Br. J. Pharmac. (1972), **45**, 463–469.

Effect of glucocorticoids on histamine metabolism in mice

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Summary

1. Histamine formation and catabolism were studied *in vivo* in standardized experiments; for the former, animals pretreated with inhibitors of histamine catabolism were killed 10 min after intravenous injection of ^{14}C-L-histidine; for the latter, no inhibitors were used and animals were killed 2·5 min after intravenous injection of ^{14}C-histamine. *In vitro* assays of histidine decarboxylase activity were also made.

2. Pretreatment of adrenalectomized mice with cortisol for one day caused a major alteration in histamine formation; stomach and intestine were abnormally high in the ^{14}C-histamine: total ^{14}C ratio, while most other tested tissues were lower. Brain and thymus were unaffected. Cortisol treatment caused a marked increase in urinary excretion of both ^{14}C-histamine and total ^{14}C.

3. Following injection of a rapidly absorbed corticoid, significant effects on urine and stomach were demonstrable at 30 min and 4 h, respectively.

4. In cortisol-treated adrenalectomized mice histidine decarboxylase activity, relative to controls, was decreased in lung but virtually unaffected in kidney, heart, muscle and liver. In stomach, activity was increased but the statistical significance was low.

5. In mice injected with ^{14}C-histamine, cortisol pretreatment caused a small drop in tissue levels of ^{14}C-histamine, ^{14}C-methylhistamine and total ^{14}C, but increased levels in urine. In all cases, however, the ratio of the two amines to total ^{14}C was not significantly different from controls.

6. From the foregoing experiments it was concluded that the results in (2) could be largely attributed to entry into urine of ^{14}C-histamine and total ^{14}C, thus reducing the availability of these substances in blood for extraction by tissues. In stomach, the cortisol-induced increase in histamine formation may involve some process other than increased histidine decarboxylase activity.

7. The activation of histidine decarboxylase in liver and lung of intact mice by Freund's adjuvant, or by endotoxin, was reduced by corticoid treatment.

8. In a single short experiment on the effect of cortisol on histamine formation in adrenalectomized rats, results from stomach, lung and heart, the only tissues assayed, were similar to those from the mouse experiment.

Introduction

A possible influence of adrenal glucocorticoid hormones on histamine metabolism was first proposed by Rose & Browne (1938) who found histamine levels of certain tissues to be elevated in adrenalectomized rats. Goth, Allman, Merritt &

Holman (1951), and Halpern, Biozzi, Briot & Benacerraf (1953) published indirect evidence that cortisone retarded formation of histamine; later, reduced binding of ^{14}C-histamine was found in skin of cortisone-treated rats injected with ^{14}C-L-histidine (Schayer, Smiley & Davis, 1954). Early work in this field has been reviewed (Schayer, 1966).

With improved means for investigating histamine formation and catabolism *in vivo* (Reilly & Schayer, 1968a, 1968b, 1970, 1971a, 1971b) we have extended studies to mice.

Methods

Female albino CF-1 mice (19–25 g) and female CFN rats, approximately 100 g, from Carworth, Inc., New City, New York, were used. ^{14}C-L-Histidine 58 mCi/mmol, and ^{14}C-histamine, 54 mCi/mmol, were purchased from Amersham/Searle, Des Plaines, Illinois.

For intravenous administration of ^{14}C-compounds, mice were injected in the tail vein. Rats were anaesthetized with pentobarbitone and injected in the femoral vein.

