*Br. J. Pharmac.* (1973), **48**, 577–589.

# Concentration of histamine in different parts of the brain and hypophysis of rabbit: effect of treatment with histidine, certain other amino acids and histamine

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**Summary**

1. Estimates have been obtained by biological assay of the histamine concentration in different parts of the rabbit brain and hypophysis.

2. Mean values (ng/g) for the brain were: hypothalamus, 660; central grey matter and medial thalamus, 275; tegmental region of mid-brain, the hindbrain and caudate nucleus, 140 to 170; hippocampus and cerebral cortex, 90 to 110; cerebellum (vermis), 60.

3. The mean value (ng/g) for the anterior lobe of the hypophysis was 650; for the posterior lobe, 400.

4. In conscious rabbits, intravenous infusion of histidine in the dose range 62 to 1,500 mg/kg, raised significantly ($P<0.01$) the concentration of histamine in all regions of the brain examined, the pattern of distribution remaining unchanged. The largest increases occurred in the mid brain (90 to 320%) and in the hypothalamus (50 to 250%); in these areas the higher doses produced higher concentrations. Elsewhere in the brain the concentration rose in response to the lowest dose of histidine, but was not increased when higher doses were given. Concentrations in the anterior lobe of the hypophysis were unaltered.

5. The infusion of histidine, unlike that of amino acid precursors, of the monoamines, produced no obvious disturbance in the animals.

6. The rise in brain histamine after dosage with histidine persisted for several hours, depending on the dose; with 500 mg/kg, the rise was virtually unchanged after 16 hours.

7. Histamine (5 mg/kg by intravenous infusion) raised the concentration of histamine in the hypophysis but not in the brain.

8. After the infusion of DOPA, $\alpha$-methyldopa or 5-hydroxytryptophan, the histamine concentration rose in the mid-brain but not in other parts of the brain.

9. These amino acids, when infused singly with histidine, did not interfere with the histidine-induced rise of brain histamine.

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## Introduction

The present work is in continuation of earlier studies on the dog (Adam, 1961) and cat (Adam & Hye, 1966). Our object was (1) to obtain estimates of the concentration of histamine in different parts of the brain and (2) to test whether treatment of the rabbit with the parent amino acid, histidine, would raise the concentration of the amine in the hypophysis and selected regions of the brain. Earlier studies on rabbit brain histamine have been reviewed by Green (1970).

When 3,4-dihydroxyphenylalanine (DOPA) is given intravenously in the rabbit, the concentration of dopamine rises in the brain (Bertler & Rosengren, 1959); similarly, 5-hydroxytryptophan (5-HTP) leads to the formation of 5-hydroxytryptamine (5-HT) (Udenfriend, Weissbach & Bogdanski, 1957). Since dopamine and 5-HT are known to release each other from storage sites in rabbit brain (Bertler & Rosengren, 1959; Brodie, Comer, Costa & Dlabac, 1966), it was of interest to examine various amino acid precursors of the monoamines for their effect on brain histamine. The amino acids were DOPA, $\alpha$-methyldopa, 5-HTP and tryptophan, and each was also given simultaneously with histidine. Brain decarboxylase for L-aromatic amino acids is believed to be non-specific and to have a greater affinity for 5-HTP and DOPA than for histidine (Lovenberg, Weisssbach & Udenfriend, 1962).

## Methods

### *Dissection*

New Zealand White male albino rabbits, 1·5 to 4·3 kg, were anaesthetized with pentobarbitone sodium injected slowly into the marginal ear vein; the required dose varied from 34 to 78 mg/kg (mean 53 mg/kg). The animals were then bled out through polyethylene cannulae inserted in the carotid arteries. In a number of experiments the head was first perfused for 10 min with Ringer–Locke solution (30 ml/min) through both carotid arteries. The aim was to remove as much residual blood as possible from the cerebral vessels since rabbit platelets, being rich in histamine (see Vugman & Rocha e Silva, 1966), might contribute to the amount of amine extractable from the tissue samples. The brain and hypophysis were quickly removed, placed on an ice-cold plate and dissected. The brain was cut in the mediansagittal plane and the samples taken for histamine estimation were weighed and extracted without delay.

### *Tissue samples*

'Anterior lobe' was mainly the pars distalis of adenohypophysis; the 'posterior lobe' was mainly the infundibular process.

