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In vivo studies on histamine catabolism and its inhibition

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Summary

1. Histamine catabolism *in vivo* was studied in mice subjected to various forms of pretreatment; tissues from mice killed 2·5 min after intravenous injection of ^{14}C-histamine were assayed for ^{14}C-histamine, ^{14}C-methylhistamine and total ^{14}C.

2. Pretreatment of mice with aminoguanidine, an inhibitor of diamine oxidase, strongly increased levels of ^{14}C-histamine in intestine; pretreatment with aminoguanidine plus a monoamine oxidase inhibitor strongly increased levels of ^{14}C-methylhistamine in liver. Effects in other tissues are reported and discussed.

3. Pretreatment of mice with non-isotopic methylhistamine increased levels of ^{14}C-histamine in liver. Methylhistamine is the first known inhibitor of histamine-methylation *in vivo*.

4. Pretreatment of mice with inhibitors of protein synthesis, drugs which reduce the basal activity of histidine decarboxylase and which block its activation, failed to affect histamine catabolism.

5. Pretreatment of mice with endotoxin or with Freund's adjuvant, irritants known to cause activation of histidine decarboxylase, failed to affect histamine catabolism.

6. There was no evidence of parallelism between the histamine-destroying enzymes and the histamine-forming enzyme, histidine decarboxylase, either in distribution or ability to undergo changes in activity. No support was obtained for the view that histamine-catabolizing enzymes play a role in the local control of responses to newly formed histamine.

Introduction

Pathways of histamine catabolism *in vivo* have been identified mainly by assay of urine for excretory products of labelled histamine. In all tested species there are two major pathways: (*a*) Oxidation, catalysed by diamine oxidase, to imidazoleacetic acid; the latter may be excreted unchanged, or as a conjugate. This pathway is almost completely inhibited by aminoguanidine. (*b*) Methylation of the imidazole ring to form methylhistamine; the latter is mainly oxidized to methylimidazoleacetic acid, the major excretory product in mice. Iproniazid and its derivatives inhibit oxidation of methylhistamine *in vivo*. There is no known inhibitor of histamine methylation (Schayer, 1959, 1966).

Our interest in histamine-catabolizing enzymes has been renewed by recent evidence that the histamine-forming enzyme, histidine decarboxylase, has inducible characteristics ; its activity can be increased in every tested tissue of mice (Schayer, 1962), it has a rather rapid turnover (Schayer & Reilly, 1968 ; Kobayashi & Maudsley, 1968), and *in vitro* findings on its distribution have been confirmed *in vivo* (Reilly & Schayer, 1968a, b, 1969). The question arises on the possible role of histamine-catabolizing enzymes in local control of this histamine which is formed at variable rates.

We now report evidence, obtained by *in vivo* experiments, on the identity of tissues capable of destroying histamine, the nature of the enzyme systems concerned, the approximate rates of turnover of histamine-catabolizing enzymes, and the response of these enzymes to stimuli known to enhance histamine formation.

Methods

Female albino CF-1 mice (18–21 g) from Carworth, Inc., New City, New York, were used. ^{14}C-histamine, specific activity 54 mCi/mmol, was purchased from Nuclear-Chicago ; solutions were prepared in silicone-treated glassware in saline (0·9% NaCl solution) containing hydrochloric acid, 0·0001 N ; assay by the BSH method (see below) showed ^{14}C-histamine to account for all radioactivity. Since the specific activity of the ^{14}C-histamine was 60 times greater than that used in earlier studies (Reilly & Schayer, 1968a, b), much less histamine was injected. All experiments were standardized ; following various forms of pretreatment, the mice were injected intravenously with ^{14}C-histamine, killed 2·5 min later, and chilled on crushed ice ; tissue samples were then taken.

