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CONCENTRATION OF HISTAMINE IN DIFFERENT PARTS OF BRAIN AND HYPOPHYSIS OF CAT AND ITS MODIFICATION BY DRUGS

BY

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In a previous study (Adam, 1961), it was shown that the regional distribution of histamine in dog's brain follows closely the pattern described for noradrenaline (Vogt, 1954) and 5-hydroxytryptamine (5-HT) (Amin, Crawford & Gaddum, 1954). Further, it was suggested that in the hypophysis histamine derives in part from mast cells, in contrast to the brain, where the amine is contained in some other type of cell. Recent findings on the subcellular distribution of histamine in these tissues have confirmed this view (Michaelson & Dowe, 1963). The subject of brain histamine has been reviewed by Green (1964).

The object of the present investigation was two-fold: (1) to obtain values for the concentration of histamine extractable from different parts of the cat's brain and hypophysis, and (2) to see if the concentration in the hypophysis and adjoining parts of the brain could be altered by drugs. The drugs were chosen mainly from those which are known to influence the metabolism of histamine (White, 1961) or of other amines in the brain (Shore, 1962).

In the course of this work, we have confirmed the presence of mast cells in the cat's hypophysis (Gray, 1935) and attempted to study their number and distribution in the gland. We have also tested for the presence of certain pharmacologically active N-alkyl derivatives of histamine (Werle & Palm, 1952) in extracts of the brain and hypophysis. Some of the results presented in this paper were communicated at a meeting of the Physiological Society (Adam & Hye, 1964).

METHODS

Dissection

Cats of either sex weighing 2.5 to 4.5 kg were anaesthetized with ether and bled out. The brain was immediately removed together with the attached hypophysis, and dissected in the cold. From eight to 16 samples were removed at each dissection. Samples were collected from 30 different areas to study the regional distribution of histamine. The effect of drugs was studied in only eight of these areas: three in the hypophysis, four in the hypothalamus and one in the thalamus. The samples from these areas are described as follows:

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"Anterior lobe" was mainly the pars distalis of the adenohypophyis; "posterior lobe," the infundibular process and pars intermedia. "Hypophysial stalk" contained the median eminence, the greater part of the infundibular stem and the pars tuberalis (Rioch, Wislocki & O'Leary, 1940).

The hypothalamus (excluding the infundibulum) on both sides of the brain was divided into four parts and the corresponding parts from each side were pooled. The samples were cut in rectangular blocks to a depth of about 3 mm from the ventricular surface. "Corpora mammillaria" were obtained by making three cuts round each mammillary body. "Ventral hypothalamus" was the ventromedial part and the "dorsal hypothalamus" the dorsomedial part of the posterior hypothalamus. "Preoptic region" was taken from the anterior hypothalamus in front of a line passing from the anterior commissure to the posterior border of the optic chiasma. "Medial thalamus" was from the region of massa intermedia cut to a depth of about 3 mm.

Samples from the above areas were weighed and extracted without delay. Samples from the rest of the brain were sometimes frozen and stored at −17° C for 24 hr without detectable loss of histamine.

"Cerebral cortex" was from areas 2, 4b, 50 and 22 of the sensory cortex and from areas 24 and 30 of the cingulate gyrus (Gurewitsch & Chatschaturian, 1928). "Cerebellum" was taken from the vermis; "caudate nucleus" from the head of the nucleus; "hippocampus" was the middle third in the inferior horn.

Extraction and estimation of histamine

The method was that of Adam (1961). Briefly, tissue samples weighing from 3 to 80 mg were ground in trichloroacetic acid (6% w/v; 5 μl/mg fresh tissue); the volume was made up to 5 ml. with water and the suspension centrifuged. An aliquot of the supernatant was neutralized and buffered to *p*H 8.0. 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 2 ml. 0.25N HCl followed by 3 ml. water. The eluate, which contained about 24 mg of NaCl, was evaporated to dryness and the residue heated in 6N HCl. After complete removal of the acid, the dried residue was taken up in a modified Tyrode solution. 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 the base.

Recovery experiments

25 to 100 ng of histamine was added to samples ($\sim$ 50 mg) obtained from different parts of the brain. Control samples were taken to estimate histamine originally present in the tissues. The mean recovery was 75% with range 69 to 90% (9).