'Hypothalamus' included the corpora mammillaria and the preoptic region; a rectangular block was cut to a depth of about 3 mm from the ventral surface. 'Medial thalamus' was taken from the region of the massa intermedia and cut to a depth of about 3 mm. 'Superior and inferior corpora quadrigemina' were removed by cutting along their bases. 'Central grey matter' was the tissue surrounding the aqueduct and cut to a depth of about 2 mm. 'Tegmentum' was the region of the mid-brain inferior to the central grey and cut to a depth of about 3 mm. 'Pons-medulla' was the region of the hind-brain and included the floor of the

fourth ventricle. 'Cerebral cortex' was a sample of grey matter cut from the temporo-parietal region. 'Caudate nucleus' was the head and body of this tissue. 'Hippocampus' was the middle third of this region, taken as a transverse section. 'Floor of the fifth ventricle' was the grey matter cut to a depth of about 2 mm.

*Extraction and estimation of histamine*

The method was that of Adam (1961). The tissue sample weighing from 3 to 100 mg was ground with trichloracetic acid (TCA) (6% w/v; 5 $\mu$l/mg tissue) in a 15 ml conical centrifuge tube fitted with a glass pestle; the volume was made up to a 5 ml graduation mark with water and the suspension centrifuged at 4° C for 30 min at 2000 rpm. A portion (4·6 ml) of the supernatant fluid was neutralized and adjusted to pH 8·0 with phosphate buffer and the $Na^+$ concentration was brought to approximately 100 mEq/litre. Histamine in the extract was adsorbed on a column containing the cationic exchange resin Amberlite CG 50 (100–200 mesh) mixed with cellulose. Elution was with 0·25 N HCl followed by water. The eluate was evaporated to dryness and the residue heated in 6 N HCl; this treatment destroys the activity of a number of biologically active substances (Adam, 1961) without inactivating histamine (Barsoum & Gaddum, 1935). After complete removal of the acid, the dried residue was taken up in a modified Tyrode solution (containing NaCl 3·2 mg/ml) for assay.

When the whole brain or large areas of the brain were extracted, the tissue was ground with TCA and the volume made up with water so that 12 mg of tissue was contained in 1 ml of extractant. Five, 1 ml portions of the suspension (equivalent to 60 mg of tissue) were processed as above.

The assay was performed on the superfused guinea-pig ileum (Gaddum, 1953; Adam, Hardwick & Spencer, 1954) in comparison with a standard solution of histamine acid phosphate. The results are expressed as histamine base.

*Recovery experiments*

Histamine in quantities of 25, 50 and 100 ng was added

a) to fresh samples of cerebellum (50–60 mg) after precipitation with TCA,

b) to TCA-extract of cerebellum after removal of the precipitate and

c) to buffer solution (0·05 M, 100 mEq/litre $Na^+$, pH 8·0).

In (a) and (b) control samples of brain were taken to estimate histamine originally present in the tissue. The recovery was calculated from the difference between the amount estimated and that present in the control.

*Infusion of amino acids and histamine solutions*

Solutions were infused into conscious rabbits at a constant rate usually over a 2 h period through a polyethylene cannula (Sterivac, size 2) inserted into a marginal ear vein at one end and connected at the other to a motor-driven disposable syringe. Animals were anaesthetized with pentobarbitone sodium (approximately 50 mg/kg) 30 min after the end of infusion and then killed and the brains removed as described for untreated animals.

*Solutions for infusion*

A solution for infusion was prepared so that 16·7 ml contained the desired dose of drug per kg body weight. If hypotonic, it was made isotonic by the addition of the required amount of sodium chloride.

Histidine hydrochloride monohydrate was dissolved in pyrogen-free sterile distilled water and neutralized by the addition of a stoichiometric amount of sodium hydrogen carbonate (360 mg/g of the hydrochloride). The final concentration of histidine varied from 3·75 to 30 mg/ml, depending on the required dose which ranged from 62 to 500 mg/kg. The solution with the highest concentration was slightly hypertonic (530 mosmoles/litre). DOPA, α-methyldopa, 5-HTP and tryptophan were dissolved in sterile distilled water and the solutions neutralized with 0·1 N NaOH at a pH meter. When one of these amino acids was to be infused simultaneously with histidine, the required amounts of the two amino acids were dissolved in the same vehicle and the solution was neutralized.

Hisamine acid phosphate in the required amount was dissolved in 30 ml sterile isotonic NaCl solution and the solution infused i.v. at 0·25 ml/min for 2 hours. Each animal received a total of 5 mg histamine base/kg.

In some control experiments animals were infused with isotonic NaCl solution for 2 h, a total of 16·7 ml/kg being given.