The pooled tissues of three mice were homogenized in cold 0·4 N perchloric acid and suitable aliquots assayed for total ^{14}C and ^{14}C-histamine. ^{14}C-methylhistamine was assayed by the method of Snyder, Axelrod & Bauer (1964). Perchloric acid extracts of mouse tissues were made alkaline, and the methylhistamine extracted into chloroform. After washing the chloroform layer, methylhistamine was transferred to acid, evaporated, dissolved in ethanol and counted. We have previously found that among histamine and its known metabolites, only methylhistamine is readily extractable by chloroform (Rothschild & Schayer, 1958 ; Schayer, unpublished). We therefore regard the method for methylhistamine to be roughly quantitative. In contrast, however, we regard ^{14}C-histamine assays based solely on the determination of radioactivity in butanol extracts (Snyder *et al.*, 1964) to be unreliable in certain conditions. For example, in experiment 1, liver ^{14}C-histamine determined in this manner gave erratic results. Consequently, all ^{14}C-histamine assays reported in this paper, except those for intestine in Table 1, were made by the isotope dilution (BSH) method (Schayer, 1968). Assays of other major metabolites were not essential for the purposes of this paper and were omitted.

Endotoxin (*E. coli* lipopolysaccharide) and complete Freund's adjuvant were purchased from Difco Laboratories, aminoguanidine sulphate from K & K Laboratories, Inc., cycloheximide (Actidione) from Nutritional Biochemicals, Inc., and 1-methyl-4(β-aminoethyl) imidazole (referred to in this paper as methylhistamine) from Regis Chemical Co. We are indebted to the following pharmaceutical laboratories for generous gifts of drugs: Hoffmann-La Roche, Inc., for 1-isobutyl-2-iso-

nicotinylhydrazine (IBINH), the Warner-Lambert Research Institute for phenelzine sulphate (Nardil), Abbott Laboratories for pargyline hydrochloride (Eutonyl), Smith, Kline and French Laboratories for tranylcypromine sulphate (Parnate), and the Merck Institute for Therapeutic Research for actinomycin D and tenuazonic acid.

Results

Experiment 1 : Effect of a diamine oxidase inhibitor, aminoguanidine, and a monoamine oxidase inhibitor, IBINH, on ^{14}C-histamine catabolism in liver and intestine

In mice injected intravenously with ^{14}C-histamine, liver and intestine contain more ^{14}C than do other tissues, and appear to be the tissues most active in destroying histamine (Reilly & Schayer, 1968a). Experiment 1 examines the identity of the histamine-catabolizing enzymes of liver and intestine (Table 1).

Liver. ^{14}C-histamine levels were increased slightly but significantly by aminoguanidine treatment but not further increased by IBINH. ^{14}C-methylhistamine levels were slightly increased in mice pretreated with aminoguanidine (*B*), and strongly increased in mice pretreated with aminoguanidine plus IBINH (*C*). When ^{14}C-amine concentrations were calculated as % of total ^{14}C, the same trends were evident. Total ^{14}C was relatively low in group *C* but this finding could not be confirmed in experiment 2. ^{14}C-histamine expressed as % of total ^{14}C in the control group (*A*) was much lower in liver than in any other tissue tested. Some major histamine catabolites were not determined, so total ^{14}C was always greater than the sum of ^{14}C-histamine plus ^{14}C-methylhistamine.

Intestine. ^{14}C-histamine levels were markedly increased by pretreatment with aminoguanidine (*B*), but not further increased by IBINH treatment (*C*). ^{14}C-methylhistamine levels showed only minor responses to these inhibitors. Total ^{14}C was definitely decreased in groups *B* and *C*, a finding confirmed in experiment 2.

Experiment 2 : Effect of aminoguanidine and IBINH on ^{14}C-histamine catabolism in various tissues

Experiment 2 is similar to experiment 1 but additional tissues were assayed (Table 2). ^{14}C-histamine expressed as % of total ^{14}C was increased by aminoguanidine treatment (*B*) in all tested tissues but was not further increased by IBINH treatment (*C*). ^{14}C-methylhistamine expressed as % of total ^{14}C was only slightly affected by either drug. The results obtained for intestine confirmed those of experiment 1 in all essential respects. In liver, the d.p.m. values for total ^{14}C were in the controls (*A*) 288,000 (S.D. 78,000); in group *B* 280,000 (S.D. 28,000) and in group *C* 232,000 (S.D. 34,000) ($n=4$); groups *B* and *C* were not significantly different from controls. Thymus and kidney showed relatively high values of ^{14}C-histamine expressed as % of total ^{14}C, so no further assays were made.

