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EFFECTS OF CHRONIC TREATMENT WITH HISTAMINE ON CATECHOLAMINE CONTENT AND SYNTHESIS IN RAT BRAIN, HEART AND ADRENALS

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Abstract—Chronic treatment with increasing doses of histamine rendered rats tolerant to the hypothermic and toxic effects of this amine. The development of this tolerance was associated with a gradual decrease in endogenous epinephrine (EPI) content, increase in norepinephrine (NE) concentration and tyrosine hydroxylase activity in the adrenals. In contrast, cardiac NE depletion occurred rapidly after both acute and chronic histamine administration. Brain NE level increased and tyrosine concentration decreased, but only after establishment of histamine tolerance. The presently observed central involvement of catecholamines in histamine tolerance may represent a compensatory mechanism in response to peripheral catecholamine depletion.

TOLERANCE to large doses of histamine is known to occur in experimental animals after repeated histamine administration.[1–3] The mechanism of this phenomenon is still largely obscure, although several possible mechanisms have been proposed. Increased tissue binding of histamine was observed after treatment of mice,[3] guinea-pigs and rats[1] with repeated doses of histamine. Excretion of histamine or the histamine metabolite, imidazole acetic acid, is also increased in mice[3] and guinea-pigs[2] respectively. Lewis and Nicholls[4] examined several possible mechanisms of tolerance in rats and mice. These authors report changes in adrenal weight and catecholamine content, and suggested the possibility of an increased production and release of adrenal adrenaline during the chronic histamine treatment. We now report that repeated administration of histamine to rats produces a marked and progressive increase in tyrosine hydroxylase activity in adrenals, evidence of an increased ability of the adrenals to synthesize catecholamines. These data are consistent with an involvement of adrenal catecholamines in the mechanism of histamine tolerance.

MATERIALS AND METHODS

Male Sprague–Dawley rats (90–110 g) were used. In acute studies, 10 mg/kg of histamine as the dihydrochloride dissolved in physiological saline was injected intraperitoneally (i.p.) and the animals were killed 1 hr later. In chronic studies, three groups of animals were used. The first group was treated daily with 10 mg/kg of histamine dihydrochloride i.p. for 1 week. The second and third groups were injected with histamine dihydrochloride i.p. for 2 and 5 weeks respectively, according to the method of Ambrus *et al.*[1] The dose schedule consisted of treatment with 50 mg/kg i.p. for 3 days, then the dose was doubled (100 mg/kg) for the next 3 days, and increased by 100 mg/kg every 3 days until the end of the treatment. The highest dose administered was 400 mg/kg at the end of the 2-week experiment and 1100 mg/kg at the end of

5 weeks. No deaths occurred in either experiment. Control rats were injected with physiological saline (0·1 ml/100 g of body weight). In chronic studies, all rats were sacrificed 24 hr after the last injection.

The development of tolerance was established in similarly treated groups of animals by the absence of changes in rectal temperature taken before and 1 hr after i.p. histamine administration (50 mg/kg). The temperature was measured by a rectal probe (Tele-Thermometer, Yellow Springs Instrument Co., Inc.). In view of the importance of environmental temperature on rectal temperature changes induced by drugs,[5] all measurements were made in a room kept at 21–22°.

In order to investigate whether the observed changes could be blocked by an antihistaminic agent, tripelenamine (Pyribenzamine, Ciba) was administered for 1 week in a dose of 10 mg/kg i.p. 1 hr prior to histamine (10 mg/kg i.p.) injection.

