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THE EFFECT OF ENZYME INHIBITORS ON THE EXCRETION OF FREE HISTAMINE IN HUMAN URINE

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There is abundant evidence that most of the histamine entering or formed in the body tissues is destroyed by enzymic action (Tabor, 1954; Schayer, 1956). The enzyme mainly responsible for its destruction has been called histaminase (Best, 1929) and diamine oxidase (Zeller, 1938), but recently Schayer, Kennedy, and Smiley (1953) have demonstrated that more than one enzyme system may be involved. Using radioactive histamine and various enzyme-inhibiting drugs, these workers found that the enzyme system corresponding to diamine oxidase could be inhibited *in vivo* by aminoguanidine and by isoniazid. This enzyme system is important in the rat, but in the mouse they found that another enzyme, inhibited by iproniazid, is more important; they called this enzyme histamine-metabolizing enzyme II. Schayer and his colleagues did not extend their observations to human beings, and the present work was undertaken to investigate the effect of the enzyme-inhibiting drugs in man and, if possible, to determine the relative importance of the two enzymes in the human body. Since ^{14}C-labelled histamine cannot be used in experiments on man, the drugs were tested by assessing their effects on the excretion of free histamine in the urine after the injection of non-isotopic histamine.

Preliminary Observations on Toxicity of Enzyme Inhibitors

Aminoguanidine, in the form of aminoguanidine bicarbonate, is a tasteless white powder almost insoluble in water. Since no report could be found of its administration to human beings, tests of its toxicity to animals were first carried out. When given as an aqueous suspension to albino mice, aminoguanidine bicarbonate had an acute oral LD50 of 3 to 4 mg./g., whereas a solution of aminoguanidine chloride had an LD50 of 2 mg./g. A dose of 0.05 mg./g. of aminoguanidine bicarbonate was given daily by mouth to 20 mice for two periods of five days, separated by an interval of two days. No deaths occurred and no toxic signs were observed. A solution of aminoguanidine chloride injected intravenously into a cat under chloralose anaesthesia had little effect on the blood pressure until doses of 20 to 100 mg./kg. were reached, when a brief fall in blood pressure (5 to 10 mm. Hg) occurred, followed by a rise of 10 to 15 mm. Hg lasting 2 to 3 min., and accompanied by a slight reduction in cardiac and respiratory rates. Even doses up to 100 mg./kg. had no effect on the depressor response to 1 μg. of histamine.

Aminoguanidine was given by mouth to two groups of three guinea-pigs, in doses of 200 mg./kg. daily for 7 days to the first group, and 1,000 mg./kg. on a single occasion to the second. There was no significant effect on the haemoglobin level, red cell count or total and differential white cell counts, estimated before, during, and at seven days after administration of the compound.

Isoniazid and iproniazid were given to human subjects by mouth in powder form as "Rimifon" (Roche Products) and "Marsilid" (Roche Products) respectively. A relatively small dose was given at first, and increased in successive experiments. When 25 mg. isoniazid/kg. was given, vomiting and slight haematemesis occurred one hour later, and it was therefore considered inadvisable to give a larger dose. The vomiting was probably not a central effect, however, but rather a sign of gastric intolerance occasioned by the conditions of the experiment (the subject was fasting), since Schmidt, Hoffmann, and Hughes (1953) found that monkeys could tolerate 40 mg. isoniazid/kg. in a single dose and there is evidence that human tolerance is similar to that of monkeys (Sullivan, Barclay, and Karnofsky, 1954).

No toxic effects were noted when iproniazid was given in doses up to 25 mg./kg., but since it is generally regarded as the more toxic drug in man (Selikoff, Robitzek, and Ornstein, 1952), the maximum dose of this drug was also limited to 25 mg./kg.

METHODS

The methods used and the general plan of the experiments have been described (Mitchell, 1956). Free histamine was extracted from urine by the method of Roberts and Adam (1950) and was assayed on a strip of guinea-pig ileum suspended in atropinized Tyrode solution. All values for histamine are given in terms of histamine base, unless stated otherwise.