Experiment 3 : Effect of monoamine oxidase inhibitors on ^{14}C-methylhistamine levels in liver

IBINH was used as a monoamine oxidase inhibitor in experiments 1 and 2 because it had been found (Schayer & Karjala, 1956) to be the drug most effective in blocking oxidation of methylhistamine to methylimidazole-acetic acid. Many

TABLE 1. *^{14}C-histamine, ^{14}C-methylhistamine and total ^{14}C in liver and intestine of mice 2·5 min after intravenous injection of ^{14}C-histamine; effects of aminoguanidine and IBINH*

Tissue and treatment	^{14}C-histamine (d.p.m./g)	^{14}C methylhistamine (d.p.m./g)	Total ^{14}C (d.p.m./g)	^{14}C-histamine (% tot. ^{14}C)	^{14}C-methyl-histamine (% tot. ^{14}C)
Liver					
A, Control	812(194)	852(180)	172,000(20,300)	0·5(0·1)	0·5(0·05)
B, Aminoguan.	2,300(594)	2,000(526)	182,000(29,900)	1·3(0·4)	1·1(0·4)
C, Aminoguan. and IBINH	3,320(833)	28,800(2,060)	134,000(6,700)	2·5(0·6)	21·5(2·0)
	(*A-B*, $P<0·001$)	(*A-B*, $P<0·01$)	(*A-B*, NS)	(*A-B*, $P<0·01$)	(*A-B*, $P<0·01$)
	(*A-C*, $P<0·001$)	(*A-C*, $P<0·001$)	(*A-C*, $P<0·01$)	(*A-C*, $P<0·001$)	(*A-C*, $P<0·001$)
	(*B-C*, NS)	(*B-C*, $P<0·001$)	(*B-C*, $P<0·01$)	(*B-C*, $P<0·01$)	(*B-C*, $P<0·001$)
Intestine					
A, Control	5,870(696)	2,130(203)	119,000(13,900)	5·0(1·1)	1·8(0·3)
B, Aminoguan.	22,500(2,430)	2,960(288)	67,400(9,100)	33·4(5·8)	4·4(0·8)
C, Aminoguan. and IBINH	17,800(2,790)	3,740(543)	63,400(12,000)	28·1(7·1)	5·9(2·0)
	(*A-B*, $P<0·001$)	(*A-B*, $P<0·001$)	(*A-B*, $P<0·001$)	(*A-B*, $P<0·001$)	(*A-B*, $P<0·001$)
	(*A-C*, $P<0·001$)	(*A-C*, $P<0·001$)	(*A-C*, $P<0·001$)	(*A-C*, $P<0·001$)	(*A-C*, $P<0·01$)
	(*B-C*, NS)	(*B-C*, $P<0·05$)	(*B-C*, NS)	(*B-C*, NS)	(*B-C*, NS)

Saline, aminoguanidine sulphate, 200 μg base, or aminoguanidine plus IBINH, 1·5 mg, injected intraperitoneally approximately 20 min before injection of ^{14}C-histamine, 0·50 μCi. Values are means, with standard deviations in brackets, of five assays per group. They are expressed as disintegrations/min per g tissue (d.p.m./g) or as % of total ^{14}C. All assays in all tables were performed on pooled tissues of three mice.

TABLE 2. *^{14}C-histamine, ^{14}C-methylhistamine and total ^{14}C in tissues of mice 2·5 min after intravenous injection of ^{14}C-histamine; effects of aminoguanidine and IBINH*

