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THE METABOLISM OF HISTAMINE BY GUINEA-PIG AND RAT LUNG *IN VITRO*

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Rose, Karaday & Browne (1940) found that histamine was destroyed when it was incubated with rat lung, but this destruction was prevented if the tissue had been previously heated to 70° C for 5 min. The rate at which histamine was destroyed was found to be related to the amount of lung with which it was incubated. Zeller (1942), Cotzias & Dole (1952), Valette, Cohen & Burkard (1956) and other investigators have shown that rat lung is fairly rich in histaminase. On the other hand, Zeller (1942), Cotzias & Dole (1952), Lindell & Westling (1953), Valette *et al.* (1956) and others have found little or no histamine-metabolizing enzyme in guinea-pig lung, but Brown, Tomchick & Axelrod (1959) have found that this tissue contains imidazole-*N*-methyl transferase, an enzyme which metabolizes histamine to form 4-(2-aminoethyl)-1-methylimidazole.

The present experiments were carried out to investigate more fully the abilities of the lungs of guinea-pigs and rats to metabolize histamine.

METHODS

Animals. In all experiments male albino guinea-pigs and male Wistar rats were used. Guinea-pigs were supplied by B.D.H. (Godalming, Surrey), Messrs. Tuck & Sons (Rayleigh, Essex), Messrs. Alderwood (9 Ivor Street, Camden Town, London, N.W.1) or by the animal house of Chelsea College of Science & Technology. Rats were obtained from the Chester Beatty Research Institute (Fulham Road, London, S.W.3) or from the animal houses at Chelsea College of Science & Technology (Chester Beatty strain) and King's College Hospital Medical School.

Guinea-pigs were fed Diet S.G.1, guinea-pig pellets, and rats were fed Diet 41B, Rat Cube Cakes (both supplied by J. Rank, Blue Cross Animal Foods).

Preparation of chopped lung. Guinea-pigs and rats were stunned and exsanguinated. The thorax was opened and the heart and lungs were removed together. Cuts were made in the right ventricle and the left auricle and a cannula was tied into the pulmonary artery. Tyrode solution at 37° C was perfused through the lungs until most of the blood had been removed. The lungs were cut off, blotted and chopped one way and then at right angles with a mechanical chopper (McIlwain & Buddle, 1953) to give small rods of tissue. These were incubated in 20 ml. of Tyrode solution at room temperature for 20 min and complete separation of the rods was ensured by cutting the tissue with scissors for a few minutes at the start of incubation. The fluid was then filtered off and the tissue washed on the filter paper with Tyrode solution. Excess fluid was removed by gently squeezing the tissue in the filter paper.

Homogenized tissue. Lung tissue was cut into small pieces with scissors, washed in Tyrode solution for 20 min and filtered. Samples were then homogenized in a Potter-Elvehjem homogenizer containing Tyrode

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solution. Histamine released as a result of this treatment was assayed either immediately or after cooling to 2° C and warming to room temperature just before assay. When the total content of histamine in the homogenate had to be known, tissue histamine was released either by boiling for 1 min or by the extraction method described below.

Incubation of tissue. Weighed samples of chopped or homogenized lung were shaken gently at 37° C with 200 μg of histamine acid phosphate in 5 ml. of Tyrode solution, unless otherwise indicated. In some experiments on chopped tissue the incubates (except the controls) contained the enzyme inhibitors aminoguanidine and/or iproniazid; tetracycline hydrochloride (10 μg/ml.) was included in some experiments to prevent bacterial activity.

After 1 to 2 hr incubation, the tubes were centrifuged and the supernatant fluids were poured off. The tissue samples were washed with 5 ml. of Tyrode solution and then re-incubated at 37° C for 10 min with another 5 ml. of Tyrode solution to remove any histamine that had diffused into the tissue. The supernatant fluids from each tube were combined and made up to 100 ml. with Tyrode solution. The tissue samples were then dried at 110° C.

Extraction of histamine. Tissue histamine was extracted by a modification of the method used by Feldberg & Talesnik (1953). The tissue was cut into small pieces and homogenized in a Potter-Elvehjem homogenizer containing 3 ml. of N-hydrochloric acid. The solution was then boiled for 2 min and allowed to cool N-sodium hydroxide solution was added to give a *p*H of 7.3 and the suspension was centrifuged. The supernatant fluid was poured off and the residue was washed with distilled water. The supernatant fluids and washings were made up to 20 ml. with distilled water, and the tissue residue was dried at 110° C.

