Biochemical Pharmacology, Vol. 23, pp. 637–641. Pergamon Press, 1974. Printed in Great Britain.

EFFECT OF ASCORBIC ACID ON DETOXIFICATION OF HISTAMINE IN RATS AND GUINEA PIGS UNDER DRUG TREATED CONDITIONS

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(*Received* 16 *April* 1973; *accepted* 30 *July* 1973)

Abstract Administration of a variety of drugs led to an increased histamine formation or release in the system, as evidenced by an enhanced histidine decarboxylase activity of gastric mucosa and increased urinary histamine level. In the rat, administration of ascorbic acid along with the drugs decreased the urinary histamine level, indicating detoxification of histamine *in vivo*. In the guinea pig, histamine-producing or histamine-releasing drugs resulted in a decreased urinary ascorbic acid level, indicating greater utilization of the vitamin.

RECENTLY, we have shown that autooxidation of ascorbic acid in the presence of histamine results in rupture of the imidazole ring leading to the biological inactivation of histamine, and we have indicated a function of ascorbic acid for detoxification of histamine *in vivo*.[1] There are some scattered reports in the literature that administration of some typical drugs like adrenaline, acetylsalicylic acid, phenylbutazone, chlorpromazine, morphine, pethidine and tetracyclines lead to an excess formation or release of histamine in the system.[2–5] An excess of histamine, on the other hand, would be toxic to the body and should be relegated to the sphere of pathology.[6] We were, therefore, interested to study the effect of ascorbic acid on detoxification of histamine after drug administration.

MATERIALS AND METHODS

Inbred female rats of Charles Foster strain of average body weight 160 g, raised and maintained in our laboratory colony, were used throughout the experiment. Male short hair guinea pigs, weighing on average 200 g were acclimatized to our animal colony condition and stock diet for 3–4 days. The stock diet* used for both rats and guinea pig had the following composition in g/100 g diet: wheat flour: 63; sucrose: 10; casein: 15; groundnut oil: 5; shark liver oil: 2; USP XVII salt mixture: 4; A.O.A.C.† vitamin mixture: 1. The guinea pigs received 5 mg ascorbic acid/day orally in the form of an aqueous solution. For normal growth and maintenance of the guinea pigs, 2 mg ascorbic acid/guinea pig per day was sufficient. However, to see the effect of drugs, 5 mg ascorbic acid/animal was given to produce a comparatively higher and steady basal urinary level of the vitamin.

* Ascorbic acid free diet.

† See *Official Methods of Analysis of the Association of Official Agricultural Chemists*, 10th edition, p. 785 (Ed. W. Horwitz), Washington, D.C. (1965).

For histamine, urine was collected in 4 ml of 6N HCl and the histamine was extracted and estimated fluorimetrically using *O*-phthalaldehyde by the method of Shore *et al.*[7] Urinary ascorbic acid was estimated titrimetrically using 2,6-dichlorophenol-indophenol.[8] The histamine-forming capacity of the gastric mucosa (HFC) was assayed in the 35,000 *g* supernatant fraction according to the fluorimetric method of Skidmore and Whitehouse.[9] Plasma histaminase was estimated following the method of Kapeller-Adler.[10]

The HFC of the gastric mucosa and the plasma histaminase values were determined approximately 48 hr after drug treatment. These values were maximum at that stage and did not increase on prolongation of the treatment. The food was withdrawn from both the control and the treated groups approximately 18 hr before sacrifice to nullify the effect of any drug induced inanition on the HFC of gastric mucosa.[3] To see the effect of ascorbic acid on the urinary excretion of histamine under drug treatment, the vitamin was administered on the 4th day of the treatment when the urinary level of histamine reached a maximum. The control groups of rats received no ascorbic acid. The histamine was estimated on the 5th day. In some other control experiments using chlorpromazine, phenobarbitone, penicillin and streptomycin, ascorbic acid was also given from the start of the treatment.