Tissue and treatment	^{14}C-histamine (d.p.m./g)	^{14}C-methylhistamine (d.p.m./g)	Total ^{14}C (d.p.m./g)	^{14}C-histamine (% tot. ^{14}C)	^{14}C-methyl-histamine (% tot. ^{14}C)
Blood					
A, Control	9,500(920)	7,960(264)	40,500(3,420)	23·5(2·4)	19·7(1·9)
B, Aminoguan.	16,700(2,040)	7,830(1,530)	39,800(4,190)	42·0(3·6)	19·7(2·4)
C, Aminoguan. and IBINH	19,500(1,770)	11,400(1,780)	48,300(4,920)	40·4(1·5)	23·6(3·5)
	(*A-B*, $P<0·001$)	(*A-B*, NS)	(*A-B*, NS)	(*A-B*, $P<0·001$)	(all NS)
	(*A-C*, $P<0·001$)	(*A-C*, $P<0·01$)	(*A-C*, $P<0·05$)	(*A-C*, $P<0·001$)	
	(*B-C*, NS)	(*B-C*, $P<0·05$)	(*B-C*, $P<0·05$)	(*B-C*, NS)	
Lymph node					
A, Control	2,670(610)	12,500(2,190)	67,000(5,070)	4·0(0·7)	18·7(2·4)
B, Aminoguan.	7,260(3,170)	13,500(2,030)	58,300(6,450)	12·5(3·9)	23·2(1·0)
C, Aminoguan. and IBINH	10,700(2,710)	20,000(4,230)	70,800(8,850)	15·1(2·9)	28·2(3·3)
	(*A-B*, $P<0·05$)	(*A-B*, NS)	(all NS)	(*A-B*, $P<0·01$)	(*A-B*, $P<0·01$)
	(*A-C*, $P<0·01$)	(*A-C*, $P<0·05$)		(*A-C*, $P<0·001$)	(*A-C*, $P<0·01$)
	(*B-C*, NS)	(*B-C*, $P<0·05$)		(*B-C*, NS)	(*B-C*, $P<0·025$)
Intestine					
A, Control	7,620(2,560)	3,400(960)	207,000(9,800)	3·7(1·1)	1·6(0·5)
B, Aminoguan.	44,600(5,950)	5,400(1,100)	123,000(10,100)	36·3(3·5)	4·4(0·6)
C, Aminoguan. and IBINH	43,300(12,900)	6,890(1,490)	129,000(6,400)	33·6(10·8)	5·3(1·2)
	(*A-B*, $P<0·001$)	(*A-B*, $P<0·05$)	(*A-B*, $P<0·001$)	(*A-B*, $P<0·001$)	(*A-B*, $P<0·01$)
	(*A-C*, $P<0·01$)	(*A-C*, $P<0·025$)	(*A-C*, $P<0·001$)	(*A-C*, $P<0·01$)	(*A-C*, $P<0·01$)
	(*B-C*, NS)	(*B-C*, NS)	(*B-C*, NS)	(*B-C*, NS)	(*B-C*, NS)
Heart					
A, Control	5,200(1,760)	50,400(12,500)	174,000(30,100)	3·0(0·8)	29·0(2·5)
B, Aminoguan.	20,500(6,970)	70,700(12,900)	236,000(36,500)	8·7(2·1)	30·0(1·4)
C, Aminoguan. and IBINH	15,900(5,660)	63,900(8,100)	193,000(31,000)	8·2(1·7)	33·1(1·3)
	(*A-B*, $P<0·01$)	(all NS)	(*A-B*, $P<0·05$)	(*A-B*, $P<0·01$)	(*A-B*, NS)
	(*A-C*, $P<0·025$)		(*A-C*, NS)	(*A-C*, $P<0·01$)	(*A-C*, $P<0·025$)
	(*B-C*, NS)		(*B-C*, NS)	(*B-C*, NS)	(*B-C*, $P<0·01$)
Thymus					
A, Control	16,200(3,210)		56,800(12,000)	28·5(0·5)	
Kidney					
A, Control	38,800(8,830)		258,000(38,800)	15·0(2·2)	

As in Table 1, but 1·0 μCi ^{14}C-histamine, instead of 0·5 μ Ci, was injected. Values are means, with standard deviations in brackets, of four assays per group (control intestine, ^{14}C-histamine, three assays).

potent inhibitors of monoamine oxidase were introduced subsequently, and three of them were compared with IBINH for effect on ^{14}C-methylhistamine levels (Table 3). Aminoguanidine had only a slight effect, but all monoamine oxidase inhibitors caused a marked rise in ^{14}C-methylhistamine expressed as % of total ^{14}C. With the exception of phenelzine at the lower dose, there was no significant difference in the effectiveness of the drugs.

Experiment 4: Effect of "specific" monoamine oxidase inhibitors on ^{14}C-histamine catabolism in vivo

In experiments 1 and 2 the effect of IBINH was not tested alone; it is a moderately active diamine oxidase inhibitor as well as a monoamine oxidase inhibitor, so its effect, given alone, cannot be interpreted unequivocally. The two monoamine oxidase inhibitors found to be most potent in experiment 3, pargyline and tranylcypromine, had little effect on ^{14}C-histamine expressed as % of total ^{14}C in either intestine or liver, but strongly increased liver ^{14}C-methylhistamine (Table 4).