Paper chromatography

Trichloroacetic acid extracts of the following tissues were prepared by the method as described above: (1) hypophysis (whole gland), (2) hypothalamus (right and left halves), (3) cerebellum (from vermis) and (4) medial thalamus. Histamine (as the dihydrochloride), β-N-methylhistamine and β-N-dimethylhistamine, 200 ng of each, were added to the extract of cerebellum for recovery. The marker was prepared by adding 2 μg of each compound to the extract of medial thalamus. The buffered supernatants were applied to columns and the dried eluates were treated with 6N HCl in the usual way. The dried residue was extracted with 3×1 ml acid ethanol (0.1% HCl in ethanol, v/v) and the extract centrifuged at 2,000 r.p.m. at 4° for 20 min. An aliquot of the supernatant was evaporated to dryness. The residue, which now contained less than 1 mg NaCl, was taken up in 3×0.1 ml. methanol and spotted on strips (3 cm wide) of Whatman No. 1 paper.

The solvent system had the following composition: *iso*propanol-ammonia (0.880)-water (150:8:26 by vol.). Separation was by ascending chromatography for 17 hr at room temperature. Compounds on the marker strip were located with fresh diazotized sulphanilic acid. The remaining strips were cut transversely at intervals of 0.5 or 1.0 cm. Elution was carried out by immersing each piece of paper in 5 ml. of 0.02N HCl for 1 hr. The eluate was evaporated, care being taken to remove the

last traces of the acid. Tyrode solution, 2 ml., was placed in the tube and, after shaking, the solution was assayed by superfusion. Estimates of the recovery of the β-N-methyl derivatives were calculated as histamine from potency ratios which were obtained on the superfused guinea-pig ileum, using a 2 and 2 design of assay (Schild, 1942). On this preparation the potency of β-N-methylhistamine was found to be 75.8% of that of histamine with fiducial limits ($P=0.99$) 69.7 to 81.8%; the potency of β-N-dimethylhistamine was 46.7% with fiducial limits 41.5 to 52.0% (Schild, 1947; Lin, Alphin, Henderson, Benslay & Chen, 1962).

Histology

The hypophysis and the hypothalamus of a single cat were examined for mast cells. The brain was fixed *in situ* by perfusion technique (Cammermeyer, 1960): 500 ml. Ringer-Locke solution was followed by 600 ml. Duboscq-Brasil fixative solution (Gatenby & Beams, 1950). The brain was removed 1 hr later and a block of tissue containing the hypophysis and the hypothalamus was taken and left overnight in the fixative solution. The tissue block was dehydrated in graded ethanol and clarified in benzene and subsequently embedded in paraffin wax *in vacuo*. The entire block was cut in the sagittal plane into sections 5 μ thick, which were serially numbered. Every tenth section was stained with aqueous toluidine blue (Gurr; 0.5% w/v), the dye being modified according to Robinson & Bacsish (1958). Excess dye was removed by rinsing in ethanol (95% v/v) and then in methanol, until the mast cells stood out against a pale background.

Sections stained with toluidine blue were systematically examined for mast cells. The number of mast cells in each section was counted and the regional position of each cell noted on a diagram representing a section of the gland near the median plane. Other sections were stained with haematoxylin and eosin.

Drugs used

Histamine acid phosphate and histamine dihydrochloride (British Drug Houses); β-N-methyl histamine dihydrobromide and β-N-dimethyl histamine dihydrochloride (Eli Lilly); compound 48/80 (Burroughs Wellcome and Co.); reserpine base (Ciba); chlorpromazine hydrochloride and thioproperazine methane sulphonate (May and Baker Ltd.); trifluopromazine hydrochloride (Squibb and Sons); iproniazid phosphate (Roche); pargyline hydrochloride (Abbott); morphine hydrochloride (B.P.); bulbocapnine hydrochloride (Merck); pentobarbitone sodium (B.P.). The concentrations of chlorpromazine HCl, trifluopromazine HCl, bulbocapnine HCl, morphine HCl and pentobarbitone Na are expressed as the salt; the concentrations of all other drugs refer to the base.

Compound 48/80 was dissolved in 0.9% saline and injected daily intraperitoneally in increasing doses until a total of 30 mg/kg was given in about two weeks. From the third day onward the animals received 30 ml. of 0.9% saline daily by I.P. injection. Histamine was estimated in samples of skin taken from the ear before and after the course of treatment.