At the end of different treatments, the rats were sacrificed by decapitation; brain, heart and adrenals were homogenized in 8, 5 and 2 ml of 0·28 M sucrose respectively. For catecholamine determinations, 3, 2·5 and 1 ml of brain, heart and adrenals homogenates, respectively, were treated with 5 ml of 0·30 M perchloric acid for brain and heart and with 4 ml for adrenals. After centrifugation, catecholamines were isolated from the supernatants by the use of Alumina columns,[6] and norepinephrine (NE), epinephrine (EPI) and dopamine (DA) assayed fluorimetrically by the method of Laverty and Taylor.[7] The remaining portions of homogenates were treated with 5 ml of 0·37 M trichloroacetic acid and used for the assay of tyrosine by the method of Waalkes and Udenfriend.[8] Tyrosine hydroxylase activity was estimated in the sucrose homogenates by the procedure of McGeer *et al.*,[9] and phenylethanolamine *N*-methyltransferase by the method of Axelrod.[10]

RESULTS

Effects of chronic histamine treatment on rectal temperature in rats. A single i.p. injection of 50 mg/kg of histamine produced a significant lowering of rectal temperature

TABLE 1. TOLERANCE TO THE HYPOTHERMIC EFFECTS OF HISTAMINE PRODUCED BY PRETREATMENT WITH INCREASING DOSES OF HISTAMINE IN RATS

Treatment*	Body temperature before challenge by 50 mg/kg histamine (°C)	Body temp. 1 hr after challenge by 50 mg/kg histamine (°C)	Change in temperature (°C)
Control	37·0 ± 0·2 (n = 9)	36·1 ± 0·2† (n = 9)	−0·9
1 Week (10 mg/kg/day)	37·0 ± 0·2 (n = 9)	36·2 ± 0·3† (n = 5)	−0·8
2 Weeks (tolerance dose)	36·9 ± 0·3 (n = 4)	36·5 ± 0·3 (n = 4)	−0·4
5 Weeks (tolerance dose)	37·0 ± 0·1 (n = 5)	36·8 ± 0·2 (n = 5)	−0·2

* Three groups of animals were used; the first group was treated with 10 mg/kg histamine i.p. for 1 week; the second and third groups were injected with histamine i.p. for 2 weeks and 5 weeks respectively, according to the method of Ambrus *et al.*[1] The rats were sacrificed 24 hr after the last injection. Mean ±S.E.M. is represented; number of experiments in parentheses.

† Significantly different from control values at $P < 0·05$.

in control animals. Treatment with 10 mg/kg of histamine i.p. for 1 week produced no evidence of tolerance to the hypothermic effect of histamine. However, after 2 weeks of treatment with the "tolerance schedule" of histamine, the hypothermic response to a 50 mg/kg i.p. dose of histamine was reduced and after 5 weeks of "tolerance dose" it was almost completely abolished. The results are shown in Table 1.

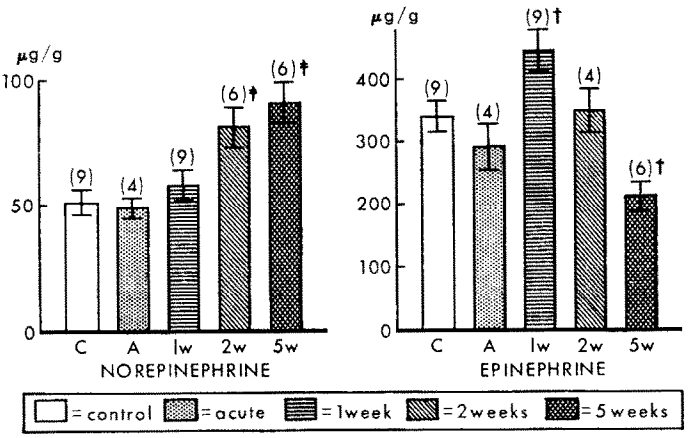

FIG. 1. Effects of histamine treatment (see Methods) on norepinephrine and epinephrine concentration in rat adrenals. Mean ±S.E.M. is represented; number of experiments in parentheses. † Significantly different from control values at $P < 0.01$; ‡ significantly different from control values at $P < 0.005$.