Experiments 5 and 6: Effect of non-isotopic methylhistamine on ^{14}C-histamine catabolism in vivo

No effective *in vivo* inhibitor of the histamine-methylating enzyme is known. In experiments 5 and 6, relatively large doses of methylhistamine were tested for influence on histamine-methylation in liver (Table 5). Methylhistamine pretreatment increased liver ^{14}C-histamine levels markedly and ^{14}C-methylhistamine levels moderately. Total ^{14}C was not significantly affected.

Experiments 7 and 8: Effects of inhibitors of protein synthesis and of RNA synthesis on ^{14}C-histamine catabolism in vivo

Inhibitors of protein synthesis—for example puromycin and tenuazonic acid—reduce the basal level of histidine decarboxylase activity within a few hours

TABLE 3. *^{14}C-methylhistamine and total ^{14}C in liver of mice 2·5 min after intravenous injection of ^{14}C-histamine; effects of monoamine oxidase inhibitors plus aminoguanidine*

Tissue and treatment	^{14}C-methyl-histamine (d.p.m./g)	Total ^{14}C (d.p.m./g)	^{14}C-methyl-histamine (% tot. ^{14}C)
Liver			
Control	1,140(450)	109,000(18,500)	1·0(0·3)
Aminoguanidine	3,290(692)	108,000(31,200)	3·0(1·1)
Aminoguan. and IBINH	29,200(5,500)	94,000(19,600)	31·1(7·0)
Aminoguan. and tranyl-cypromine 200 μg	35,200(7,250)	96,000(10,900)	36·7(5·9)
Aminoguan. and tranyl-cypromine 600 μg	39,400(6,800)	104,000(13,100)	37·9(1·9)
Aminoguan. and phenelzine 200 μg	16,800(3,250)	107,000(9,800)	15·7(2·3)
Aminoguan. and phenelzine 600 μg	38,000(7,610)	101,000(16,500)	37·6(3·0)
Aminoguan. and pargyline 200 μg	35,100(8,900)	96,000(18,400)	36.6(4·1)
Aminoguan. and pargyline 600 μg	29,900(7,360)	85,000(12,400)	35·2(3·8)

Aminoguanidine plus drugs (200 μg or 600 μg salt) injected intraperitoneally approximately 20 min before injection of ^{14}C-histamine, 0·4 μCi; aminoguanidine and IBINH as in Table 1. Values are means, with standard deviations in brackets, of four assays per group.

TABLE 4. *^{14}C-histamine, ^{14}C-methylhistamine and total ^{14}C in liver and intestine of mice 2·5 min after intravenous injection of ^{14}C-histamine; effects of monoamine oxidase inhibitors*

Tissue and treatment	^{14}C-histamine (d.p.m./g)	^{14}C-methylhistamine (d.p.m./g)	Total ^{14}C (d.p.m./g)	^{14}C-histamine (% tot. ^{14}C)	^{14}C-methylhistamine (% tot. ^{14}C)
Intestine					
Control	2,900(571)	Not	96,500(7,550)	3·0(0·4)	—
Pargyline	2,000(217)	assayed	94,000(12,500)	2·1(0·3)	
Tranylcypromine	2,020(331)		81,000(7,530)	2·5(0·3)	
Liver					
Control	864(136)	674(65)	137,000(11,000)	0·6(0·06)	0·5(0·1)
Pargyline	1,090(233)	10,800(3,130)	113,000(16,600)	1·0(0·13)	9·6(2·3)
Tranylcypromine	1,300(235)	15,400(1,990)	102,000(9,540)	1·3(0·17)	15·1(1·3)

Saline or drugs, 200 μg salt, injected intraperitoneally approximately 20 min before injection of ^{14}C-histamine, 0·5 μCi. Values are means, with standard deviations in brackets, of four assays per group (control liver, three assays).