*Injection of glycogen*

Liver glycogen, 100 mg/kg was injected i.v. as a 50 mg/ml solution in water into rabbits anaesthetized with pentobarbitone. The animals were bled 10 min after the injection.

*Collection of blood samples*

The shaved skin near the marginal vein of one ear was coated with a thin layer of soft paraffin. Heparin (500 i.u./kg) was given into the vein of the other ear. The coated vein was then nicked with a sharp blade and a sample of 0·05 ml blood was collected from the surface of the ear with a siliconed blood pipette. The samples of blood collected before and after various treatments of the animals were used for the estimation of histamine by the method described for tissue samples.

*Chemicals used*

L-histidine hydrochloride monohydrate, L-3,4-dihydroxyphenylalanine (DOPA), DL-5-hydroxytryptophan (5-HTP), L-tryptophan (Koch-Light); L-3-(3,4-dihydroxyphenyl)-2-methylalanine (α-methyldopa) (Merck, Sharp and Dohme); histamine acid phosphate (BDH); rabbit liver glycogen (BDH); pentobarbitone sodium (Veterinary, Abbot); Heparin (Evans Medical). Other reagents used were of analytical grade. Water was glass-distilled, or was Water for Injection, BP.

The presence of histamine as an impurity in commercial samples of histidine was tested biologically. Samples of histidine (50 and 100 μg), treated according to the method of Adam (1961) did not contain detectable amounts (<1 ng) of histamine.

## Results

### *Recovery of histamine by the method*

The mean percentage recovery from brain was 68 ± 25 (S.D.) (18 experiments), from supernatant fluid 97 ± 4 (6) and from buffer solution 91 ± 5 (23). When brain tissue was extracted 3 times with TCA (Hye, 1964) more than 99% of the histamine extractable from the tissue was present in the first extract. Hence it appears that most of the loss occurs during the extraction step, a portion of the histamine being either destroyed or irreversibly bound to the precipitate.

The present results have not been corrected for the loss of histamine.

### *Distribution of histamine in brain and hypophysis of rabbit*

Estimates of the concentration of histamine in the hypophysis and various regions of the brain of 46 rabbits are given in Table 1. In some cases the tissues had been freed of blood by perfusion of the head with Ringer-Locke solution. This procedure, however, did not alter significantly the values for histamine, except in the cerebellum (Table 2). Since residual blood in a tissue sample did not contribute significantly to the estimate, routine perfusion of the head was not considered

TABLE 1. *Histamine concentration in rabbit brain and hypophysis (estimates in ng of base/g of tissue)*

| Tissue | Mean | Range | S.E. of mean |
|---|---|---|---|
| Hypophysis | | | |
| Anterior lobe | 650 | 340–1250 (31) | 36 |
| Posterior lobe | 400 | 180–580 (15) | 35 |
| Whole brain | 130 | 120–140 (3) | — |
| Diencephalon | | | |
| Hypothalamus | 660 | 470–910 (25) | 27 |
| Medial thalamus | 270 | 140–440 (19) | 18 |
| Mid-brain (whole) | 190 | 180–200 (3) | — |
| Superior colliculus | 210 | 150–300 (10) | 17 |
| Inferior colliculus | 170 | 100–120 (10) | 11 |
| Tegmental region | 170 | 110–260 (12) | 12 |
| Central grey matter | 280 | 160–400 (22) | 15 |
| Hind-brain (whole) | 80 | 60–90 (3) | — |
| Pons-medulla | 140 | 60–360 (20) | 15 |
| Floor IVth ventricle | 180 | 110–360 (8) | 28 |
| Cerebrum (whole) | 120 | 90–170 (4) | — |
| Cerebral cortex | 110 | 50–170 (23) | 7 |
| Caudate nucleus | 150 | 90–220 (12) | 12 |
| Hippocampus | 90 | 50–150 (10) | 8 |
| Cerebellum (whole) | 80 | 60–90 (4) | — |
| Vermis | 60 | 30–90 (12) | 5 |
| Choroid plexus | 610 | 30–1800 (8) | — |

Number of animals in parentheses.