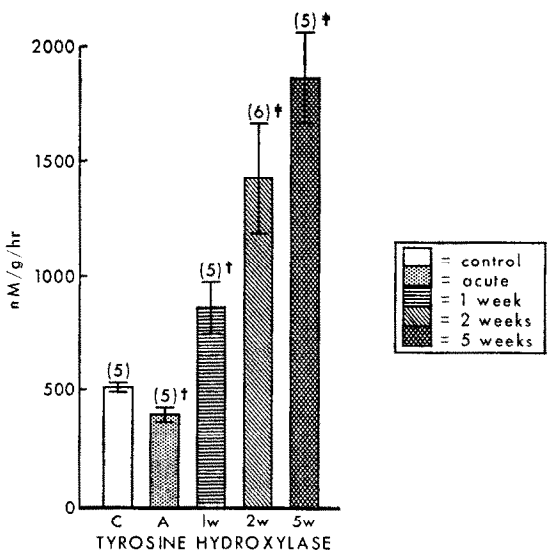

FIG. 2. Effects of histamine treatment (see Methods) on rat adrenal tyrosine hydroxylase activity. Mean ±S.E.M. is represented; number of experiments in parentheses. † Significantly different from control values at $P < 0.01$; ‡ significantly different from control values at $P < 0.005$.

Adrenal catecholamines and tyrosine hydroxylase activity. The effects of acute and chronic histamine administration on adrenal catecholamines and tyrosine hydroxylase are summarized in Figs. 1 and 2. Treatment with a single dose of histamine (10 mg/kg

i.p.) produced a small and not statistically significant drop in EPI content (85 per cent of control value); tyrosine hydroxylase (Fig. 2) was significantly inhibited (74 per cent of control) and the NE concentration remained unchanged (Fig. 1). After 1 week of treatment with 10 mg/kg histamine i.p., both the EPI content (131 per cent of control) and the tyrosine hydroxylase activity (166 per cent of control) were significantly increased.

Higher doses of histamine for longer periods of time (the tolerance schedule of histamine for 2 and 5 weeks) produced a progressive increase in adrenal NE content (to 178 per cent of control) and tyrosine hydroxylase activity (to 375 per cent of control). Conversely, the EPI content of the adrenals was progressively decreased (to 62 per cent of its control value). There was a slight increase in adrenal weight (11 per cent) after the full 5 weeks of histamine treatment. The body weight of experimental rats was significantly ($P < 0{\cdot}001$) lower (259 ± 7 g) than that of controls (323 ± 7 g).

TABLE 2. EFFECT OF CHRONIC TREATMENT WITH HISTAMINE ON RAT ADRENAL TYROSINE HYDROXYLASE AND PHENYLETHANOLAMINE-*N*-METHYL TRANSFERASE

Treatment	No. of animals	Adrenal wt (mg)	(%)	Tyrosine hydroxylase (nmoles/g wet tissue/hr)	(%)	Phenylethanolamine *N*-methyl transferase (nmoles/g wet tissue/hr)	(%)
Control	4	$42{\cdot}8 \pm 1{\cdot}3$	100	510 ± 30	100	1064 ± 18	100
2 Weeks (tolerance schedule)	4	$51{\cdot}6 \pm 1{\cdot}0$*	121	1330 ± 210*	261	1214 ± 14*	114

* Significantly different from control value at $P < 0{\cdot}005$.

Adrenal phenylethanolamine-N-methyltransferase (PNMT). The effects of chronic (2 weeks) treatment with histamine on PNMT and tyrosine hydroxylase were tested in another experiment and the results are indicated in Table 2. As can be seen, PNMT activity was significantly increased (+ 14 per cent), but the increase in tyrosine hydroxylase activity was much greater (+ 161 per cent). The increase in adrenal weight of experimental animals reached the level of statistical significance (+ 21 per cent) in this small group of animals.

Cardiac catecholamine content. Acute or 1-week chronic treatment with histamine (10 mg/kg i.p.) significantly lowered the NE content in the heart, as indicated in Table 3. The tolerance dose schedule for 2 and 5 weeks produced a progressive decrease in cardiac NE concentration to 74 and 69 per cent of control respectively. After 5 weeks of treatment, there was a statistically significant ($P < 0{\cdot}02$) drop in heart weight (84 per cent of control). The weight of control hearts was $1{\cdot}18 \pm 0{\cdot}04$ g and of experimental $0{\cdot}99 \pm 0{\cdot}05$ g.