TABLE 5. *^{14}C-histamine, ^{14}C-methylhistamine and total ^{14}C in liver of mice 2·5 min after intravenous injection of ^{14}C-histamine; effect of non-isotopic methylhistamine (MeH)*

Tissue and treatment	^{14}C-histamine (d.p.m./g)	^{14}C-methylhistamine (d.p.m./g)	Total ^{14}C (d.p.m./g)	^{14}C-histamine (% tot. ^{14}C)	^{14}C-methylhistamine (% tot. ^{14}C)
Liver : experiment 5					
Aminoguanidine	1,870(156)	4,820(1,890)	128,000(5,450)	1·5(0·1)	3·8(1·4)
Aminoguan. and MeH 6 mg	78,600(9,520)	15,800(3,440)	122,000(12,500)	64·4(10·9)	13·0(2·6)
Aminoguan. and MeH 2 mg	41,000(8,250)	Not assayed	117,000(13,600)	35·0(9·2)	—
Experiment 6					
Aminoguanidine	1,950(173)	2,870(1,170)	117,000(12,200)	1·7(0·06)	2·5(0·9)
Aminoguan. and MeH 10 mg	89,500(10,100)	7,840(3,390)	126,000(6,400)	71·0(4·6)	6·2(2·9)

Aminoguanidine sulphate, 200 μg base, or aminoguanidine plus methylhistamine base, injected approximately 20 min before injection of ^{14}C-histamine, 0·5 μCi. Values are means, with standard deviation in brackets, of four assays per group.

TABLE 6. *^{14}C-histamine, ^{14}C-methylhistamine and total ^{14}C in liver and intestine of mice 2·5 min after intravenous injection of ^{14}C-histamine; effects of tenuazonic acid, cycloheximide and actinomycin D*

Tissue and treatment	^{14}C-histamine (d.p.m./g)	^{14}C-methylhistamine (d.p.m./g)	Total ^{14}C (d.p.m./g)	^{14}C-histamine (% tot. ^{14}C)	^{14}C-methyl-histamine (% tot. ^{14}C)
Experiment 7, intestine					
Control	2,480(727)	Not	101,000(8,500)	2·5(0·6)	—
Tenuazonic acid	2,980(221)	assayed	155,000(11,800)	1·9(0·06)	
Cycloheximide	4,560(2,080)		154,000(13,500)	3·0(1·1)	
Actinomycin	3,040(1,140)		115,000(6,200)	2·6(0·8)	
Experiment 8, liver					
Control	Not	24,500(6,200)	82,000(14,500)	—	29·9(3·2)
Tenuazonic acid	assayed	39,800(4,600)	136,000(13,000)		29·3(1·0)
Cycloheximide		42,300(3,000)	150,000(7,500)		28·2(2·9)
Actinomycin		25,300(7,100)	105,000(19,200)		24·1(3·9)

Saline, tenuazonic acid 2 mg, cycloheximide 1 mg, or actinomycin D 7 μg, injected intraperitoneally 3 times, at 0, 65 and 150 min. ^{14}C-histamine, 0·5 μCi, injected at 240 min. In experiment 8, aminoguanidine and IBINH were also given, as in experiment 1, to cause accumulation of ^{14}C-methylhistamine in liver. Values are means with standard deviations in brackets, of four assays per group.

(Schayer & Reilly, 1968), a fact which suggests that activity of this enzyme is in a fairly rapid state of turnover. These drugs, and actinomycin D, an inhibitor of RNA synthesis, were now tested for effect on ^{14}C-histamine and total ^{14}C levels of intestine (experiment 7) and on ^{14}C-methylhistamine and total ^{14}C levels of liver (experiment 8), the purpose being an attempt to evaluate turnover time of histamine-catabolizing activities (Table 6).

Values of ^{14}C-histamine or of ^{14}C-methylhistamine, both expressed as % of total ^{14}C, were not significantly affected by any of the drugs. Total ^{14}C, however, was increased in intestine and liver ($P<0{\cdot}01$) by tenuazonic acid and cycloheximide and less so by actinomycin D.

Experiments 9 and 10 : Effects of endotoxin and Freund's adjuvant on ^{14}C-methylhistamine in liver

Endotoxin is the most potent known activator of histidine decarboxylase, maximal activation occurring in approximately 4–6 h. Freund's complete adjuvant causes a more gradual activation, the maximum occurring about 3 days after injection. The effect of these irritants on histamine catabolism was tested in mice which had been pretreated with aminoguanidine and IBINH to cause methylhistamine accumulation in liver (Table 7). Neither endotoxin nor Freund's adjuvant had a significant effect on ^{14}C-methylhistamine expressed as % of total ^{14}C.