Boiling under reflux. Weighed samples of guinea-pig (Tuck strain) and rat lung were incubated for 2 hr at 37° C in 5 ml. of Tyrode solution containing histamine acid phosphate (200 μg). Half of each supernatant fluid was boiled under reflux with N-hydrochloric acid for 1 hr, cooled and neutralized with N-sodium hydroxide solution. The histamine was extracted from one-half of each tissue residue, while the other half was boiled under reflux for 1 hr with N-hydrochloric acid, cooled, neutralized with N-sodium hydroxide solution and centrifuged.

Assay of histamine. Incubates and extracts were assayed against a solution of histamine acid phosphate containing tetracycline, aminoguanidine and/or iproniazid when appropriate, on guinea-pig isolated ileum suspended in Tyrode solution containing atropine (10^{-7}). A 2×2 assay design was used and the doses were randomized in the form of a Latin Square. The $P=0.05$ limits of error of the assays ranged from -3 and $+3\%$ to -16 and $+19\%$. In most experiments the limits of error were less than 10%. Mepyramine maleate (10^{-8}) was used to confirm that the activity of the extracts was due to histamine.

Drugs. These were histamine acid phosphate (Burroughs Wellcome), aminoguanidine bicarbonate (L. Light & Co.), iproniazid phosphate (Marsilid, Roche Products) and tetracycline hydrochloride (Achromycin, Lederle).

RESULTS

When histamine was incubated with the lungs of rats or of some strains of guinea-pig, the amount recovered from the supernatant fluid was substantially reduced (Table 1); this effect was unaltered by the presence of tetracycline (10 μg/ml.). Less reduction occurred with the lungs of other strains of guinea-pig (Table 1).

The amount of histamine extracted from lung which had been incubated with histamine for 2 hr was similar to that extracted from lung incubated for 2 hr with Tyrode solution alone (Table 2). The inactivation of histamine by guinea-pig (Tuck strain) and rat lung was greatly reduced either at 2° C or in an atmosphere of nitrogen, and was abolished at *p*H 3.5 or after heating the tissue just to 100° C and washing it before incubation to remove any released histamine (Fig. 1). Inactivation of histamine by guinea-pig (Tuck strain) lung was greater when the amount of tissue incubated was increased (Fig. 2), when incubation was longer (Fig. 3), and when the concentration of substrate was higher (Fig. 3).

TABLE 1

REDUCTION OF THE AMOUNT OF HISTAMINE AFTER INCUBATING CHOPPED LUNG WITH 200 μg OF HISTAMINE ACID PHOSPHATE IN TYRODE SOLUTION

Histamine loss refers to μg of the acid phosphate per mg of dry tissue per hr. Values on the far right are means

Species	Supplier	Experiment	Histamine loss (μg/100 mg/hr)	
Rat	Chelsea	1	92	
		2	145	
		3	48	115
		4	94	
		5	134	
		6	178	
Guinea-pig	B.D.H.	1	72	
		2	68	
		3	57	70
		4	84	
	Tuck	1	87	
		2	67	77
		3	28	
	Alderwood	1	20	
		2	34	
		3	9	20
		4	21	
		5	17	
	Chelsea	1	25	
		2	17	
		3	27	
		4	0	
		5	17	
		6	15	17
		7	15	
		8	13	
		9	25	
		10	19	

TABLE 2

REDUCTION OF THE AMOUNT OF HISTAMINE AFTER INCUBATING CHOPPED GUINEA-PIG AND RAT LUNG WITH HISTAMINE ACID PHOSPHATE

The reduction is not due to uptake of histamine by the tissue

Species	Experiment	Weight of dry tissue (mg)	Volume of incubate (ml.)	Histamine acid phosphate		
				Added (μg)	Reduction (μg)	Total in tissue (μg)
Guinea-pig	1	102	4	160	30·5	28·6
		102	4	—	—	25·0
	2	139	4	160	59	121
		133	4	—	—	124
	3	116	4	160	36	41·8
		114	4	—	—	40·0
	4	140	4	160	37	91
		156	4	—	—	96
Rat	1	51	4	160	62	9·2
		53	4	—	—	8·5
	2	40	5	250	106	6·2
		43	5	—	—	5·5