Tests were carried out to make sure that small amounts of the drugs excreted in the urine did not

interfere with the extraction or assay of histamine. When aminoguanidine bicarbonate, isoniazid or iproniazid was added to urine before extraction to give a concentration of 50 mg. or 100 mg./50 ml., there was no significant difference between estimates of the free histamine content of the original urine and of the duplicate sample to which the drug had been added.

The experiments were carried out on healthy men between 25 and 35 years of age. At least four weeks elapsed between experiments on any individual. In each experiment, urine was collected at intervals of 2 hr. over a period of 8 hr. (from 5.30 a.m. to 1.30 p.m.) and the amount of free histamine determined in each of the four samples. The subject fasted for 10 hr. before the test and throughout its duration, but was allowed to drink water.

In the first series of experiments, aminoguanidine was given to each of three subjects (X, Y, and Z) on two occasions, in a dose of 10 mg./kg. of body weight in the first test, and 20 mg./kg. in the second. The drug was given by mouth half an hour before the end of the second collection period (at 9 a.m.).

The next series of experiments was carried out on the same three men (subjects X, Y, and Z). A total of 10 μg. of histamine acid phosphate/kg. was injected subcutaneously during the third collection period, in four equal doses at intervals of half an hour, starting at 9.30 a.m. This "histamine excretion test" was performed three times on each subject. In the first test no additional drug was given, while in the second and third aminoguanidine was given at 9 a.m. in doses of 5 mg. and 10 mg./kg. respectively.

In the final series of experiments the histamine excretion test was carried out as before, seven tests being made on one subject. Isoniazid was given by mouth at 9 a.m., in a dose of 5 mg./kg. in the first test, 10 mg./kg. in the second, and 25 mg./kg. in the third. In the next four experiments, iproniazid was given in the same way in doses of 5, 10, 15, and 25 mg./kg.

In all the experiments, extraction of free histamine was started within an hour of collection of the last specimen of urine.

RESULTS

The amounts of free histamine excreted during the first and second two-hour periods in all the experiments ranged from 0.28 μg. to 1.20 μg., a range similar to that of 0.37 μg. to 1.10 μg. found in other healthy men (Mitchell, 1956). These values represent the basal excretion of free histamine when the subject is fasting.

The results of the experiments with aminoguanidine are shown in Table I. In the first six tests, when aminoguanidine was given without subsequent injection of histamine, the mean values indicate a small increase in the excretion of histamine in periods III and IV. This increase was

TABLE I

THE EFFECT OF AMINOGUANIDINE ON THE EXCRETION OF METABOLIC FREE HISTAMINE AND ON THE EXCRETION OF SUBCUTANEOUSLY INJECTED HISTAMINE IN THE URINE OF THREE HEALTHY MEN

Oral Dose of Aminoguanidine Bicarbonate Given at 9 a.m. (mg./kg.)	Total Dose of Histamine Acid Phosphate Injected during Period III (μg./kg.)	Subject and Body Weight (kg.)	Free Histamine in Urine in μg. Histamine Base/2-hr. Period				Extra Histamine Excreted in Period III as % of Total Dose Injected
			I 5.30–7.30	II 7.30–9.30	III 9.30–11.30	IV 11.30–1.30	
10	Nil	X. 75	0·46	0·51	0·88	0·72	—
		Y. 66	0·43	0·40	0·62	1·06	—
		Z. 72	0·39	0·42	0·35	0·80	—
		Mean	0·43	0·44	0·62	0·86	—
20	,,	X. 75	0·74	0·72	1·61	1·33	—
		Y. 66	—*	0·72	1·82	0·93	—
		Z. 72	0·66	0·74	0·94	1·00	—
		Mean	0·70	0·73	1·46	1·09	—
Nil	10	X. 75	0·54	0·59	2·59	0·63	1·18
		Y. 66	0·80	0·83	1·87	0·76	0·70
		Z. 72	0·38	0·42	1·77	0·70	0·83
		Mean	0·57	0·61	2·08	0·70	0·90
5	10	X. 75	0·35	0·40	4·66	0·93	2·46
		Y. 66	0·85	0·60	3·80	0·70	2·03
		Z. 72	0·60	0·87	4·28	0·98	2·15
		Mean	0·60	0·62	4·25	0·87	2·21
10	10	X. 75	0·55	0·73	4·86	1·43	2·45
		Y. 66	—*	1·20	6·14	0·90	3·27
		Z. 72	0·37	0·36	6·00	0·63	3·42
		Mean	0·46	0·76	5·67	0·99	3·05

* Volume of sample inadequate.