TABLE 2. *Effect of perfusing the head with Ringer-Locke solution on the concentration of histamine in hypophysis and brain of the rabbit*

| Region | Mean conc. of histamine ng/g tissue ± S.E. (no. of observations) | |
|---|---|---|
| | Head not perfused | Head perfused |
| Anterior lobe of hypophysis | 670 ± 40 (23) | 590 ± 60 (8) |
| Hypothalamus | 650 ± 30 (17) | 700 ± 40 (8) |
| Medial thalamus | 250 ± 20 (11) | 290 ± 30 (8) |
| Central grey matter | 300 ± 20 (15) | 240 ± 20 (7) |
| Cerebral cortex | 120 ± 10 (11) | 100 ± 10 (12) |
| Cerebellum (vermis) | 60 ± 5 (12) | 30* ± 5 (9) |

* Significantly different, $P < 0.01$.

necessary. The possible effect of residual blood on histamine values was also tested in the following way. Each of two rabbits received an intravenous injection of glycogen in a dose which produced a rapid lowering of blood histamine concentration (Rocha e Silva, 1950; Waalkes & Coburn, 1959). At the time of killing the animals, 10 min after glycogen injection, the blood histamine had fallen to 14% of that before glycogen. By contrast, histamine values for the hypothalamus, central grey matter and cerebellum and in the anterior lobe of the hypophysis were 80–100% of the mean control values and lay within the normal limits.

Some of the control values for brain and hypophyseal histamine were obtained from rabbits infused with 0·9% w/v NaCl solution (saline). Under these conditions the mean concentrations in the anterior lobe, 690 ng/g ± 60 S.E. (13 expts.) and the hypothalamus, 690 ± 60 (7) were not significantly different from those obtained from non-infused rabbits (anterior lobe 690 ± 100 (6); hypothalamus 670 ± 50 (6)).

*Hypophysis*

The mean weight of the anterior lobe was 21·6 mg (range 11 to 50 (31)), and the mean histamine content 14 ng (range 8 to 27). The mean weight of the posterior lobe was 5·5 mg (range 3 to 8·5 (15)), and the mean histamine content 2 ng (range 1 to 13).

*Brain*

The histamine concentration showed a regional variation. The highest concentration was in the hypothalamus (mean concentration 660 ng/g). The next highest concentration, less than half of that in the hypothalamus, was found in the central grey matter of the mid-brain and in the medial thalamus. The caudate nucleus, areas of the hindbrain, the remaining regions of the mid-brain, the cerebral cortex and hippocampus all provided low values. In the cerebellar vermis the mean concentration was only 60 ng/g. Thus the difference between the hypothalamus and cerebellum was about ten-fold.

*Histamine in whole blood of untreated rabbit*

The mean estimate was 3·9 μg/ml (S.D. 1·4, range 1·6 to 8·8, 127 estimates). The i.v. infusion of saline (16·7 ml/kg during 2 h) did not alter the concentration of histamine in blood (Table 3). There was no significant positive correlation between the concentration of histamine in blood and the quantity of amine extractable from hypophysis or different regions of the brain.

*Effect of histidine on the concentration of histamine in hypophysis, brain and blood of conscious rabbits*

Behaviourally, the rabbits appeared to be unaffected by histidine and there was no change in rectal temperature.

In the dose range 62–1500 mg/kg, histidine did not alter significantly the concentration of histamine in the anterior lobe of the hypophysis or in blood. However, the concentration of histamine increased in many parts of the brain (Table 3). The increase was, proportionally, greatest in the central grey and

TABLE 3. *Effect of histidine infusion on the concentration of histamine in hypophysis, brain and blood of rabbit*

| Region | Control | Dose of histidine in mg/kg | | | | |
|---|---|---|---|---|---|---|
| | | 1500 | 500 | 250 | 125 | 62 |
| Anterior lobe of hypophysis | 650± 36 (31) | 720± 70 (6) | 800± 80 (11) | 830±120 (5) | 610 (2) | 880±147 (4) |
| Hypothalamus | 660± 27 (25) | 2310±120 (5) | 1480±130 (5) | 1360±120 (5) | 950 (2) | 1070± 72 (4) |
| Medial thalamus | 270± 18 (19) | 680± 40 (6) | 560± 70 (5) | | | |
| Superior colliculus | 210± 17 (10) | 770 (3) | 440± 30 (5) | | | |
| Inferior colliculus | 170± 11 (10) | 500 (2) | 440± 30 (5) | | | |
| Tegmentum | 170± 12 (12) | 640 (2) | 440± 30 (5) | 460± 20 (5) | 370 (2) | 320± 40 (4) |
| Central grey | 280± 15 (22) | 1160± 50 (5) | 960± 70 (6) | 800± 70 (5) | 540 (2) | 520± 53 (4) |
| Pons-medulla | 140± 15 (20) | 290 (2) | 260± 30 (5) | 280± 20 (5) | 240 (2) | 200± 19 (4) |
| Floor of IVth ventricle | 180± 28 (8) | 580 (2) | 440± 90 (5) | | | |
| Caudate nucleus | 150± 12 (12) | 280± 40 (5) | 330± 30 (5) | 290± 20 (5) | 260 (2) | 250± 24 (4) |
| Cerebral cortex | 110± 7 (23) | 270± 10 (5) | 270± 20 (5) | | | |
| Hippocampus | 90± 8 (10) | 310 (3) | 170± 20 (5) | | | |
| Cerebellum (vermis) | 60± 5 (12) | 110 (3) | 130± 30 (5) | | | |
| Blood, before infusion | 4060±530 (11)* | 3500±380 (5) | 3300±400 (10) | 3800±700 (5) | | |
| Blood, towards end of infusion | 4000±530 (11)* | 4200±340 (5) | 2900±500 (10) | 3700±800 (5) | | |