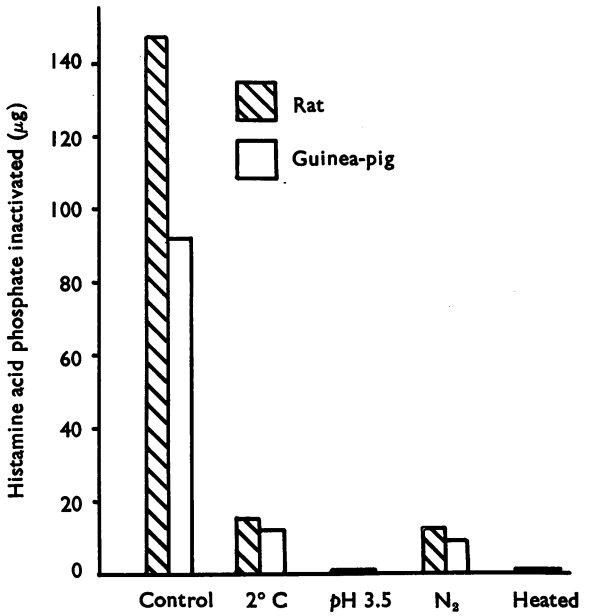

Fig. 1. The inhibition of histamine inactivation by guinea-pig and rat lung. See text for details.

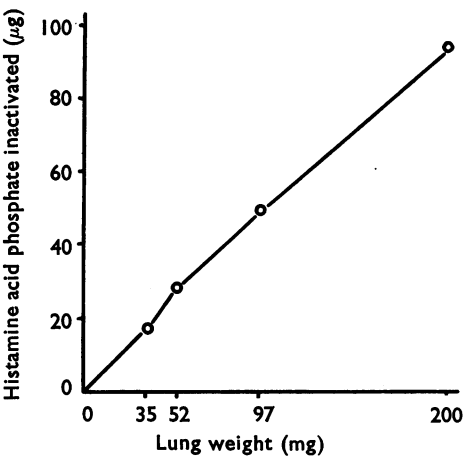

Fig. 2. The inactivation of histamine by different weights (mg of dry weight) of guinea-pig lung incubated with 200 μg of histamine acid phosphate for 1 hr.

Characterization of the histamine-metabolizing enzymes

(a) *Acetylation.* There was no increase in activity of the supernatant fluids or tissue residues after boiling under reflux with hydrochloric acid, thus indicating that no acetylhistamine was formed by guinea-pig or rat lung.

(b) *Aminoguanidine and iproniazid.* Aminoguanidine and iproniazid reduced the inactivation of histamine by the tissues (Table 3), but both were more effective on rat lung than on

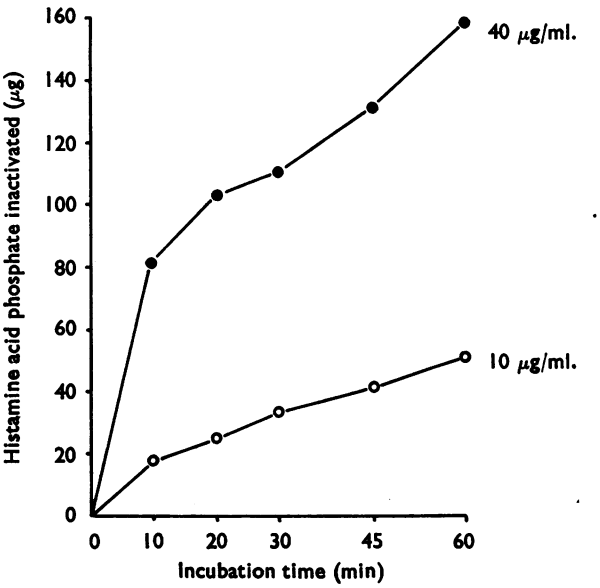

Fig. 3. The effect of incubation time and substrate concentration on the inactivation of histamine (concentrations on right) by guinea-pig lung.

TABLE 3

THE EFFECT OF AMINOGUANIDINE AND IPRONIAZID ON THE INACTIVATION OF HISTAMINE BY SAMPLES OF LUNG

The samples were incubated for 2 hr in 5 ml. of Tyrode solution containing 200 μg of histamine acid phosphate

Inhibitor	Concentration (M)	Inhibition (%) of histamine metabolism for Tuck guinea-pigs 1	2	3	4	5	Mean	Rats 1	2	3	4	5	Mean
Aminoguanidine	10^{-3}				78	77	78				89	93	91
	10^{-4}				57	62	60				76	87	82
	10^{-5}	59	30	41			43	51	36	60			49
	10^{-6}	42	20	35			32	18	4	20			14
	10^{-7}	24	2	20			15	17	12	0			10
Iproniazid	2×10^{-3}	79	62	76			72	90	94	75			86
	10^{-4}	60	59	83	55		64	94	82	83	82		85
	10^{-5}	36	37	37			37	78	58	72			69
Aminoguanidine + iproniazid	$10^{-5}+10^{-4}$			100	74		87			91	88		90
	$10^{-6}+10^{-4}$		80				80						
	$10^{-6}+10^{-5}$								78				78