Reserpine was dissolved in 20% w/v ascorbic acid before use. In one series of experiments cats received a single dose of reserpine in the range 0.1 to 10 mg/kg, and were killed at 18 hr. In a second series the dose was 0.5 mg/kg and the animals were killed at different time intervals from 6 to 120 hr.

Chlorpromazine HCl and trifluopromazine HCl were dissolved in saline and injected intramuscularly; all other drugs were injected intraperitoneally. Where the drug was given in divided doses, the last dose was administered 2 to 3 hr before removal of the brain. During the period of treatment the animals were allowed to move freely in a room kept at 20 to 25°, and were given food and water *ad libitum*.

Further details of the methods used in the present work are contained in a thesis by one of us (Hye, 1964).

RESULTS

Chromatographic separation of active substance

In the strip containing the extract of hypothalamus, the activity corresponded to the area of the histamine spot (R_F 0.42) on the marker. The total activity was estimated to

be equivalent to 72 ng of histamine. Activity was not detected in those areas which corresponded to the spots of β-N-methylhistamine (R_F 0.57) and β-N-dimethylhistamine (R_F 0.67) on the marker. A similar result was obtained with the extract of hypophysis, when the total activity in the area corresponding to the histamine spot on the marker was 44 ng.

In the strip containing the three amines, three peaks of activity were detected which corresponded to the spots of the reference compounds on the marker. The recovery of histamine was estimated to be 68% ; of β-N-methylhistamine, 56% and β-N-dimethylhistamine, 60% of the amount added to the extract (200 ng).

It was concluded that since the extracts of hypothalamus and hypophysis did not contain measurable amounts of either β-N-methylhistamine or of β-N-dimethylhistamine, the activity estimated in these tissues was attributable mainly to histamine. Calculation showed that the presence of these alkyl derivatives would have been detected if their contribution to the activity in the extracts had been 5% or more of that due to histamine.

Histological findings

In sections stained with toluidine blue, mast cells were present in 48 out of the 140 sections examined. Mast cells were seen only in the hypophysis where they were situated close to blood vessels. None were found in the hypothalamus. Their regional distribution within the gland is shown diagrammatically in Fig. 1. The total number counted in the anterior lobe (pars distalis) was 98 ; in the posterior lobe 543, of which only four were in the pars intermedia ; in the hypophysial stalk 380, of which 310 were in the pars tuberalis.

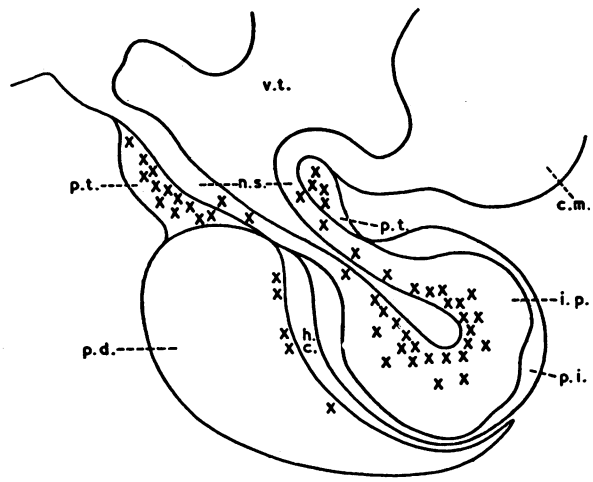

Fig. 1. Distribution of mast cells in cat's hypophysis. Each cross represents about 200 mast cells in the whole gland. Key: c.m. = corpora mammillaria ; v.t. = third ventricle ; h.c. = hypophysial cavity (cleft) ; n.s. = neural stalk ; i.p. = infundibular process ; p.t. = pars tuberalis ; p.d. = pars distalis ; p.i. = pars intermedia.

Estimates of the total number of mast cells in the different parts of the hypophysis were obtained by multiplying the total number counted in the series of sections by 10. The estimated total number of mast cells for the whole gland was about 10,000.

Distribution of histamine in brain and hypophysis

Estimates of the concentration in samples taken from 25 cats are presented in Table 1. Estimates of the amount of histamine contained in the different parts of the hypophysis, the hypothalamus and in the medial thalamus are shown in Table 2.