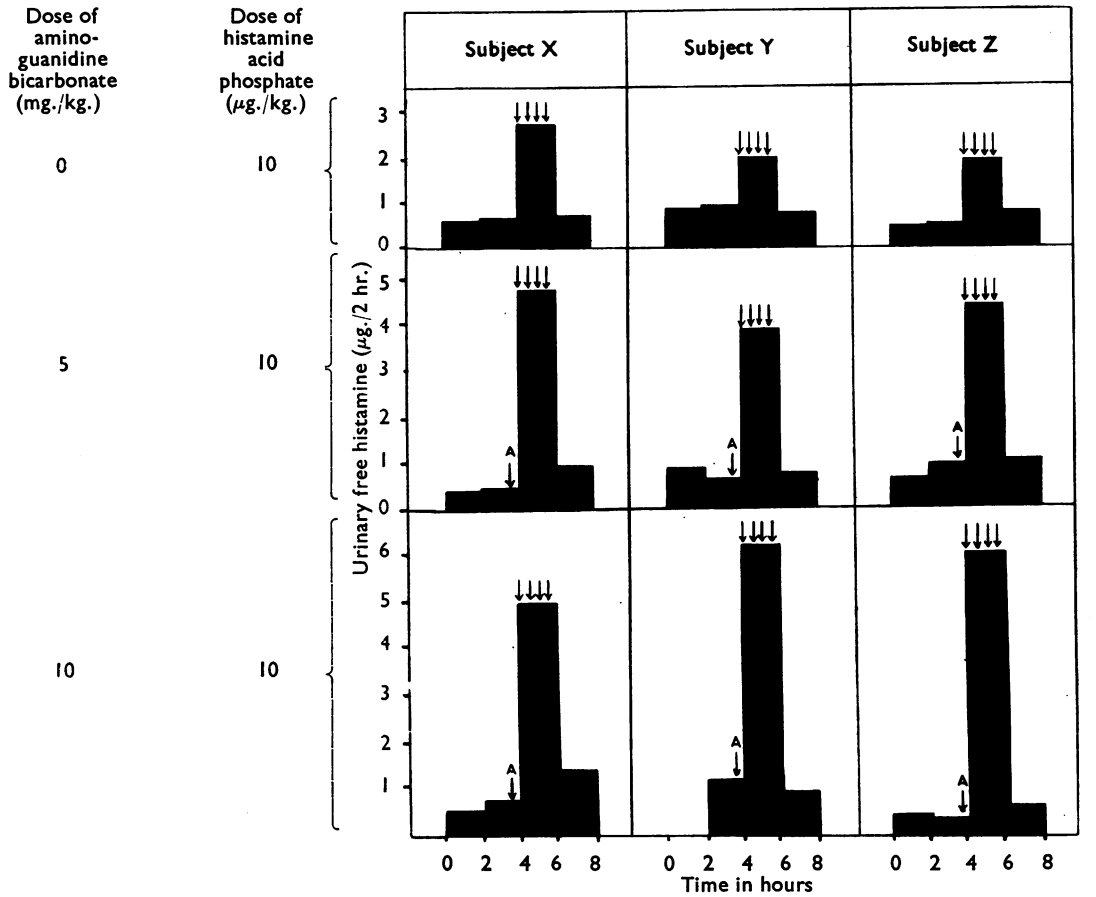

FIG. 1.—The effect of aminoguanidine on the urinary excretion of subcutaneously injected histamine by three healthy men. Total dose of histamine acid phosphate injected in four doses as indicated by the sets of 4 arrows. Aminoguanidine given in one oral dose at arrows marked A.

barely significant, but in all six experiments the rate of excretion in period IV was higher than in either control period in the same test. Such consistency can be considered significant.