The drugs used were chlorpromazine, phenobarbitone, sulphadiazine, sulphamerazine and sulphaguanidine from May & Baker; asprin, phenacetin and streptomycin from Pfizer; tetracycline, pethidine and morphine from Dey's Medical Company; chlorcyclizine and epinephrine from Burroughs Wellcome; penicillin and griseofulvin from Glaxo Laboratories; trifluoperazine from Smith, Kline & French; phenylbutazone and oxyphenbutazone from Suhrid Geigy; chloramphenicol from Parke Davis and tolazoline from CIBA.

Histamine dihydrochloride and histidine dihydrochloride were from Sigma Chemical Company, U.S.A. L-ascorbic acid (G.R.) was from S. Merck (India).

RESULTS AND DISCUSSION

The results presented in Table 1 show that administration of a number of drugs led to a marked increase in the urinary excretion of histamine by rats. Kahlson[6] and Gaddum[11] have shown that the urinary level of histamine is an index of histamine production or release in the body. Table 1 also shows that the increased urinary excretion induced by drugs was in some cases probably due to enhanced formation of histamine, as evidenced by the increased histamine-forming capacity (HFC). The HFC of the gastric mucosa has been given because it represents about half the total histamine formed in the body. Antibiotics and other compounds (Table 1) which did not induce HFC but led to an enhanced urinary excretion of histamine, may be considered as histamine releasers in the system.[5] The plasma histaminase from normal rats (3·1 $\pm$ 0·2 ImU/100 ml plasma) was not significantly changed by treatment with any of the drugs used.

Table 1 further indicates that irrespective of the nature of the drug examined, whenever there was an elevation in the urinary level of histamine, administration of ascorbic acid along with the drug resulted in a significant fall in the urinary histamine level. The decrease in the urinary level of histamine by ascorbic acid was not due to its indirect effect on inhibition of histidine decarboxylase or enhancement of histaminase activity. The activity of none of these enzymes was affected by

TABLE 1. HFC AND EFFECT OF ASCORBIC ACID ON THE URINARY HISTAMINE LEVEL OF RATS UNDER DRUG TREATED CONDITIONS

Treatment	Dosage used/rat per day*	HFC of gastric mucosa†	Urinary histamine‡	Urinary excretion of histamine after ascorbic acid administration§
None	—	135 ± 6	2·8 ± 0·2	1·9 ± 0·2
Phenobarbitone	2·5 mg	342 ± 5	13·5 ± 0·2	8·0 ± 0·1
Chloretone	20 mg	318 ± 5	12·8 ± 0·2	7·9 ± 0·2
Chlorpromazine	5 mg b.d.	398 ± 6	13·4 ± 0·1	7·8 ± 0·1
Meprobamate	10 mg b.d.	304 ± 6	8·2 ± 0·2	4·3 ± 0·1
Aspirin	10 mg b.d.	238 ± 4	6·1 ± 0·2	3·7 ± 0·1
Phenacetin	10 mg b.d.	242 ± 6	6·1 ± 0·2	3·4 ± 0·1
Pethidine	2 mg (i.m.) b.d.	208 ± 3	5·9 ± 0·2	3·1 ± 0·2
Morphine	0·33 mg (i.m.)	218 ± 4	5·8 ± 0·2	3·2 ± 0·2
Epinephrine	0·05 mg (i.m.)	235 ± 3	6·1 ± 0·1	3·3 ± 0·2
Phenylbutazone	5 mg b.d.	332 ± 5	‖	‖
Oxyphenbutazone	5 mg b.d.	308 ± 3	6·3 ± 0·1	3·5 ± 0·1
Penicillin	10,000 I.U. (i.m.) b.d.	107 ± 6	19·8 ± 0·3	6·9 ± 0·3
Streptomycin	20 mg (i.m.)	115 ± 5	21·8 ± 0·2	7·3 ± 0·2
Chloramphenicol	20 mg b.d.	196 ± 3	11·4 ± 0·3	4·3 ± 0·1
Tetracyline	20 mg b.d.	126 ± 4	14·6 ± 0·2	5·7 ± 0·2
Griseofulvin	10 mg b.d.	108 ± 4	21·4 ± 0·2	8·3 ± 0·3
Trifluoperazine	1 mg	111 ± 4	5·6 ± 0·1	2·9 ± 0·1
Chlorcyclizine	1 mg	128 ± 3	5·9 ± 0·1	2·4 ± 0·1
Histamine	1 mg (i.p.)	139 ± 4	11·1 ± 0·3	3·0 ± 0·1
Sulphadiazine	25 mg b.d.	136 ± 6	2·2 ± 0·2	1·8 ± 0·2
Sulphamerazine	25 mg b.d.		2·4 ± 0·1	2·1 ± 0·1
Tolazoline	4 mg	141 ± 3	2·6 ± 0·2	1·9 ± 0·1
Reserpine	0·05 mg b.d.	105 ± 6	2·2 ± 0·1	2·1 ± 0·1
Prednisone	10 mg	—	2·5 ± 0·1	1·9 ± 0·2
Cortisone acetate	4 mg	134 ± 5	2·3 ± 0·1	1·8 ± 0·1