Hypophysis. The concentration for each part of the gland varied over a wide range and was highest in the hypophysial stalk. It was calculated that an average gland

TABLE 1

HISTAMINE CONCENTRATION IN CAT'S HYPOPHYSIS AND BRAIN

Estimates expressed in ng of base/g of tissue. Number of animals in parentheses

Tissue sample	Mean	Range	S.E. of mean
Hypophysis			
Anterior lobe	2,400	840–6,500 (25)	280
Posterior lobe	1,700	560–3,400 (25)	140
Hypophysial stalk	5,200	1,200–9,200 (14)	670
Hypothalamus			
Corpora mammillaria	1,150	660–1,800 (15)	90
Ventral hypothalamus	800	400–1,300 (15)	70
Dorsal hypothalamus	480	290–810 (15)	40
Preoptic region	430	230–730 (15)	30
Thalamus			
Medial	250	110–410 (21)	20
Ventrolateral	75	60–110 (8)	10
Dorsolateral	350	180–610 (9)	40
Med. geniculate body	370	180–740 (12)	50
Lat. geniculate body	180	90–400 (12)	30
Midbrain			
Superior colliculus	150	90–200 (10)	11
Inferior colliculus	120	70–200 (10)	10
Central grey matter	160	80–240 (13)	12
Reg. of red nucleus	100	50–160 (8)	12
Pons and medulla			
Floor of 4th ventricle	70	50–110 (8)	7
Reg. of reticular formation	50	40–60 (4)	—
Brachium pontis	—	<30, <30 (2)	—
Nc. cuneatus	—	<30 (1)	—
Nc. gracialis	—	<30 (1)	—
Area postrema	—	1,500, 1,200, <280, <300, <280 (5)	—
Telencephalon			
Cerebral cortex	90	40–130 (8)	15
Caudate nucleus	50	30–70 (9)	5
Hippocampus	60	40–90 (3)	—
Corpus callosum	—	<80, <90 (2)	—
Other parts			
Cerebellum	—	<20, <30 (7)	—
Pineal body	—	380 (1)	—
Optic nerve	—	<60–<70 (3)	—
Choroid plexus	—	220, 200 (2)	—

TABLE 2

ESTIMATES OF HISTAMINE CONTAINED IN CAT'S HYPOPHYSIS AND ADJOINING PARTS OF BRAIN

Number of animals in parentheses

Tissue sample	Mean weight (mg)	Range	Mean content (ng)	Range
Anterior lobe	18·8	13–28	45	16–120 (25)
Posterior lobe	12·7	8–19	22	8–41 (25)
Hypophysial stalk	3·3	1·5–4·0	17	6–38 (14)
Corpora mammillaria	16·1	12–21	19	11–27 (15)
Ventral hypothalamus	34·2	29–46	28	14–49 (15)
Dorsal hypothalamus	30·6	22–43	15	7–22 (15)
Preoptic region	19·5	16–25	9	6–16 (15)
Medial thalamus	35·6	23–54	9	5–16 (21)

weighing 35 mg is likely to contain about 84 ng of histamine, of which 54% would be in the anterior lobe, 26% in the posterior lobe and 20% in the hypophysial stalk.

Hypothalamus. The mean values for the brain were lower and less variable than those for the hypophysis. The highest concentrations were found in the corpora mammillaria and in the ventral hypothalamus. In the dorsal hypothalamus and in the preoptic region the concentration was about half that of the ventral hypothalamus.

Thalamus. The mean values for the various parts were all lower than those for the hypothalamus. The concentration was highest in the medial geniculate body and in the dorsolateral part; intermediate in the medial thalamus and lowest in the lateral geniculate body and ventrolateral part. Values for the medial thalamus were about half those of the dorsal hypothalamus.

Rest of brain. Concentrations in the midbrain were mostly below 200 ng/g; elsewhere in the brain, less than 100 ng/g. Histamine was not detected in the cerebellum, corpus callosum or in the optic nerve. Only two out of five samples of the area postrema contained detectable amounts of histamine. (The mean wet weight of this tissue was 4.2 mg (range 3.1–5.2).)

Effect of drugs

The effect of drugs on the concentration of histamine in the hypophysis and adjoining parts of the brain is shown in Table 3.

Several of the drugs tested altered the concentration of histamine in the brain but not in the hypophysis. Compound 48/80 was the only drug to alter the concentration in the gland.

