinary histamine†	Urinary histamine after administration of L-ascorbic acid‡
None	138 ± 5	2·9 ± 0·2	2·2 ± 0·2
Vaccines and toxoids			
Cholera vaccine	212 ± 4	6·3 ± 0·1	3·1 ± 0·2
TAB vaccine	232 ± 3	7·1 ± 0·2	3·6 ± 0·2
TABC vaccine	218 ± 6	6·6 ± 0·2	3·4 ± 0·1
Whooping cough vaccine	278 ± 4	9·9 ± 0·4	4·1 ± 0·1
Diphtheria toxoid	145 ± 3	10·2 ± 0·2	6·0 ± 0·1
Tetanus toxoid	152 ± 6	12·5 ± 0·3	7·0 ± 0·2
Diphtheria pertussis	141 ± 3	9·8 ± 0·4	5·9 ± 0·2
Dietary stress			
Wheat flour	148 ± 4	13·9 ± 0·4	6·1 ± 0·1
Rice flour	132 ± 6	12·8 ± 0·3	5·1 ± 0·2
Corn flour	129 ± 4	14·5 ± 0·4	7·8 ± 0·3
Fasting (48 hr)	105 ± 5	11·1 ± 0·2	5·8 ± 0·2
Physical stress			
Cold (4 ± 1°)	312 ± 7	13·4 ± 0·4	7·8 ± 0·3
Heat (39 ± 1°)	376 ± 8	21·0 ± 0·4	10·0 ± 0·3
Pregnancy (17–20 days)	138 ± 5	40·0 ± 1·0	17·5 ± 0·8
Histamine (1 mg i.p.)	139 ± 4	11·1 ± 0·3	4·0 ± 0·1

* Values given for HFC (ng histamine formed mg protein in 90 min) are mean of six observations ± S.E.M. Gastric mucosa from three rats were pooled for three separate observations. P values for HFC between normal and treated: $P < 0·01$ for whooping cough vaccine, cold and heat; $P < 0·02$ for TAB vaccine; $P < 0·05$ for cholera vaccine and TABC vaccine; P values for others were not significant.

† For urinary histamine (μg/rat per day) each value is a mean of six observations ± S.E.M. from six rats.

‡ 100 mg/rat per day given orally; for other details see "Materials and Methods".

before,[2] the effect of ascorbic acid may be attributed to detoxication of histamine *in vivo*. The possibility that, besides detoxication of histamine, ascorbic acid may have a role in decreasing the release of histamine in the stress condition is yet to be ascertained.

Apparently there is a difference between the antihistaminic property of ascorbic acid and that of the antihistaminic drugs. Ascorbic acid leads to rupture of the imidazole ring making the histamine biologically inactive.[1] On the other hand, the anthihistamines competitively bind the receptor sites of the cell membrane protecting the cell from histamine action.[9]

As observed in the case of drug treatment,[2] when ascorbic acid administration was continued in the stress condition the urinary level of histamine remained low throughout the experimental period. Discontinuation of ascorbic acid resulted in a sharp rise in the urinary histamine level. On the other hand, ascorbic acid administration at any stage of stress condition resulted in a sharp fall in the level of histamine. Figure 1 shows that the urinary level of histamine of rat increased with increasing temperature of the room above 25° and attained a maximum value at 40°. Figure 1 further shows that administration of ascorbic acid kept the urinary histamine level significantly lower.

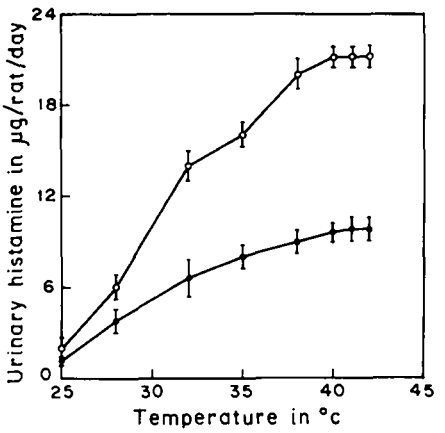

FIG. 1. Urinary excretion of histamine from rat kept at different room temperatures. (○), without ascorbic acid administration; (●), after feeding 100 mg ascorbic acid/rat per day. The vertical bars represent S.E.M.

The stress conditions also led to a significant increase in the urinary histamine of the guinea pigs fed an ascorbic acid-free diet (Fig. 2). As observed in rat (Table 1), this increase in the urinary histamine was either due to an increase in the tissue HFC or greater release of histamine. The HFC of 35,000 *g* supernatant fraction from gastric mucosa of normal guinea pigs was 9·2 ± 0·4 ng histamine formed/mg protein per 90 min, and the value rose to 14·5 ± 0·2–25·3 ± 0·6 after administration of vaccines, or heat or cold treatment. The HFC was not enhanced in dietary stress, pregnancy or after toxoid administration. In any case, when 10 mg ascorbic acid was administered to the guinea pigs in the stress conditions, the increased urinary histamine was brought back almost to the normal value (Fig. 2).

It has been shown[1] that during histamine destruction L-ascorbic acid undergoes autooxidation, indicating utilization of the vitamin. It was also observed that the histamine producing or histamine releasing stress conditions or intraperitoneal injec-

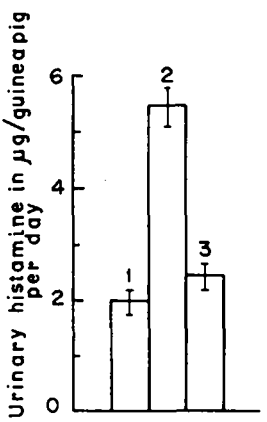

FIG. 2. Urinary excretion of histamine from guinea pigs fed scorbutogenic diet. 1, control; 2, received tetanus toxoid; 3, received tetanus toxoid and 10 mg ascorbic acid/guinea pig per day. Similar results were obtained using other toxoids or dietary stress. The vertical bars represent S.E.M.

tion of histamine (250 μg/animal twice daily) resulted in a decrease of urinary excretion of ascorbic acid by the guinea pigs from 0·40 ± 0·03 to 0·14 ± 0·01 -0·28 ± 0·01 ($P < 0·01$). As mentioned before,[2] this decrease in the urinary level of ascorbic acid would indicate a greater utilization of the vitamin. The fall in the urinary excretion of ascorbic acid in the guinea pig in the stress condition, or after histamine injection, could be brought back to the normal value by an extra supplementation of 10 mg ascorbic acid.

The results presented in this communication support our previous inference[1] that any beneficial effect of large doses of ascorbic acid observed in various stress conditions is due to its detoxification of excess histamine produced in response to the stress.

Acknowledgement —The work was partly financed by the United States PL-480 Grant No. Fg-In-416.

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