Brain catecholamines and tyrosine hydroxylase activity. Brain NE, DA and tyrosine content and tyrosine hydroxylase activity were assayed after various histamine treatments. The results summarized in Tables 4 and 5 indicate that acute administration of

TABLE 3. EFFECT OF HISTAMINE TREATMENT ON HEART NOREPINEPHRINE CONTENT

Treatment*	Norepinephrine					
	Control (μg)	Experimental (μg)	% of Control	Control (μg/g)	Experimental (μg/g)	% of Control
Acute (10 mg/kg)	0·505 ± 0·025 (5)	0·405 ± 0·020† (5)	80	0·582 ± 0·029 (5)	0·475 ± 0·024† (5)	82
1 Week (10 mg/kg/day)	0·503 ± 0·022 (10)	0·373 ± 0·029† (10)	74	0·574 ± 0·025 (10)	0·438 ± 0·034† (10)	76
2 weeks (tolerance schedule)	0·505 ± 0·037 (4)	0·351 ± 0·034† (6)	70	0·567 ± 0·032 (4)	0·420 ± 0·031† (6)	74
5 weeks (tolerance schedule)	0·539 ± 0·012 (5)	0·311 ± 0·024‡ (5)	58	0·458 ± 0·010 (5)	0·314 ± 0·024‡ (5)	69

* In acute studies, 10 mg/kg of histamine was injected i.p. and the animals were killed 1 hr later In chronic studies, three groups of animals were used; the first group was treated with 10 mg/kg of histamine i p. for 1 week; the second and third groups were injected with histamine i.p. for 2 and 5 weeks respectively, according to the method of Ambrus *et al.*[1] The rats were sacrificed 24 hr after the last injection. Mean ±S.E.M. is represented; number of experiments in parentheses.

† Significantly different from control values, $P < 0{\cdot}01$.

‡ Significantly different from control values, $P < 0{\cdot}001$.

TABLE 4. EFFECT OF HISTAMINE TREATMENT ON RAT BRAIN TYROSINE AND TYROSINE HYDROXYLASE*

	Tyrosine			Tyrosine hydroxylase		
Treatment†	Control (μg/g)	Experimental (μg/g)	% of Control	Control (nmoles/g/hr)	Experimental (nmoles/g/hr)	% of Control
Acute (10 mg/kg)	19·0 ± 2·6	21·3 ± 3·0	112	16·2 ± 0·6	15·2 ± 0·9	94
1 Week (10 mg/kg/day)	18·5 ± 1·3	20·3 ± 1·5	110	15·2 ± 0·5	15·8 ± 0·6	104
2 Weeks (tolerance schedule)	19·2 ± 2·1	13·0 ± 0·7‡	68	15·8 ± 0·4	16·3 ± 0·5	103
5 Weeks (tolerance schedule)	18·3 ± 1·8	11·7 ± 0·4‡	64	15·0 ± 0·6	14·4 ± 0·3	96

* Mean ±S.E.M. for groups of four to ten animals is represented.
† For histamine dosage, see Table 3.
‡ Significantly different from control value at $P < 0·02$.

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TABLE 5. EFFECT OF HISTAMINE TREATMENT ON RAT BRAIN CATECHOLAMINES*

Treatment†	Brain wt			Norepinephrine			Dopamine		
	Control (g)	Experimental (g)	% of Control	Control (μg/g)	Experimental (μg/g)	% of Control	Control (μg/g)	Experimental (μg/g)	% of Control
Acute (10 mg/kg)	1·846 ± 0·025	1·779 ± 0·033	96	0·493 ± 0·031	0·469 ± 0·039	95	0·585 ± 0·019	0·591 ± 0·017	101
1 week (10 mg/kg/day)	1·814 ± 0·018	1·821 ± 0·026	100	0·472 ± 0·023	0·480 ± 0·030	102	0·603 ± 0·041	0·561 ± 0·030	93
2 Weeks (tolerance schedule)	1·827 ± 0·031	1·776 ± 0·026	97	0·450 ± 0·013	0·464 ± 0·014	103	0·588 ± 0·019	0·599 ± 0·032	102
5 Weeks (tolerance schedule)	1·857 ± 0·010	1·795 ± 0·027	97	0·470 ± 0·023	0·571 ± 0·022‡	121	0·592 ± 0·045	0·648 ± 0·077	109