Compound 48/80. The mean concentration of histamine in the ear skin before treatment was 55 μg/g (range 30–68 (5)). After treatment, the concentration fell significantly ($P<0.01$) to 20 μg/g (range 4–27 (5)), or to 34% of the control value. Similarly in the hypophysial stalk the mean concentration fell to 33% and in the posterior lobe to 43% of the corresponding control values; the fall in each case was significant ($P<0.01$). The concentration in the anterior lobe or in the brain was not significantly changed.

TABLE 3

EFFECT OF DRUGS ON CONCENTRATION OF HISTAMINE IN HYPOPHYSIS AND RELATED PARTS OF BRAIN IN CAT

Mean estimate ± S.E., or with range, expressed in ng of base/g of fresh tissue. Number of animals in parentheses

Drug	Total dose (mg/kg)	Duration	Hypophysis			Hypothalamus				Thalamus Medial (massa intermedia)
			Anterior lobe	Posterior lobe	Hypophysial stalk	Corpora mammillaria	Ventral	Dorsal	Preoptic region	
Controls			2,400 ±280 (25)	1,700 ±140 (25)	5,200 ±670 (14)	1,150 ±90 (15)	800 ±70 (15)	480 ±40 (15)	430 ±30 (15)	250 ±20 (21)
Compound 48/80	10–36 I.P.	7–21 days	2,600 ±760 (5)	730* ±140 (5)	1,700* ±380 (5)	1,190 ±90 (5)	770 ±70 (5)	420 ±60 (5)	390 ±40 (5)	280 ±30 (5)
Reserpine	0·5–10 I.P. (single dose)	18 hr	1,700 ±630 (9)	2,300 ±540 (10)	4,500 ±830 (10)	400* ±25 (10)	270* ±20 (10)	200* ±6 (10)	190* ±6 (10)	160* ±12 (10)
Chlorpromazine	3 × 50 I.M.	24 hr	2,200 ±500 (7)	1,400 ±140 (7)	3,700 ±320 (7)	1,700* ±180 (7)	1,300* ±130 (7)	720* ±70 (7)	650* ±90 (7)	330* ±20 (7)
Trifluopromazine	3 × 50 I.M.	24 hr	—	—	—	1,800* ±240 (6)	1,260* ±190 (6)	650* ±80 (6)	650* ±90 (6)	—
Thioproperazine	3 × 50 I.P.	24 hr	—	—	—	1,690 1,400–1,900 (3)	1,150 1,100–1,200 (3)	630 540–740 (3)	580 540–610 (3)	—
Iproniazid	5 × 25 I.P.	5 days	1,700 ±185 (7)	1,200 ±170 (7)	2,850 ±530 (7)	2,000* ±340 (7)	1,250* ±150 (7)	690* ±90 (7)	670* ±130 (7)	320 ±40 (7)
Pargyline	5 × 20 I.P.	5 days	—	—	—	1,800 970–2,800 (4)	890 500–1,400 (4)	470 400–640 (4)	410 370–450 (4)	—
Bulbocapnine	3 × 30 I.P.	4 hr 24 hr	—	—	—	1,500 1,760	770 1,200	420 600	440 540	280 270
Morphine	3 × 30 I.P.	24 hr	1,850, 5,330	1,050, 1,930	2,930, 4,600	1,490, 1,240	900, 990	560, 350	480, 550	280, 200
Pentobarbitone	60 I.P.	1 hr	670 500–820 (3)	940 690–1,360 (3)	3,380 2,630–4,300 (3)	1,300 1,240–1,420 (3)	1,030 970–1,090 (3)	590 560–610 (3)	660 580–700 (3)	480 390–720 (3)

* Significantly different from the mean of the control, $P<0·05$.

Reserpine. In the dose range 0.5 to 10.0 mg/kg, reserpine reduced the histamine concentration in the hypothalamus and medial thalamus. The effect was independent of dose within this range. In the four parts of the hypothalamus the fall was highly significant ($P<0.001$): in the ventral hypothalamus the concentration fell to 34% of the control value, in the corpora mammillaria to 35%, in the dorsal hypothalamus to 42% and in the preoptic region to 44%. In the medial thalamus the fall was only to 64% of the control ($P<0.01$). When the dose of reserpine was 0.1 or 0.25 mg/kg, the effect was seen only in the corpora mammillaria and ventral hypothalamus.

After a single dose of 0.5 mg/kg reserpine, the maximum fall in the hypothalamus occurred at 18 hr (Fig. 2). Recovery of the concentration was slow and still incomplete at 120 hr.