For analysis of tissues from *in vivo* experiments the pooled tissues of three mice, or of two rats, were homogenized in cold 0·4 M perchloric acid. Aliquots were assayed for total ^{14}C by direct count, for ^{14}C-histamine by isotope dilution as benzenesulphonylhistamine as previously described (Reilly & Schayer, 1968a) and for ^{14}C-methylhistamine by the method of Snyder, Axelrod & Bauer (1964). Histidine

TABLE 1. *Effect of* 1 *day cortisol treatment on histamine formation in adrenalectomized mice;* ^{14}*C-histamine and total* ^{14}*C in tissues of mice* 10 *min after intravenous injection of* ^{14}*C-L-histidine*

Tissue and treatment		I ^{14}C-histamine (d.p.m./g)	II Total ^{14}C (d.p.m./g) ($\times 10^{-3}$)	I/II* ($\times 10^{3}$)
Blood	Control	3,930± 781	3,410±607	1·16±0·08
	Cortisol	1,290± 56	1,760± 96	0·74±0·03†
Stomach	Control	22,600± 978	1,270± 32	17·8 ±1·0
	Cortisol	54,800± 367	1,160± 69	47·4 ±3·2‡
Intestine	Control	4,210± 143	1,630± 23	2·58±0·09
	Cortisol	6,550± 595	1,390± 62	4·72±0·40‡
Lung	Control	5,010± 185	1,460± 47	3·44±0·15
	Cortisol	1,710± 94	1,090± 46	1·57±0·03‡
Liver	Control	6,310± 176	4,540±310	1·42±0·10
	Cortisol	2,670± 171	4,440±170	0·60±0·04‡
Muscle	Control	1,080± 23	1,100± 35	0·99±0·03
	Cortisol	807± 38	1,190± 30	0·68±0·04‡
Brain	Control	651± 21	818± 25	0·80±0·03
	Cortisol	659± 25	844± 45	0·79±0·05
Heart	Control	8,420± 269	1,630± 96	5·20±0·23
	Cortisol	4,590± 445	1,450± 49	3·16±0·27‡
Kidney	Control	13,300±1,080	3,180±205	4·18±0·23
	Cortisol	5,280± 421	2,550±123	2·06±0·09‡
Thymus	Control	3,200± 273	863± 51	3·72±0·33
	Cortisol	2,270± 92	614± 24	3·70±0·17
Urine	Control	6,470±1,660	1,230±271	5·04±0·35
	Cortisol	57,500±3,910	10,000±741	5·75±0·28

Day 0, all mice adrenalectomized. Day 1, saline or cortisol acetate 1 mg, injected under skin of back. Day 2, all injected intraperitoneally with aminoguanidine 0·2 mg, and methylhistamine 6 mg, 20 min later injected intravenously with ^{14}C-L-histidine 19 μCi, and killed 10 min later. Values are means± S.E.M. of five assays per group (cortisol groups of heart, intestine and muscle, four assays). * Heading denotes d.p.m. ^{14}C-histamine per thousand d.p.m. total ^{14}C. Difference between groups in column I/II: † $P<0·01$; ‡ $P<0·001$; others N.S.

decarboxylase activity was measured by the isotope dilution method (Schayer, 1968, 1971).

Cortisol and prednisolone phosphate were gifts from the Merck Institute for Therapeutic Research (Rahway, N.J.). Methylhistamine was purchased from the Regis Chemical Co. (Chicago, Ill.), aminoguanidine sulphate from K and K Laboratories (Plainview, N.Y.), Freund's complete adjuvant and *E. coli* lipopolysaccharide (endotoxin) from Difco Laboratories (Detroit, Mich.).

Results

Effect of cortisol on formation of ^{14}C-histamine in mice injected with ^{14}C-L-histidine

Adrenalectomized mice were used to eliminate the influences of released endogenous corticoids, and of adrenaline; the latter can activate histidine decarboxylase in some mouse tissues. All mice were pretreated with inhibitors of histamine catabolism. The results (Table 1) show that cortisol increased ^{14}C-histamine in stomach and intestine, but reduced it in all other tested tissues except brain. There was relatively little effect on total ^{14}C except for a large drop in the blood level. Cortisol caused a massive urinary excretion of ^{14}C-histamine and total ^{14}C.