Inhibitor	Concentration (M)	Chelsea guinea-pigs 1	2	3	4	5	6	Mean
Aminoguanidine	10^{-4}	0	33	13	31	27	37	24

guinea-pig lung. Thus, at a concentration of 10^{-4} M, each inhibitor reduced the ability of rat lung to inactivate histamine by approximately 80% and that of guinea-pig lung (Tuck strain) by approximately 60%. In addition, aminoguanidine was more active on " Tuck " guinea-pigs than on " Chelsea " guinea-pigs (Table 3). In the presence of 10^{-3} M-aminoguanidine, the activity of rat lung was inhibited by 91% and that of guinea-pig lung (Tuck strain) by 78%.

Inactivation of histamine by homogenized lung. Compared with the chopped tissue, homogenization of guinea-pig lung (Chelsea College strain) increased by 20 to 51% (average

TABLE 4

THE EFFECT OF HOMOGENIZATION ON THE INACTIVATION OF HISTAMINE BY LUNGS OF GUINEA-PIGS (CHELSEA) AND RATS

Tissue samples (400 to 800 mg moist weight) were incubated for 60 to 160 min in Tyrode solution containing 200 μg of histamine acid phosphate. Bracketed values also have means

			Histamine acid phosphate			
Species	Expt.	Time that tissue was homogenized (min)	Released (μg)	Inactivated (μg)	Increase of inactivation during homogenization (%)	
Guinea-pig	1	0	—	39		
		2	4	43+4	20	
	2	0	—	50		
		2	9	67+9	51	37
	3	0	—	10		
		2	14	0+14	40	
Rat	1	0	—	61		
		2		115	88	
	2	0	—	57		
		2		144	153	
	3	0	—	58		146
		2		94	62	
	4	0	—	14		
		0·5		62		
		7		53	279	

TABLE 5

THE RELEASE OF HISTAMINE (AS HISTAMINE ACID PHOSPHATE) FROM 500-MG SAMPLES OF GUINEA-PIG AND RAT LUNG, AND ITS INACTIVATION BY THE TISSUE

Homogenization was at room temperature

			Histamine acid phosphate					
Species	Expt.	Time that tissue was homogenized (min)	Released from homogenate (μg)	Total content in homogenate (μg)	Total content of unhomogenized sample (μg)	Inactivated in homogenate (μg)	Metabolized (μg/min)	Metabolized (% of total /min)
Guinea-pig	1	3	—	11·8	13·6	1·8	0·6	4·4
	2	4	25·4	32·0	38·8	6·8	1·7	4·4
	3	5	10·0	20·0	21·4	1·4	0·28	1·3
	4	5	16·2	20·9	26·2	5·3	1·06	4·0
	5	7	13·7	22·4	26·8	4·4	0·63	2·3
Rat	1	3	—	9·4	10·5	1·1	0·37	3·5
	2	3	—	16·8	20·0	3·2	1·07	5·3
	3	10	—	6·5	10·0	3·5	0·35	3·5
	4	10	—	3·5	5·1	1·6	0·16	3·1
	5	10	—	13·8	33·0	19·2	1·92	5·8

39%) its ability to inactivate histamine (Table 4), while the histamine-metabolizing activity of rat lung was increased by 62 to 279% (average 146%).

Metabolism of tissue histamine during homogenization. Some of the tissue histamine is released when lung is homogenized. Experiments were carried out to determine how much of the tissue histamine was metabolized when 500 mg (moist weight) samples of guinea-pig and rat lung were homogenized for 3 to 10 min at room temperature. The amount of histamine extracted from the homogenate was 1.2 to 19.2 μg less than that extracted from 500-mg samples of the chopped untreated tissue (Table 5). The rate of histamine metabolism varied from 0.6 to 1.7 μg/min with guinea-pig lung and from 0.16 to 1.02 μg/min with rat lung. Thus the percentage of the total tissue histamine metabolized per minute of homogenization was 1.3 to 4.4% with guinea-pig lung and 3.1 to 5.8% with rat lung.