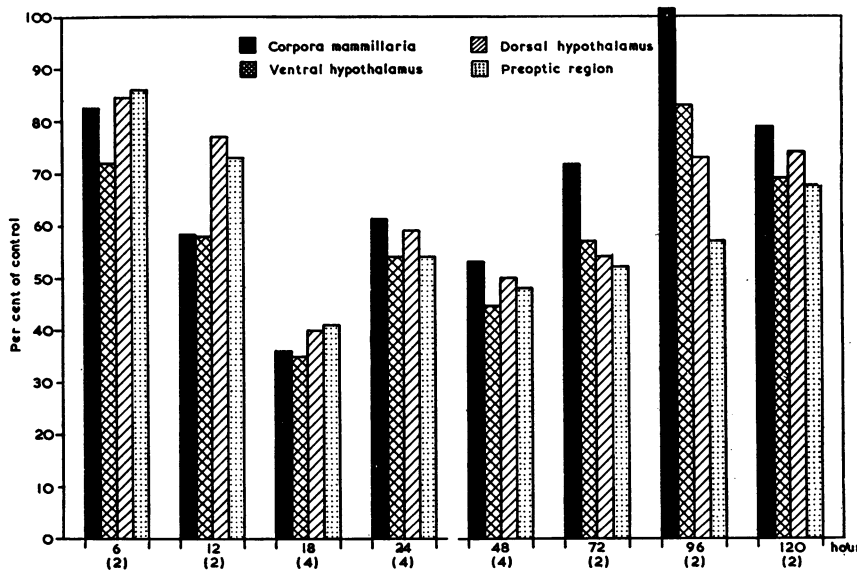

Fig. 2. Partial depletion and recovery of histamine in cat's hypothalamus after single dose of reserpine (0.5 mg/kg I.P.). Horizontal line at 100% represents control values for concentration of histamine (Table 1). Columns represent mean concentration of histamine expressed as percentage of control in four regions of hypothalamus at different time intervals after injection of reserpine. ( )=number of animals.

Phenothiazines. After treatment with chlorpromazine (3×50 mg/kg), the mean concentration in each of the four parts of the hypothalamus rose significantly ($P<0.01$) to values that lay between 148 and 163% of the controls. In the medial thalamus the concentration rose to 132% of the control ($P<0.05$).

When the dose was reduced to 3×25 mg/kg in 24 hr, there was no detectable change in the brain histamine (single experiment). When the dose was increased to 5×25 mg/kg in 48 hr, the histamine concentration rose by more than three-fold in the corpora mammillaria and the ventral hypothalamus; in the rest of the hypothalamus and in the medial thalamus the concentration was more than doubled (single experiment).

Trifluopromazine and thioproperazine in doses similar to those of chlorpromazine also increased the histamine concentration in the hypothalamus.

Monoamine oxidase (MAO) inhibitors. After treatment with iproniazid (25 mg/kg daily for five days), the mean concentration in each of the four parts of the hypothalamus rose significantly ($P<0.01$) to values that lay between 146 and 174% of the controls. In the medial thalamus the concentration rose to 128% of the control ($P<0.05$). On this dosage, however, iproniazid produced changes in the blood which became darker in colour probably owing to the formation of methaemoglobin. When the same total dose (125 mg/kg) of iproniazid was given over 24 hr, there was no detectable rise (single experiment). Pargyline, a non-hydrazine type of MAO inhibitor, increased the concentration mainly in the corpora mammillaria.

Other drugs. After treatment with bulbocapnine (3×30 mg/kg in 24 hr) the concentration rose in the four areas of the hypothalamus. When the same dose was given in 4 hr, there was no change in the concentration.

The concentration in the hypothalamus and hypophysis was unchanged after treatment with morphine.

When anaesthesia was induced with pentobarbitone and maintained for 1 hr, the concentration rose in the preoptic region and in the medial thalamus.

DISCUSSION

Histamine in hypophysis and brain

The pharmacological identification of the activity measured in the assay has already been discussed (Adam, 1961). In the present work, chromatographic analysis of the active material in the dried, acid-treated eluate has confirmed that the activity in the test is probably due only to histamine and not to a mixture with β-N-methylhistamine and/or β-N-dimethylhistamine. These derivatives of histamine, which have been reported to occur in human urine (Kapeller-Adler & Iggo, 1957) have not been detected in peripheral nerve or in sympathetic ganglion (Werle & Palm, 1952); nor do they appear to be present in extracts of the hypophysis or hypothalamus.