Time study of cortisol effect

As the data of Table 1 show a major change in histamine formation 24 h after cortisol acetate, shorter periods, 0·5 and 4 h, were used. In order to obtain a more rapid response, a soluble corticoid, prednisolone phosphate, plus cortisol (free alcohol) were injected intraperitoneally. To conserve ^{14}C-L-histidine, the dose was reduced to 1·9 μCi, and only stomach and urine assayed (Table 2). In stomach, ^{14}C-histamine, columns I and I/II, was increased at 0·5 and 4 h but only the latter was significant. For urine, ^{14}C-histamine and total ^{14}C were markedly increased at both time intervals but their ratios remained the same as in controls.

TABLE 2. *Histamine formation in adrenalectomized mice tested* 0·5 *and* 4 *h after injection of glucocorticoids*

		I ^{14}C-histamine (d.p.m./g)	II Total ^{14}C (d.p.m./g) ($\times 10^{-3}$)	I/II ($\times 10^{3}$)
Stomach	A. Control	2,660±337	125±3·8	21·2±2·5
	B. Corticoid, 0·5 h	3,100±226	117±8·0	28·4±4·3
	C. Corticoid, 4 h	4,670±317	140±8·5	33·3±1·2
		(A–B, NS)	(A–B, NS)	(A–B, NS)
		(A–C, $P<0·01$)	(A–C, NS)	(A–C, $P<0·01$)
Urine	A. Control	232± 63	95±29	2·67±0·29
	B. Corticoid, 0·5 h	1,180±235	432±83	2·75±0·08
	C. Corticoid, 4 h	3,460±222	1,200±71	2·88±0·06
		(A–B, $P<0·025$)	(A–B, $P<0·025$)	(A–B, NS)
		(A–C, $P<0·001$)	(A–C, $P<0·001$)	(A–C, NS)

Two days after adrenalectomy mice injected intraperitoneally with saline or mixture of prednisolone phosphate 0·2 mg, and cortisol (free alcohol) 0·5 mg. All mice injected intraperitoneally with aminoguanidine 0·2 mg, and methylhistamine 6 mg, 25 min before they were killed, and intravenously with ^{14}C-L-histidine 1·9 μCi, 10 min before they were killed. Values are means ± S.E.M. of five assays per group (Group A urine and B stomach, 4 assays).

Effect of cortisol on basal level of histidine decarboxylase activity in tissue of adrenalectomized mice

Cortisol treatment reduced lung histidine decarboxylase activity, but had no significant effect on enzyme from kidney, heart, liver and muscle (Table 3). Stomach histidine decarboxylase activity was increased in each of three tests but the statistical significance was low.

Effect of cortisol on catabolism of injected ^{14}C-histamine

Adrenalectomized mice were injected with saline or cortisol acetate 1 mg, under the skin of the back on Day 1, and on Day 2 injected intravenously with ^{14}C-histamine 0·5 μCi. Mice were killed 2·5 min later, urine and tissues taken and assayed for ^{14}C-histamine, ^{14}C-methylhistamine and total ^{14}C. In liver, intestine, lung, muscle, heart, kidney and stomach, levels of ^{14}C-histamine, ^{14}C-methylhistamine and total ^{14}C were slightly subnormal, but all ^{14}C compounds were increased in urine. However, for urine and tissues, the ratios of ^{14}C-amines to total ^{14}C were not significantly different in control and cortisol groups; accordingly, there is no evidence that the rate of *in vivo* catabolism of histamine is materially influenced by cortisol*.

* The complete data of this experiment will be provided on request.

Effect of glucocorticoids on irritant-induced activation of histidine decarboxylase in mouse lung and liver

Freund's complete adjuvant and endotoxin were used to activate histidine decarboxylase; intact mice were used since these stimuli kill adrenalectomized mice. In experiment 1 (Table 4) cortisol given on Day 2 to mice pretreated with Freund's adjuvant not only prevented further activation of histidine decarboxylase by Day 3, but reduced activity to a level below the controls. In experiment 2, designed to test the effect of corticoids injected post-stimulus, the degree of endotoxin activation in lung and liver was reduced.