Rabbits infused with 0·9% w/v NaCl solution. Histidine in a total dose of 62–500 mg/kg was infused i.v. over a period of 2 h; rabbits were anaesthetized and killed 30 min after the infusion. The dose 1500 mg/kg was infused as 3 doses of 500 mg/kg each over a period of 2 h, given 12 h apart; animals were anaesthetized and killed 30 min after the last infusion. In the dose range 250–1500 mg/kg the increases in different parts of the brain were highly significant ($P<0·01-<0·001$) as compared with the control.

Mean concentration of histamine in ng/g tissue or ng/ml blood±S.E. (no. of observations)

tegmental regions of the mid-brain (90–320%). In the hypothalamus an increase of 50–250% was noted. In these regions the increases were highly significant (comparison with normal concentrations by Student's $t$ test, $P<0{\cdot}01-<0{\cdot}001$) with higher concentrations being produced with the larger doses. Other areas such as pons-medulla and the caudate nucleus also showed a rise of histamine concentration which, however, reached a maximum with doses at the lower end of the range.

The results of control experiment in which a large amount of histidine (20 $\mu$g) was added to each of several brain samples (60 mg) indicate that histamine was probably not formed during the extraction procedure.

### *The possible contribution of peripherally-formed histamine to histidine-induced rise of brain histamine*

Since histamine may have been formed outside the brain during the infusion of histidine, five experiments were performed to test for the possible entry of histamine from blood into brain. A dose of 5 mg histamine/kg was infused i.v. over a 2 h period; the animals were anaesthetized and killed 30 min later.

The results (Table 4) showed that both lobes of the hypophysis took up histamine from the circulation and concentration rose about seven-fold. In the hypothalamus and medial thalamus the concentration did not rise detectably, but in the cerebral cortex and cerebellum significant ($P<0{\cdot}05$ and $<0{\cdot}01$) increases of about 50 and 130% respectively were noted. Blood samples taken towards the end of infusion showed an increase of about 20% in the histamine content.

TABLE 4. *Effect of intravenous infusion of histamine (5 mg/kg over 2 h) on the concentration of histamine in hypophysis, brain and blood of rabbit*

| Tissue | Concentration of histamine (ng/g tissue or ng/ml blood) Control | Histamine-infused |
|---|---|---|
| Hypophysis | | |
| Anterior lobe | 590± 60 (8) | 5040*±370 (5) |
| Posterior lobe | 400± 35 (15)+ | 3340*±490 (5) |
| Hypothalamus | 700± 40 (8) | 690 ±110 (5) |
| Medial thalamus | 290± 30 (8) | 290 ± 50 (5) |
| Cerebral cortex | 100± 10 (12) | 160*± 20 (5) |
| Cerebellum (vermis) | 30± 6 (9) | 70*± 20 (5) |
| Blood, before infusion | 3100±470 (3) | |
| Blood, towards end of infusion | | 3800 ±740 (3) |

Rabbits were anaesthetized and killed 30 min after the end of the infusion. The head was perfused with Ringer-Locke solution before the removal of the brain. Mean estimates±S.E. (no. of rabbits). * Significantly different from control, $P<0{\cdot}001$ for hypophysis $<0{\cdot}05$ for cerebral cortex $<0{\cdot}01$ for cerebellum. Values obtained after head perfusion, excepting posterior lobe +.