Discussion

Several working assumptions are used to interpret results of the experiments of this paper: (*a*) a low value of ^{14}C-histamine expressed as % of total ^{14}C indicates the presence of one or more histamine-catabolizing enzymes ; (*b*) a marked increase in ^{14}C-histamine caused by aminoguanidine indicates the presence of diamine oxidase ; (*c*) a strong accumulation of ^{14}C-methylhistamine caused by monoamine oxidase inhibitors indicates the presence of the histamine-methylating enzyme. Obviously, the contribution of each tissue to histamine catabolism cannot be interpreted unequivocally for, to some extent, metabolites formed in one tissue may be accumulated by others.

We killed mice 2·5 min after intravenous injection of ^{14}C-histamine, that is, at a time when tissues had been perfused but blood levels of ^{14}C-histamine remained

TABLE 7. *^{14}C-methylhistamine and total ^{14}C in liver of mice 2·5 min after intravenous injection of ^{14}C-histamine; effects of pretreatment with endotoxin or Freund's complete adjuvant*

Tissue and treatment	^{14}C-methylhistamine (d.p.m./g)	Total ^{14}C (d.p.m./g)	^{14}C-methyl-histamine (% tot. ^{14}C)
Experiment 9, Liver			
Control	20,700(5,770)	79,000(5,290)	26·2(5·5)
Endotoxin	14,200(3,850)	63,000(5,520)	22·5(6·8)
Experiment 10, Liver			
Control	39,400(8,300)	109,000(9,800)	36·1(5·6)
Freund	48,800(5,500)	137,000(16,100)	35·6(1·9)

Freund's complete adjuvant, 0·25 ml, injected intraperitoneally 4 days before ^{14}C-histamine, 0·5 μCi; endotoxin, 100 μg, injected 4 h before ^{14}C-histamine. Aminoguanidine and IBINH given as in experiment 1. Values are means, with standard deviations in brackets, of five assays per group.

high ; hence a sharply lower tissue level might be a valid indication of local catabolism. If longer intervals between injection and death were used, the progressive destruction of histamine, and the dissemination of catabolites, might produce a more uniform pattern throughout the body and complicate interpretation.

Our test for diamine oxidase activity uses aminoguanidine in doses which cause nearly complete inhibition *in vivo* (Schayer, Smiley & Kennedy, 1954) ; this drug has no other known action of comparable strength. Aminoguanidine strongly increases intestinal ^{14}C-histamine levels (Tables 1 and 2) and so diamine oxidase probably dominates intestinal histamine catabolism in mice ; a similar conclusion based on *in vitro* studies was drawn by Kim, Backus, Harris & Rourke (1969).

Since aminoguanidine also reduces intestinal total ^{14}C it is possible that : (*a*) newly formed endogenous histamine accumulates, partially fills binding sites, and reduces uptake of injected ^{14}C-histamine, or (*b*) aminoguanidine may occupy histamine-binding sites in the intestine and reduce uptake of injected ^{14}C-histamine.

Of the other tissues tested (Table 2), lymph node, heart and liver show small to moderate increases in ^{14}C-histamine in aminoguanidine-treated mice and may therefore possess some diamine oxidase activity, but this increase could arise, at least in part, from the increased blood level. As there is no evidence for significant diamine oxidase activity in blood of normal mice, the intestine is probably the major source of increased blood histamine.

Evidence for *in vivo* histamine methylation by a tissue requires evidence, first, of local catabolism and, second, of an accumulation of ^{14}C-methylhistamine not attributable to blood. In liver, monoamine oxidase inhibitors increase ^{14}C-methylhistamine levels while the diamine oxidase inhibitor, aminoguanidine, has little effect (Tables 1, 3 and 4). These facts suggest that monoamine oxidase is the catalyst of methylhistamine oxidation and not diamine oxidase, as suggested by Kapeller-Adler (1965). However, ^{14}C-methylhistamine is not significantly increased when doses of tranylcypromine and pargyline are raised from 200 μg to 600 μg per mouse. Therefore, under these conditions, some enzyme activity concerned with methylhistamine destruction may not be reached by the monoamine oxidase inhibitors, or not be affected by them.