TABLE 3. *Effect of cortisol on histidine decarboxylase (HD) activity of mouse tissues*

Procedure	Tissue	Group	Relative HD activity*
Experiment 1. Day 0, all mice adrenalectomized. Day 1, saline or cortisol acetate 1 mg injected under skin of back. Day 2, killed and tissues assayed for histidine decarboxylase activity. 5 assays per group.	Stomach	Control	100±35
		Cortisol	161±29
	Lung	Control	100± 5·4
		Cortisol	64± 5·2†
	Kidney	Control	100± 5·7
		Cortisol	129±13
	Heart	Control	100±13
		Cortisol	103± 4·4
Experiment 2. Same as experiment 1 except one group killed 2 days after cortisol acetate. 5 assays per group.	Muscle	Control	100± 6·6
		Cortisol	118± 7·0
	Liver	Control	100±29
		Cortisol	93± 9·4
	Stomach	Control	100± 8·4
		Cortisol	141±22
	Stomach (2 days)	Control	100±10
		Cortisol	152±24

* To simplify comparison of data, histidine decarboxylase activities are expressed relative to mean of controls=100. † $P<0·01$; all others NS.

Effect of cortisol on formation of ^{14}C-histamine in stomach, lung and heart of adrenalectomized rats

Rats were adrenalectomized on Day 0. On Day 1 groups received saline or cortisol acetate 5 mg, under the back skin. On Day 2, all rats were injected intraperitoneally with aminoguanidine 1 mg, at zero time, intravenously with ^{14}C-L-histidine 19 μCi, at 20 min, and killed at 30 minutes. Results (d.p.m. ^{14}C-histamine per 10^3 d.p.m. total ^{14}C) of 5 assays per group, were for stomach, control $34{\cdot}0 \pm 3{\cdot}9$ and cortisol $48{\cdot}7 \pm 4{\cdot}4$ ($P<0{\cdot}05$); lung, control $5{\cdot}02 \pm 0{\cdot}28$ and cortisol $3{\cdot}28 \pm 0{\cdot}16$ ($P<0{\cdot}001$); heart, control $3{\cdot}07 \pm 0{\cdot}14$ and cortisol $2{\cdot}68 \pm 0{\cdot}14$ ($P<0{\cdot}1$). Findings were similar to those in mice.

Discussion

Pretreatment of mice with cortisol caused major changes in the distribution of ^{14}C-histamine formed during 10 minutes after intravenous injection of ^{14}C-L-histidine (Table 1); the most obvious changes were in stomach and urine. Among the possible mechanisms involved might be cortisol-induced changes in tissue histidine decarboxylase activity or in uptake of blood-borne histamine by tissues. Changes in histamine catabolizing ability of tissues cannot be considered of major importance because all mice were pretreated with inhibitors of histamine catabolism. Further interpretation of Table 1 requires introduction of evidence from other experiments.

Data of Table 2 show that the effect of glucocorticoids on urinary ^{14}C excretion, and on ^{14}C-histamine in stomach, may be detectable within 0·5 h of corticoid injection and are pronounced at 4 hours.

TABLE 4. *Effect of glucocorticoids on activation of histidine decarboxylase by Freund's adjuvant and by endotoxin*