### *Persistence of raised histamine concentration in brain after histidine infusion*

Sixteen hours after the infusion of histidine, at a dose of 500 mg/kg, the concentration of histamine in different parts of the brain was still as high as that at 30 min (Figure 1). At 16 h after the infusion of 250 mg/kg the concentration was slightly lower than at 30 min; at 32 h the concentration in each of the regions of the brain examined lay within the limits of the mean for untreated controls. At 16 h after the infusion of 62 mg/kg, the values were not different from those of untreated controls.

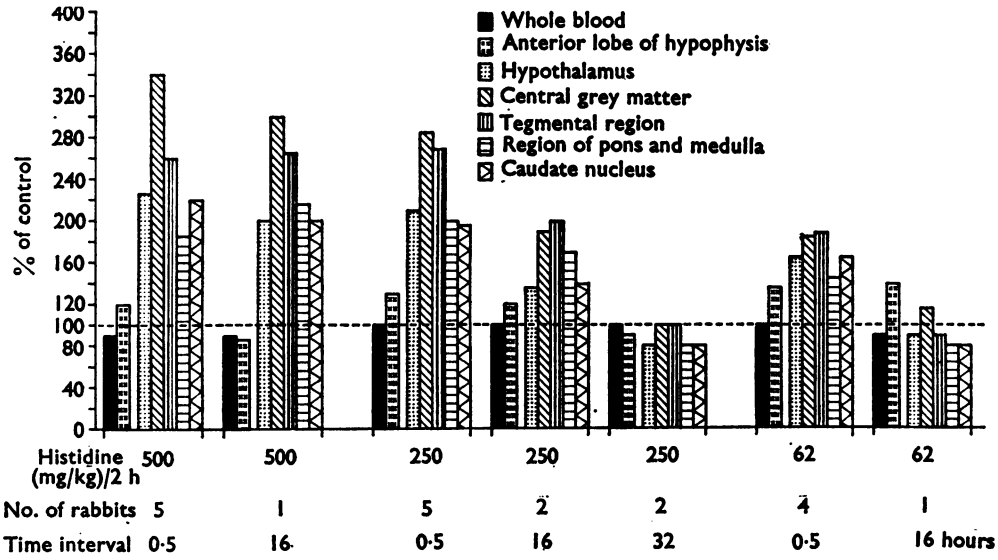


FIG. 1. Persistence of raised histamine levels in brain after the i.v. infusion of various doses of histidine. The columns represent the mean concentrations expressed as percentages of the untreated control. Rabbits were anaesthetized at 0·5, 16 or 32 h after the end of infusion and then killed.

### *Effect of intravenous infusion of histidine in the anaesthetized rabbit*

The infusion of histidine (250 and 500 mg/kg for 2 h) in rabbits anaesthetized with sodium pentobarbitone raised the concentration of histimine in the hypothalamus, central grey matter, tegmental region and pons-medulla; the rise was comparable with that seen when histidine was infused in the conscious rabbits.

TABLE 5. *The effect of α-methyldopa, DOPA, 5-hydroxytryptophan (5-HTP) and tryptophan infusions on the histamine concentration in rabbit brain*

| | | Concentration of histamine (ng/g tissue) | | | | |
|---|---|---|---|---|---|---|
| Amino acid | Dose, mg/kg (no. of expts) | Hypothalamus | Central grey | Tegmentum | Pons-medulla | Caudate nucleus |
| DOPA | 60 (1) | 720 | 470* | 260* | 160 | 190 |
| | 100 (1) | 710 | 440* | 230 | 170 | 270* |
| α-Methyldopa | 200 (1) | 790 | 370 | 250 | 180 | 160 |
| | 4×200+ (2) | 670 | 470* | 340* | 200 | 210 |
| | | 730 | 380 | 270* | 270 | 150 |
| 5-HTP | 65 (3) | 800 | 360† | 200† | 160 | 140 |
| | | 580 | 290 | 250 | 140 | 120 |
| | | 530 | 450* | 240 | 100 | 120 |
| | 75 (2) | 720 | 470* | 240 | 160 | 190 |
| | | 1360* | 760* | 410* | 300* | 310* |
| Tryptophan | 200 (2) | 770 | 360 | 230 | 230 | 130 |
| | | 610 | 300 | 200 | 140 | 140 |
| Control (means, TABLE 1) | | 660 | 280 | 170 | 140 | 150 |
| Upper 95% fiducial limits of controls | | 932 | 425 | 260 | 280 | 240 |

All infusions over a 2-h period except in + where the dose of amino acid was given over 30 min and repeated 4 times with an interval of 12 h between doses. * Values above the upper 95% fiducial limits of controls. † Mean from these 5 experiments significantly ($P<0.005$) different from controls.