* Mean ±S.E.M. for groups of four to ten animals is represented.
† For histamine dosage, see Table 3.
‡ Significantly different from control value at $P < 0·02$.

histamine (10 mg/kg i.p.) or 1 week of histamine treatment (10 mg/kg i.p./day) produced no significant effects. However, 2 weeks of treatment induced a significant decrease (68 per cent of control) in brain tyrosine content. After a further 3 weeks of treatment, tyrosine content was significantly decreased (64 per cent of control). The brain NE content of treated animals was significantly higher than in control animals both when calculated as μg/whole brain or as μg/g of tissue.

TABLE 6. EFFECT OF HISTAMINE AND TRIPELENAMINE ON RAT TISSUE CATECHOLAMINES AND TYROSINE HYDROXYLASE*

Treatment	Heart norepinephrine (μg/g)	Adrenal		
		Epinephrine (μg/g)	Norepinephrine (μg/g)	Tyrosine hydroxylase (nmole/g/hr)
Control	0·522 ± 0·023 (10)	340 ± 23 (9)	51 ± 5 (9)	522 ± 22 (5)
Histamine (10 mg/kg i.p. for 7 days)	0·399 ± 0·031† (10)	444 ± 33† (9)	58 ± 6 (9)	866 ± 108† (5)
Tripelenamine (10 mg/kg i.p.)	0·512 ± 0·028 (5)	332 ± 31 (5)	59 ± 8 (5)	542 ± 57 (5)
Histamine + tripelenamine	0·492 ± 0·056 (5)	344 ± 18 (5)	59 ± 9 (5)	541 ± 41 (5)

* Mean ± S.E.M. is represented; number of experiments in parentheses.
† Significantly different from control values, $P < 0·01$.

Effects of tripelenamine pretreatment. Chronically injected histamine (10 mg/kg i.p. for 7 days) produced a marked increase in adrenal EPI content and tyrosine hydroxylase activity and a significant reduction in the cardiac NE concentration. The results summarized in Table 6 demonstrate that these histamine-induced changes were prevented by the antihistaminic agent, tripelenamine, administered prior to histamine treatment.

DISCUSSION

Effects of acute and chronic treatment with histamine on rat adrenals. Acute treatment with histamine did not induce significant changes in catecholamine content in adrenals measured 1 hr after the injection, although a slight inhibition of tyrosine hydroxylase activity was observed. This inhibition may readily be explained on the basis of a direct effect, since histamine produced a similar inhibition of adrenal tyrosine hydroxylase when added to adrenal homogenates.

After 1 week of histamine treatment (10 mg/kg i.p./day), both the EPI content and the tyrosine hydroxylase activity in adrenals were significantly increased, indicating a possibility of an increased synthesis of catecholamines. Larger doses of histamine for longer periods of time resulted in production of tolerance to the temperature-lowering effects, which was associated with a progressive fall in EPI content, rise in NE concentration, and increase in tyrosine hydroxylase activity.

The marked depletion of adrenal EPI observed presently could represent a reflex protective response to high doses of histamine or a direct effect of histamine on the adrenal gland. Epinephrine is a well established physiological histamine antagonist in anaphylactic shock.[11] The increased NE content in the presence of a reduced EPI level may be a consequence of the observed greatly increased tyrosine hydroxylase activity compared with a very small increase in PNMT activity.

Because histamine release of catecholamines from adrenal medulla is believed to be a short-lasting phenomenon,[12] the endogenous catecholamine content may vary depending on the time after histamine administration at which the adrenal catecholamine content was determined. Hökfelt,[13] for example, observed a significant depletion of NE and a rise in EPI 3 hr after a single subcutaneous injection of histamine (500 μg/kg). In our chronic studies, the animals were sacrificed 24 hr after the last histamine administration in order to avoid acute effects. The difference between our results and those reported by Lewis and Nicholls[4] could have been due possibly to a different time of killing after histamine treatment, to the different strain of rats used or to both.