* Unless otherwise stated in parentheses as i.p. or i.m. (intraperitoneal or intramuscular) all drugs were administered orally either as an aqueous solution or as a suspension in groundnut oil; b.d. denotes twice daily.

† Values given for HFC (ng histamine formed mg protein per 90 min) is mean of six observation ± S.E.M. Gastric mucosa from two rats were pooled for two separate observations.

‡ For urinary histamine (μg/rat per day), each value given is a mean of six observations ± S.E.M. from six rats. P values for HFC between normal and treated: $P < 0.01$ for phenobarbitone, chloretone, chlorpromazine, meprobamate, phenylbutazone and oxyphenbutazone. $P < 0.02$ for aspirin, phenacetin and epinephrine. $P < 0.05$ for pethidine, morphine and chloramphenicol; P values for others were not significant. See "Methods and Material" for experimental details. Media for drugs used: chloretone, aspirin, phenacetin were administered as suspension in groundnut oil. Pethidine, morphine, epinephrine, penicillin, streptomycin, chlorcyclizine and histamine were administered as solutions in sterile water. The rest were administered as suspensions in water.

§ 100 mg/rat per day given orally half an hour after drug administration.

‖ Could not be estimated due to interference with fluorimetric assay.

administration of ascorbic acid either under the normal condition or the drug treated condition (Table 2). When instead of the drugs, 1 mg of histamine was injected into the rats, the increase in the urinary histamine was about four-fold but it was brought back almost to the normal value after administration of ascorbic acid (Table 1). The results would indicate a function of ascorbic acid for detoxification of histamine *in vivo*. However, the mechanism of histamine destruction by ascorbic acid is not clear. We have indicated that *in vitro* the imidazole ring is broken.[1]

TABLE 2. EFFECT OF ASCORBIC ACID ADMINISTRATION ON HFC AND PLASMA HISTAMINASE OF RATS UNDER NORMAL AND DRUG TREATED CONDITIONS

Treatment	HFC of gastric mucosa (ng histamine formed/ mg protein per 90 min)	Plasma histaminase* (ImU/100 ml plasma)
None	135 ± 6	3·1 ± 0·2
Ascorbic acid	126 ± 4	3·0 ± 0·1
Phenylbutazone	332 ± 5	3·4 ± 0·1
Phenylbutazone + ascorbic acid	331 ± 5	3·4 ± 0·1
Aspirin	238 ± 4	3·6 ± 0·2
Aspirin + ascorbic acid	244 ± 3	3·4 ± 0·2
Phenobarbitone	342 ± 5	3·4 ± 0·3
Phenobarbitone + ascorbic acid	352 ± 6	3·2 ± 0·2
Penicillin	107 ± 6	2·9 ± 0·2
Penicillin + ascorbic acid	111 ± 3	3·3 ± 0·1

* Each value given is a mean of six observations ± S.E.M. from six rats. ImU denotes international milliunit which is equivalent to destruction of 1 nm histamine/min. All other conditions and details are same as in Table 1.