### *Effect of α-methyldopa, DOPA, 5-hydroxytryptophan and tryptophan on the concentration of histamine in brain, hypophysis and blood*

The amino acids were infused i.v. over a 2 h period into conscious rabbits which

were anaesthetized and killed 30 min after the end of a single infusion or of the last of a series. DOPA, α-methyldopa and 5-HTP, but not tryptophan, produced a rise in the histamine concentration in the mid-brain region; elsewhere, in the hypothalamus, caudate nucleus or pontine-medullary regions, the amino acids appeared to be without effect (Table 5). Hypophyseal and blood concentrations were likewise unaltered.

α-Methyldopa (200 mg/kg, 3 expts.), DOPA (60 mg/kg, 1 expt.) or 5-HTP (75 mg/kg, 1 expt.) infused simultaneously with histidine (250 mg/kg) over a period of 2 h did not interfere to a detectable degree with the histidine-induced rises in brain histamine.

## Discussion

The concentration of histamine in both lobes of the hypophysis of the rabbit was found to be lower and less variable than that reported for the dog (Adam, 1961), cat (Adam & Hye, 1966) and guinea-pig (Stephen, 1968). The difference may be due to the apparent absence of mast cells in the hypophysis of rabbit (Stephen, 1968).

Histamine extractable from brain could conceivably come from mast cells, blood (platelets) or neural tissue. However, mast cells have not been found in rabbit brain (Constantinides, 1953; Stephen, 1968). The following evidence supports the view that histamine estimated in the hypophysis and brain derives mostly from the tissues and not from the blood. First, perfusion of the head with Ringer-Locke solution (Table 2) did not produce a detectable fall in the concentration of histamine in hypophysis or brain, except in the cerebellum where a reduction to 50% was observed (from 60 to 30 ng/g) and may have been due to cerebral oedema. Alternatively, it is possible that about 30 ng of histamine extractable from 1 g of tissue is blood histamine. The values reported in this paper have not been corrected for the possible contribution of histamine by blood. Secondly, histamine is unevenly distributed in brain (Table 1). The concentration of histamine in the brain or hypophysis is not correlated with that of blood; for example, the fall in blood histamine after treatment with glycogen was not associated with a decrease in brain or hypophyseal histamine.

The distribution of histamine in rabbit brain is similar to that found in dog (Adam, 1961) and cat (Adam & Hye, 1966) though the values for the hypothalamus and thalamus were lower. The present results do not confirm the high concentrations reported by Waalkes, Coburn & Terry (1959) or the even distribution of histamine in the rabbit brain reported by Shore, Burkhalter & Cohn (1959). The former group employed a spectrophotometric method of estimation; the latter group usd a fluorometric method, the limited specificity of which when applied to brain, has been discussed by Carlini & Green (1963) and Levine, Sato & Sjoerdsma (1965).

The relatively high concentration of histamine in the hypothalamus and the low concentration in the cerebral cortex of the rabbit may be related to the capacity of the different regions to decarboxylate histidine. White (1959; 1960) found in the cat that the hypothalamus has a high capacity to form histamine from histidine compared with the cortex. The distribution of histamine in the brain does

not seem to parallel the distribution of the capacity to ring-methylate histamine; according to Brown, Tomchick & Axelrod (1959), the activity is more or less the same in different parts of the rabbit brain.

The concentration of catecholamines in different regions of the rabbit brain (Matsuoka, Yoshida & Imaizumi, 1964) is roughly comparable with that of histamine; however, the concentration of histamine in the caudate nucleus is much lower than of dopamine. The concentration of 5-HT in the rabbit hypothalamus (Joyce, 1962) is comparable with that of histamine found in the present work. Values reported for 5-HT in the mid-brain and hind-brain of rabbit (Costa & Aprison, 1958; Costa, Pscheidt, van Meter & Himwich, 1960) are higher than those for histamine.