Since tyrosine hydroxylase is believed to be the rate-limiting enzyme,[14,15] measurement of the activity of this enzyme should provide a more useful indication of the ability of the adrenals to synthesize catecholamines than the determination of catecholamine levels. It is of interest that in the present study there was a progressive increase in enzyme activity which correlated well with the increased NE levels, suggesting a gradual increase in NE synthesis associated with the development of histamine tolerance. The increase in tyrosine hydroxylase activity observed presently is in line with other reports which indicated an increased activity of this enzyme in the adrenals after prolonged periods of drug-induced sympathoadrenal hyperactivity.[16,17]

The increase in adrenal weight which occurred after the 5-week tolerance schedule of histamine confirms similar reports by others,[18] who in addition observed a slight hypertrophy of adrenal cortex,[4] and is in line with Selye's observation[19] on enlargement of adrenals as a result of exposure to damaging stimuli.

The role of adrenals in histamine toxicity is not yet properly understood. There is a general agreement that histamine toxicity increases in adrenalectomized rats.[20–22] However, while some authors stressed the beneficial effects of corticosteroids[21–23] and mineralcorticoids,[24] others demonstrated that epinephrine increases the resistance to toxic doses of histamine and partially counteracts histamine shock in adrenalectomized rats.[25]

Effects of acute and chronic histamine administration on the heart. Histamine-induced changes occurred more rapidly in the heart than in the other tissues studied. Cardiac NE content was significantly lowered by both acute and chronic administration of histamine; the lowest level of NE was observed after 5 weeks on the histamine tolerance schedule. These data are consistent with the reports of studies *in vitro*, which indicated that histamine induced the release of cardiac NE.[26,27] It is of interest that a mutual relationship between histamine and catecholamines was recently reported to occur in guinea-pig hearts.[28]

In the present studies, prolonged (5 weeks) histamine treatment led also to a significant decrease in the weight of the heart (84 per cent of control), although this could be related to a decrease in the whole body weight of the experimental rats (80 per cent of control).

Effects of acute and chronic histamine administration on the brain. Acute, 1- or

2-week treatment with histamine had no effect on brain catecholamines, tyrosine content or tyrosine hydroxylase activity.

After 5 weeks of treatment on the tolerance schedule, there was a marked decrease in the tyrosine content of brain together with a significant increase in the NE level. The reduction in brain tyrosine content may simply be a result of reduced food intake, since it has been observed that a short period of starvation results in a marked reduction in the brain level of tyrosine.[29] Since the tyrosine content in normal rat brain is well above the saturation level for tyrosine hydroxylase,[30] a decrease of 37 per cent in the tyrosine content would probably not affect the ability of the brain to synthesize catecholamines. There are at present insufficient data available to suggest whether the increased brain NE content is involved with the establishment of tolerance.

Although histamine does not readily cross the blood–brain barrier from the vascular system, it is likely that small amounts of histamine do penetrate into brain, particularly when blood levels are abnormally high. Diffusion of histamine into the central nervous system was reported after intraperitoneal injections of large doses to the rat,[31] and subcutaneously administered histamine was shown to modify conditioned behaviour in the rat,[32] which may be consistent with a passage of histamine into brain. Moreover, part of the hypothermia produced by a subcutaneous injection of histamine was suggested to arise as a result of an action on the central nervous system.[33] It is therefore possible that the high blood histamine level could influence brain catecholamine concentration and metabolism.

Effects of pretreatment with tripelenamine. It is known that antihistaminic compounds are effective in antagonizing the direct and indirect histamine-induced release of adrenaline from adrenal medulla.[34] In our studies, changes in cardiac and adrenal catecholamine content and adrenal tyrosine hydroxylase activity induced after 1 week of histamine administration could be prevented by pretreatment with tripelenamine.

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