DISCUSSION

When histamine is incubated with guinea-pig and rat lung, the amount recovered from the supernatant fluid is reduced. This is due to inactivation of histamine and not to its storage in the tissue, as the amount of histamine extracted from tissue that had been incubated with histamine acid phosphate was similar to that extracted from tissue incubated with Tyrode solution alone. The histamine appears to be inactivated only by the tissue, and not by bacteria, as the presence of tetracycline in the incubate was without effect.

The ability of guinea-pig lung to inactivate histamine varies with the strain of animal. This may explain the findings by other workers (Zeller, 1942; Cotzias & Dole, 1952; Lindell & Westling, 1953; Valette *et al.*, 1956) that guinea-pig lung has little or no ability to metabolize histamine.

The enzymic nature of the inactivation of histamine by rat lung was demonstrated in 1940 by Rose *et al.* The prevention of histamine breakdown at *p*H 3.5, at 2° C, in an atmosphere of nitrogen and after heating the tissue, confirms their findings on rat lung and demonstrates the enzymic activity in guinea-pig lung. In addition, metabolism of histamine by guinea-pig lung increases with the amount of tissue incubated, with the concentration of substrate and with the length of incubation. Since there is no increase in the amount of histamine in guinea-pig and rat lung incubates or tissue extracts after refluxing with N-hydrochloric acid, it appears unlikely that histamine is inactivated by acetylation.

Aminoguanidine specifically blocks diamine oxidase (Schuler, 1952; Blaschko, Friedman, Hawes & Nilsson, 1959). It appears therefore that, since nearly all of the histamine-metabolizing activity of rat lung is inhibited by aminoguanidine, diamine oxidase is the major histamine-metabolizing enzyme in this tissue. However, since 40% of the activity of " Tuck " guinea-pig lung and 76% of the activity of " Chelsea " guinea-pig lung is not blocked by 10^{-4} M-aminoguanidine, it appears that in addition to diamine oxidase a second histamine-metabolizing enzyme is present. This would agree with the finding of Brown *et al.* (1959) that guinea-pig lung contains imidazole-*N*-methyl transferase, and with the experiments of Schayer & Karjala (1956) which showed that diamine oxidase and " histamine-metabolizing enzyme II " are present in the intact guinea-pig. Methylation of histamine to form 4-(2-aminoethyl-1-methylimidazole is not blocked by aminoguanidine or iproniazid (Schayer & Karjala, 1956). As 4-(2-aminoethyl)-1-methylimidazole has little activity on isolated guinea-pig ileum (Lee & Jones, 1949), the formation of this sub-

stance by imidazole-*N*-methyl transferase could account for the inactivation of histamine occurring in the presence of aminoguanidine and iproniazid.

Schayer (1953), Shore & Cohn (1960) and others have shown that iproniazid is a potent inhibitor of diamine oxidase as well as of monoamine oxidase. This finding is supported by the results of the present experiments.

The large increase in histamine-metabolizing activity of homogenized rat lung, compared with the small increase in guinea-pig lung, suggests that histamine reaches the inactivating enzyme or enzymes less easily in rat chopped lung than in guinea-pig chopped lung. It appears, therefore, that the histamine inactivating enzymes of guinea-pig and rat lung are bound differently or are in a different cellular location.

The total quantity of histamine extracted from the homogenate of a sample of guinea-pig or rat lung was less than that extracted from an equal weight of chopped tissue. Some of the lung histamine must, therefore, be inactivated when lung is homogenized. This rate of inactivation is often substantial as the percentage of the total tissue histamine metabolized per minute of homogenization was as much as 4.4% with guinea-pig lung and 5.8% with rat lung.

SUMMARY

1. Histamine is metabolized when incubated with guinea-pig or rat chopped lung at 37° C. This metabolism is substantial with the lungs of rats and of some strains of guinea-pig, but the lungs of guinea-pigs of other strains show much less activity.

2. Incubation of the tissues with aminoguanidine suggests that diamine oxidase accounts for at least 80% of the histamine-metabolizing activity of rat lung and, depending on the strain of the animal, for approximately 20% to at least 60% of the activity of guinea-pig lung; a second histamine-metabolizing enzyme appears to be present in guinea-pig lung. Histamine does not appear to be acetylated by these tissues.

3. Homogenization slightly increases the histamine-metabolizing activity of guinea-pig lung while that of rat lung is markedly increased.

4. When guinea-pig and rat lung are homogenized at room temperature, some of the tissue histamine is metabolized.

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