Of the other tissues tested (Tables 1 and 2), lymph node and intestine show small increases in ^{14}C-methylhistamine in mice pretreated with monoamine oxidase inhibitors, but this increase could occur, at least in part, from ^{14}C-methylhistamine transferred from liver by the blood.

Heart shows high values of ^{14}C-methylhistamine which may have been formed in the heart or extracted from blood. The failure of IBINH to increase ^{14}C-methylhistamine suggests that the heart may have little ability to oxidize methylhistamine.

Thymus and kidney show relatively high values of ^{14}C-histamine expressed as % of total ^{14}C. As these data did not suggest strong histamine-destroying ability, these tissues were not tested further. Lung, stomach and muscle (Reilly & Schayer, 1968) and spleen (unpublished) were omitted for the same reason.

So far, the findings suggest that (*a*) liver is a potent histamine-catabolizing tissue, acting mainly by methylation, and (*b*) intestine is active, but considerably less so than liver, acting mainly by diamine oxidase action. Other tissues are of less importance for histamine catabolism in mice. These conclusions are consistent with

the observation that, in mice injected with ^{14}C-histamine, methylated products account for about 70% of the catabolites in urine and products of diamine oxidase action for about 30% (Schayer & Karjala, 1956); in mice fed with ^{14}C-histamine, however, products of diamine oxidase action predominate (Schayer, 1953). Lindahl (1958) showed that histamine is methylated *in vitro* by mouse liver. Brown, Tomchick & Axelrod (1959) found that, *in vitro*, every mouse tissue tested can methylate histamine, liver showing only moderate activity. These workers, however, added S-adenosylmethionine to their incubates and, *in vivo*, this co-factor is not necessarily present in all tissues in adequate amounts or at the right site.

Methylhistamine is the first effective *in vivo* inhibitor of the histamine-methylating enzyme to be reported. Pretreatment with methylhistamine also increases ^{14}C-methylhistamine; for, although less of this metabolite may be formed than normally, destruction of the radioactive form is retarded by dilution with non-isotopic methylhistamine. Snyder & Axelrod (1964) found several drugs which slightly inhibit methylhistamine formation *in vivo*. The inhibition of histamine methylation by methylhistamine may make it possible to use urinary histamine levels as criteria of histamine formation or release. Previously, urinary histamine levels have been meaningful only in the female rat, which catabolizes histamine largely by diamine oxidase action (Schayer, Wu & Smiley, 1954; Schayer, 1959; Kahlson & Rosengren, 1968). The present findings also imply that pharmacological effects of administration of methylhistamine may, to some extent, be indirect in that they may be due to prolongation and intensification of the actions of endogenous histamine.

Protein synthesis inhibitors reduce total ^{14}C in intestine and liver. Since these drugs reduce histamine formation, they may increase the number of binding sites for exogenous histamine. Actinomycin D, which does not reduce histamine formation, has relatively little effect on total ^{14}C. Since the drugs do not significantly alter the values of ^{14}C-amines expressed as % of total ^{14}C, there is no evidence that protein synthesis inhibitors affect the activity of the histamine-methylating enzyme or of diamine oxidase. Under similar conditions histidine decarboxylase activity of lung and liver is reduced by approximately 70% in 4 h (Schayer & Reilly, 1968). Kobayashi & Maudsley (1968) found that cycloheximide strongly reduces histidine decarboxylase activity in stomach of normal rats, the half-life being about 100 min. Evidently the histamine-catabolizing enzymes have a much slower turnover than does the histamine-forming enzyme.

Endotoxin, the strongest known histidine decarboxylase activator, has no discernible effect on histamine methylation. We had previously found that endotoxin has no effect on intestinal diamine oxidase activity (Reilly & Schayer, 1968b). The data obtained with Freund's adjuvant suggest that liver, even after 4 days' exposure to increased endogenous histamine formation (Schayer & Reilly, unpublished), shows no sign of a compensatory increase in histamine-catabolizing activity.

We failed to find any indication of parallelism between the two major histamine-catabolizing enzymes and histidine decarboxylase in distribution among the tissues, in turnover rate, or in ability to undergo activation in response to irritant stimuli. Hence there is no evidence that these catabolizing enzymes play a role in the control of the duration of the local action of newly formed histamine.

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