The effect of aminoguanidine on the excretion of free histamine was more definite when histamine was injected subsequently (Table I and Fig. 1). When the histamine excretion test was carried out without giving aminoguanidine, the mean value for the extra free histamine excreted during period III was 1.49 μg., representing 0.90% of the dose administered. This mean rate is not significantly different ($P=0.35$) from the mean rate of 1.12% recorded previously for this test (Mitchell, 1956), the calculations being made as described in that paper. If the results of these two series of tests are combined, the mean percentage of the dose excreted for the seven tests is 1.02% (range 0.70% to 1.32%).

When aminoguanidine was given in a dose of 5 mg./kg. half an hour before the first injection of histamine, the mean value for the extra free histamine excreted in period III represented 2.21% of the dose. This mean rate of excretion is significantly higher ($P<0.01$) than the mean rate when no aminoguanidine was given. Aminoguanidine in a dose of 10 mg./kg. resulted in an even greater excretion of free histamine in period III, the mean value representing 3.05% of the dose administered.

When isoniazid or iproniazid in doses up to 25 mg./kg. were administered (Table II) there was no definite effect on the histamine excretion test.

DISCUSSION

Following the parenteral injection of histamine, there is an increase in urinary free histamine amounting to about 1% of the dose administered (Adam, 1950; Mitchell, 1956). Most of the

TABLE II

THE EFFECTS OF ISONIAZID AND IPRONIAZID ON THE EXCRETION OF FREE HISTAMINE IN THE URINE OF A HEALTHY MAN (BODY WEIGHT 80 KG.) AFTER THE INJECTION OF 10 μG. HISTAMINE ACID PHOSPHATE/KG. OVER A PERIOD OF 2 HR. (PERIOD III)

Drug Given as a Single Oral Dose at 9 a.m.	Dose (mg./kg.)	Free Histamine in Urine in μg. Histamine Base/2-hr. Period				Extra Histamine Excreted in Period III as % of Total Dose Injected
		I 5.30–7.30	II 7.30–9.30	III 9.30–11.30	IV 11.30–1.30	
Isoniazid (Rimifon, Roche)	5	0·40	0·45	2·46	0·63	1·11
	10	0·39	0·38	2·63	0·56	1·23
	25	0·74	0·59	3·29	0·67	1·43
Iproniazid (Marsilid, Roche)	5	0·45	0·52	2·83	0·57	1·28
	10	0·66	0·64	3·05	0·85	1·31
	15	0·35	0·41	2·38	0·90	1·09
	25	0·28	0·60	2·85	0·58	1·31

remaining 99% is thought to be destroyed by enzymes in the body (Gaddum, 1951). Previous work suggested that histamine injected subcutaneously is not destroyed at the site of injection (Mitchell, 1956) but reaches the blood stream unchanged. The fact that aminoguanidine injected intravenously had no effect on the depressor response to histamine is consistent with the findings for other histaminase inhibitors (Arunlakshana, Mongar, and Schild, 1954), and suggests that, in the cat at least, little or no enzymic destruction of exogenous histamine takes place in the plasma. This probably also applies to man, whose pattern of histaminase distribution is similar to that of the cat (Kahlson, 1956). Subcutaneously injected histamine may therefore be expected to reach the general body tissues virtually unchanged, since little is likely to be destroyed in the lungs, which in man contain very little histaminase (Zeller, Birkhauser, Mislin, and Wenk, 1939). Most of the histamine reaching the kidney will be destroyed by the rich stores of histaminase in that organ (Waton, 1956), only a fraction being excreted as free histamine. It is apparent therefore that even slight inhibition of the enzymes concerned may be expected to produce a considerable increase in urinary free histamine.

The most suitable time to give the enzyme-inhibiting drug was estimated from the known facts about isoniazid. Absorption of this drug from the stomach is rapid and complete, the peak plasma level being reached between half an hour and two hours after administration (Elmendorf, Cawthon, Muschenheim, and McDermott, 1952; Rubin and Burke, 1953). The drug was therefore given half an hour before the first injection of histamine, so that the peak plasma level would be reached some time between the first and last injections. The same observations apply to iproniazid, which also reaches peak plasma levels very rapidly after oral administration (Rubin, Drekter, Scheiner, and de Ritter, 1952).