Procedure	Tissue and group	Relative HD activity*	
Experiment 1. Intact mice. Groups killed (A) 3 days after saline i.p.; (B) 2 days after Freund's adjuvant i.p.; (C) 3 days after Freund i.p.; (D) Freund i.p. day 0, cortisol acetate 1 mg s.c. day 2, killed day 3. Four assays per group (liver D, 3 assays).	Lung		
	A. Saline	100±8·1	A–B, NS
	B. Freund, day 2	166±26	A–C, $P<0{\cdot}001$
	C. Freund, day 3	474±36	B–D, $P<0{\cdot}025$
	D. Freund+ glucocorticoid	65±6·1	C–D, $P<0{\cdot}001$
			A–D, $P<0{\cdot}025$
	Liver		
	A. Saline	100±13	A–B, NS
	B. Freund, day 2	132±12	A–C, $P<0{\cdot}001$
	C. Freund, day 3	315±22	B–D, $P<0{\cdot}05$
	D. Freund+ glucocorticoid	74±14	C–D, $P<0{\cdot}001$
			A–D, NS
Experiment 2. Intact mice. Saline or endotoxin i.v. at zero time; saline or glucocorticoids† i.p. at 5 min. Killed at 4 h. Four assays per group (Lung A, B and C, 3 assays).	Lung		
	A. Saline	100±14	A–B, NS
	B. Glucocorticoid	75±8·4	A–C, $P<0{\cdot}001$
	C. Endotoxin	715±4·2	B–D, $P<0{\cdot}001$
	D. Endotoxin+ glucocorticoid	470±40	C–D, $P<0{\cdot}01$
			A–D, $P<0{\cdot}001$
	Liver		
	A. Saline	100±17	A–B, NS
	B. Glucocorticoid	122±17	A–C, $P<0{\cdot}001$
	C. Endotoxin	729±66	B–D, $P<0{\cdot}01$
	D. Endotoxin+ glucocorticoid	402±57	C–D, $P<0{\cdot}01$
			A–D, $P<0{\cdot}01$

* To simplify comparison of data, histidine decarboxylase activities are expressed relative to mean of control=100. † Mixture of prednisolone phosphate 0·2 mg, and cortisol (free alcohol) 0·5 mg.

Histidine decarboxylase assays of cell-free tissue extracts (Table 3) indicate that corticoid treatment of mice reduced activity in lung, but not in kidney, heart, liver or muscle. Stomach histidine decarboxylase activity was increased in each of three experiments but due to variability of the data, the result of no single experiment is statistically significant.

Returning to the interpretation of Table 1 data, it seems probable that through its effect on urine formation, cortisol greatly increased removal of ^{14}C-histamine, ^{14}C-L-histidine and other ^{14}C compounds from blood, and reduced their availability to tissues for uptake or formation of ^{14}C-histamine. In lung, the ability to form histamine was also reduced. Although free ^{14}C-L-histidine was not measured in this experiment, in a similar one, using intact mice killed 10 minutes after intravenous injection of ^{14}C-L-histidine, the latter comprised more than half of the total ^{14}C in blood (Reilly & Schayer, 1968a).

The other major effect of cortisol, increased ^{14}C-histamine in stomach, could be due in part to increased histidine decarboxylase activity, but might also relate to other factors, for example, enhanced accessibility of substrate to enzyme in enterochromaffin-like cells, the main site of stomach histamine formation in mice (Hakanson & Owman, 1967). The cortisol-induced increase in intestinal ^{14}C-histamine may be due to uptake of ^{14}C-histamine formed in stomach, or possibly to the presence of enterochromaffin-like cells.

Corticoids reduce histidine decarboxylase activation by irritants (Table 4). In experiment 1, group D, enzyme activity was far below its control, group C, and even below the normal level. In part, this effect may have been due to reduced absorption of colloidal droplets from the intraperitoneal depots of Freund's adjuvant. In experiment 2, designed so that the irritant could reach its destination in phagocytic cells before corticoids were injected, group D is reduced below its control, group C, but is well above the normal level. Since virtually nothing is known of the mechanism of histidine decarboxylase activation, no further comments on the inhibitory effect of corticoids on this process are warranted. However, in so far as histamine is involved in inflammation (Schayer & Reilly, 1968; Reilly & Schayer, 1969) in adjuvant action (Schayer, 1970) and in reticulo-endothelial function (Schayer, 1964) the opposite effects of glucocorticoids may be, in part, due to suppression of increased local histamine formation.

R. W. S. is supported by U.S. Public Health Service Grant AM 10155. We are indebted to Mrs. Christine Sokolski and Mrs. Mary Castro for excellent technical assistance. In part, this work was supported by General Research Support Grant RR 05651. Statistical analyses were made by the Information Sciences Division (Supported by Grant MH 14934). Rockland State Hospital is an institution of the New York State Department of Mental Hygiene.

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(*Received March* 10, 1972)