The rise of histamine in brain seen after the infusion of histidine was probably due to decarboxylation of the amino acid after it had passed from the blood into the brain. Other factors, however, may have contributed to the result. Histidine may have been decarboxylated in the formed elements of blood, especially platelets (Schayer & Kobayashi, 1956), thereby leading to a rise of histamine in whole blood. Residual blood in cerebral vessels may have contributed to the rise of histamine in brain. This possibility was examined in various ways and the following evidence was obtained. First, an increase in whole blood histamine was not detected (Table 3); secondly, the absolute rise in brain histamine occurred unevenly but the pattern of distribution of the amine remained similar to that seen in the untreated rabbit; thirdly, injection of glycogen caused a profound fall of histamine in blood but not in brain. Finally, decarboxylation of histidine by platelets and other tissues may have raised the plasma histamine which then entered the brain; however, when histamine was infused i.v. the concentrations in the hypothalamus and thalamus did not rise detectably (Table 4).

These observations support the conclusion that most of the histamine extractable from brain of histidine-treated rabbits is histamine formed in the brain from histidine and not derived from blood.

The rise of histamine in brain seen after the infusion of histidine indicates rapid conversion of histidine to histamine and suggests that the decarboxylating enzyme in brain is not saturated by normal concentrations of the substrate. In some areas of the brain (pons-medulla, caudate nucleus) the rise in histamine in response to histidine was small, in others (hypothalamus, mid-brain) closely related to the dose of histidine. This finding may reflect an uneven distribution of decarboxylase for histidine or storage sites for the newly formed amine. The results confirm those of White (1959) who also observed that the rate of histamine formation in the hypothalamus of the cat was 10–15 times greater than in the cerebral cortex. It appears that histidine, in common with 5-HTP (Costa & Rinaldi, 1958) and DOPA (Bertler & Rosengren, 1959) is rapidly decarboxylated in rabbit brain. Similarly, after treatment with 5-HTP (Costa *et al.*, 1960; Udenfriend *et al.*, 1957) or DOPA (Bertler & Rosengren, 1959), the concentration of the corresponding amines was highest in the mid-brain, hypothalamus and caudate nucleus, and least in the cerebral cortex and cerebellum.

The increase in brain histamine after dosage with histidine persisted for a period of hours, higher doses of histidine causing more persistent increases in histamine. Whether the newly-formed histamine was mostly 'bound' or the concentration of histidine in brain remained high for a long period during which time decarboxylation

continued, remains a matter for speculation. It was evident, however, that the increase in brain histamine in response to infusion of histidine was not associated with overt pharmacological effects on behaviour.

The finding that histidine did not cause a significant rise of histamine in the anterior lobe of the hypophysis (Table 3) confirms earlier observations in the cat (Adam, Hye & Waton, 1964); formation of histamine in this region may have been too slow to be detectable under the conditions of the present experiments; alternatively, the histamine produced may have diffused into the circulation or been rapidly metabolized.

After the infusion of histamine, the concentration of the amine did not rise significantly in the hypothalamus or thalamus (Table 4). These findings agree with those reported for the uptake of histamine by brain of other species (Halpern, Neveu & Wilson, 1969; Adam *et al.*, 1964; Snyder, Axelrod & Baur, 1964; Snyder & Axelrod, 1965). Nevertheless, the concentration of histamine rose in the cerebral cortex and cerebellum (vermis), possibly because the amine diffused through the pial vessels. The rise in the concentration of histamine in both lobes of the hypophysis supports the view that the gland is outside the blood-brain barrier (Davson, 1956). The small increase in total blood histamine confirms earlier reports that free histamine disappears rapidly from circulation (Dragstedt & Mead, 1935; Rose & Browne, 1938; Alexander, 1946; Emmelin, 1951).

It was observed that $\alpha$-methyldopa, DOPA and 5-HTP raised the concentration of histamine in the mid-brain but not in other areas of the brain examined (Table 5). This rise could be due to inhibition of histamine catabolism by the monoamines formed or their catabolic products; 5-HT is known to inhibit methylation of histamine *in vitro* (Brown *et al.*, 1959; Gustafsson & Forshell, 1963) and *in vivo* (Snyder & Axelrod, 1964). These findings may imply a differentiation between the mid-brain and other regions in the metabolism of histamine.

It has been suggested that histidine decarboxylase activity in the brain is non-specific, the enzyme L-aromatic acid decarboxylase having a strong affinity for 5-HTP and DOPA but a low affinity for histidine (Lovenberg *et al.*, 1962). The finding that $\alpha$-methyldopa, DOPA and 5-HTP did not inhibit the formation of histamine from exogenous histidine may suggest that in rabbit brain the decarboxylase for histidine is a specific enzyme.

The authors are deeply grateful to Dr. T. B. B. Crawford, of the Department of Pharmacology, for helpful criticism in the preparation of this manuscript.

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(*Received January* 18, 1973)