Since nothing is known of the absorption of aminoguanidine in man, this compound was also given half an hour before the first histamine injection. It is apparent from the results in Table I that some of the drug must have been absorbed rapidly, although the results of the first six tests indicate that a slightly longer interval between the administration of the drug and the first histamine injection might have produced a greater effect. Furthermore, the results of the tests on albino mice suggest that a solution of aminoguanidine chloride might have been more rapidly and completely absorbed than the almost insoluble bicarbonate.

The effect of aminoguanidine on the excretion of injected histamine is presumably due to inhibition of histaminase in the kidney, a view supported by the experimental work of Lindell and Westling (1956) in cats. In rats a definite increase in urinary histamine occurs after the subcutaneous injection of only 0.5 mg. aminoguanidine chloride/kg. whereas 10 mg./kg. almost stops diamine oxidase (histaminase) activity (Schayer, Kennedy, and Smiley, 1953). Even allowing for the lesser efficacy of the bicarbonate and the possibility of incomplete absorption from the stomach, the comparatively small response to oral doses of 5 and 10 mg. aminoguanidine bicarbonate/kg. suggests that, in the human body, histaminase is not so readily inhibited by aminoguanidine as in the rat. Nevertheless, the results confirm that this enzyme is important in the destruction of histamine in the human body.

Schayer (1953) reported that, in rats, 10 mg. isoniazid/kg. had a slight effect on histamine excretion and 50 mg./kg. had a pronounced effect. Since isoniazid is as effective orally as parenterally, the dose of 25 mg./kg. in the human subject might have been expected to produce a definite increase in the excretion of free histamine. That it did not do so simply confirms that hista-

minase in the human body is less readily inhibited than in the rat.

Iproniazid inhibits histamine-metabolizing enzyme II, which is thought to be a composite of a methylating enzyme and monoamine oxidase (Schayer, 1956). Iproniazid in a dose of 190 to 200 mg./kg. had a pronounced inhibitory effect on monoamine oxidase in mice (Schayer, 1953) and in rats (Schayer and Smiley, 1953), but a dose of 100 mg./kg. had a slight effect. Zeller and Barsky (1952) reported inhibition of monoamine oxidase activity in rats with as little as 28 mg./kg. It appears probable, therefore, that histamine-metabolizing enzyme II is not of major importance in man, since iproniazid in doses up to 25 mg./kg. had no effect at all on histamine excretion.

The results of the animal tests confirm Alles' report (1926) that aminoguanidine is relatively non-toxic, and no toxic effects of the drug were observed in the human subjects. Aminoguanidine may, however, cause changes in the blood like those of pernicious anaemia (Lieber and Smith, 1939) and such a possibility would have to be considered if the drug were given over a period. The histamine excretion test caused no significant symptoms, but when aminoguanidine was given before the injections, headache and flushing occurred. These were, of course, histamine effects and not attributable directly to the aminoguanidine.

The low toxicity of aminoguanidine and its effect on histamine metabolism indicate that it might be of therapeutic value in any condition in which histaminase activity is excessive. In pregnancy the plasma contains an abnormally large amount of histaminase, but this may be necessary for the maintenance of normal pregnancy, and the administration of aminoguanidine is dangerous, since it may interrupt the course of pregnancy (Roberts, 1954). Apart from pregnancy, no other condition has so far been recognized in human beings in which there is excessive production of histaminase (Kapeller-Adler, 1956).

Summary

1. The oral administration of aminoguanidine to healthy men in doses of 10 to 20 mg./kg. of body weight resulted in a slight increase in the excretion of free histamine in the urine.

2. When histamine is injected subcutaneously, 1% of the dose normally appears in the urine in the free form. This rate of excretion increased to about 2% when 5 mg. of aminoguanidine/kg. was given by mouth before the injection of histamine and to about 3% when 10 mg./kg. was given. The increase is attributed to inhibition of histaminase (diamine oxidase) by the aminoguanidine.

3. No significant increase in the rate of excretion of subcutaneously injected histamine followed the oral administration of isoniazid (Rimifon) or iproniazid (Marsilid) in doses up to 25 mg./kg.

4. The results are interpreted as indicating that histaminase is the principal enzyme destroying histamine in the human body.

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