When ascorbic acid administration was continued along with the drug, the urinary level of histamine remained low for an experimental period of 7 days (Fig. 1). Withdrawal of ascorbic acid resulted in a sharp rise of the urinary histamine level. On the other hand, ascorbic acid administration at any stage of drug treatment resulted in a sharp fall of the urinary level of histamine (Fig. 1).

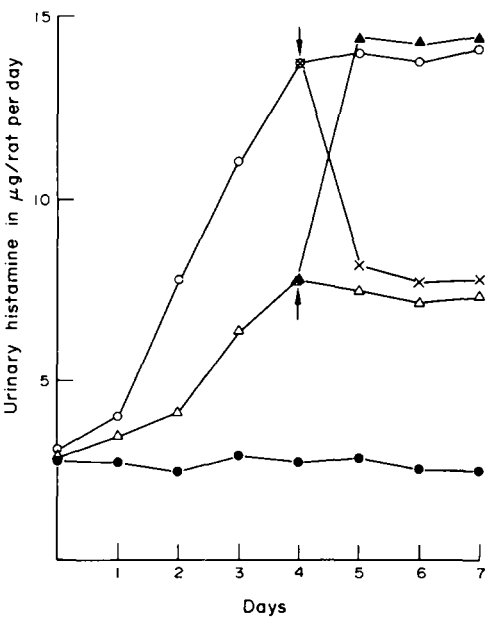

FIG. 1. Effect of ascorbic acid on the urinary excretion of histamine by rats in the drug treated condition. (●), Normal; (○), chloretone treated; (×), chloretone treated and after administration of ascorbic acid on and from the 4th day of treatment, ↓ indicates ascorbic acid administration; (△), after administration of chloretone and ascorbic acid; (▲), after administration of chloretone and ascorbic acid followed by discontinuation of ascorbic acid on and from the 4th day of chloretone treatment, ↑ indicates ascorbic acid discontinuation. Similar results were obtained with chlorpromazine, phenobarbitone, penicillin and streptomycin. Conditions are same as in Table 1.

A maximum fall in the urinary histamine level was obtained with an oral dose of ascorbic acid of 100 mg/rat per day. A dose smaller than this was less effective. On the contrary, a dose more than 100 mg/rat per day did not further decrease the urinary histamine level. Also, a divided dose of ascorbic acid (50 mg b.d. or 20 mg five times a day) produced similar results obtained with a single dose of 100 mg/rat per day.

The drugs which induced HFC in the rat tissue also induced the HFC of the guinea pig tissue. The HFC of 35,000 *g* supernatant fraction from gastric mucosa of normal guinea pigs was 9·5 ± 0·5 ng histamine formed/mg protein per 90 min, and the value rose to 16 ± 0·4 25·3 ± 0·6 after treatment with the histamine producing drugs mentioned in Table 1. As observed in the case of rats, the plasma histaminase value from normal guinea pigs (5·9 ± 0·2 ImU/100 ml plasma) was not significantly changed after drug administration. It was observed that excretion of ascorbic acid by the normal guinea pigs (0·40 ± 0·02 mg/guinea pig per day) fell significantly to 0·20 ± 0·02–0·30 ± 0·01, ($P < 0·01$) after administration of the histamine producing or histamine releasing drugs as mentioned in Table 1. A similar picture was obtained by intraperitoneal injection of histamine instead of the drugs. Since the guinea pig cannot synthesize ascorbic acid, the fall in the urinary ascorbic acid level would indicate a greater utilization of the vitamin. Compounds which did not induce HFC or release histamine did not lower the urinary level of ascorbic acid in the guinea pig.

The fall in the urinary level of ascorbic acid after treatment with drugs or histamine could be brought back to the normal value (0·40 ± 0·02 mg/guinea pig per day) by an oral administration of an extra dose of 5 mg ascorbic acid/guinea pig per day.

Histamine obviously plays some physiological role, but an increased formation or release is considered to be a pathological state.[6] Therefore, excess formation or release of histamine by drug administration should be considered as a side effect of the drugs. The results presented in this communication would indicate a function of ascorbic acid for detoxification of histamine after administration of histamine-producing or histamine-releasing drugs.

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

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