Nevertheless, our solutions for assay may have contained the ring-methylated derivative of histamine (1-methyl-4-(β-aminoethyl)imidazole, referred to subsequently as 1,4-methylhistamine) (Schayer, 1959). According to White (1966), the concentration of 1,4-methylhistamine extractable from the cat's hypothalamus is about two-thirds that of histamine. Since this derivative has been estimated to be 200 to 300 times less active than histamine on the guinea-pig's ileum (Lee & Jones, 1949; Dr. J. P. Green, personal communication; and confirmed by ourselves) its presence in our solutions would not have complicated the result of the assay.

The present estimates of the brain histamine are lower than those reported by McGeer (1964), who used a modification of the fluorimetric method of assay (Shore, Burkhalter & Cohn, 1959). The disparity is greatest in the cerebellum where, by the present method, the concentration was less than could be detected (<30 ng/g) as compared with a value of 200 ng/g found by McGeer (1964). The limitations of the fluorimetric method when applied to brain have been discussed by Carlini & Green (1963).

Estimates of the concentration of histamine in the hypophysis agree with the earlier findings of Harris, Jacobson & Kahlson (1952). They confirm the wide variation observed by these authors, which, apart from errors of dissection, could be related to variation in the number and distribution of mast cells in the gland. They are lower than those previously reported for the dog (Adam, 1961), suggesting that in the cat's hypophysis the mast cells are fewer in number or contain less histamine.

Examination of a single gland showed that the mast cells occurred chiefly in the posterior lobe and in the pars tuberalis of the adenohypophysis, where this forms part of the hypophysial stalk. Hence it is probable that histamine extractable from these tissues derives mainly from mast cells. The anterior lobe (pars distalis) differs from the other parts of the gland in that it contains more than half of the histamine extractable from the entire gland but only a small proportion of the mast cells. It is therefore probable that in this part of the gland some of the histamine resides in cells other than mast cells. The results obtained with compound 48/80 are consistent with this view. These observations confirm the earlier work of Gray (1935) who found mast cells in the posterior lobe and hypophysial stalk but not in the anterior lobe of the cat's hypophysis.

In the cat, as in the dog, the hypothalamus appears to be devoid of mast cells. According to Michaelson and Dowe (1963), histamine in dog's hypothalamus is present mainly in particles which sediment with microsomes, but the cellular origin of the particles is not known.

The pattern of distribution of histamine in the cat's brain is similar to that described for the dog, except that the amine was not always detectable in the area postrema. The progressive fall in concentration by more than 10-fold between the hypothalmus, thalamus and remainder of the brain coincides with a roughly similar fall in the decarboxylating activity of the brain for histidine; the activity being greatest in the hypothalamus and least in the cerebellum (White, 1959; 1960). Parts of the brain which contain histamine also contain 1,4-methylhistamine (White, 1966) and the methylating enzyme (imidazole N-methyl transferase: Brown, Tomchick & Axelrod, 1959; histamine methyltransferase: Lindahl, 1960). This enzyme, like MAO, is widely distributed in the brain (Axelrod, Maclean, Albers & Weissbach, 1961).

Histamine is present in parts of the brain which are known to contain monoamines. In the hypothalamus, the concentrations of the amines are comparable, but elsewhere in the brain—for example, in the region of the reticular formation—the concentration of histamine is somewhat lower than that reported for monoamines in the cat's brain (Vogt, 1954; Bogdanski, Weissbach & Udenfriend, 1957; Kuntzman, Shore, Bogdanski & Brodie, 1961).

Effect of drugs

The study of histamine in brain on a regional basis made it possible to detect changes in its concentration after treatment with drugs. Compound 48/80, which was chosen for its action on mast cells, did not alter the brain histamine, but the result is difficult to interpret since it is not known whether the drug penetrated into the brain. Of the remaining drugs, reserpine, the phenothiazines and MAO inhibitors produced significant alterations in the concentration of histamine in brain, but not in the hypophysis. The effect

of these drugs was seen more clearly in the hypothalamus than in the thalamus; and within the hypothalamus, in the ventral half and corpora mammillaria. The results for the hypothalamus are summarized in Fig. 3, where change in concentration is expressed as a percentage of the control value.

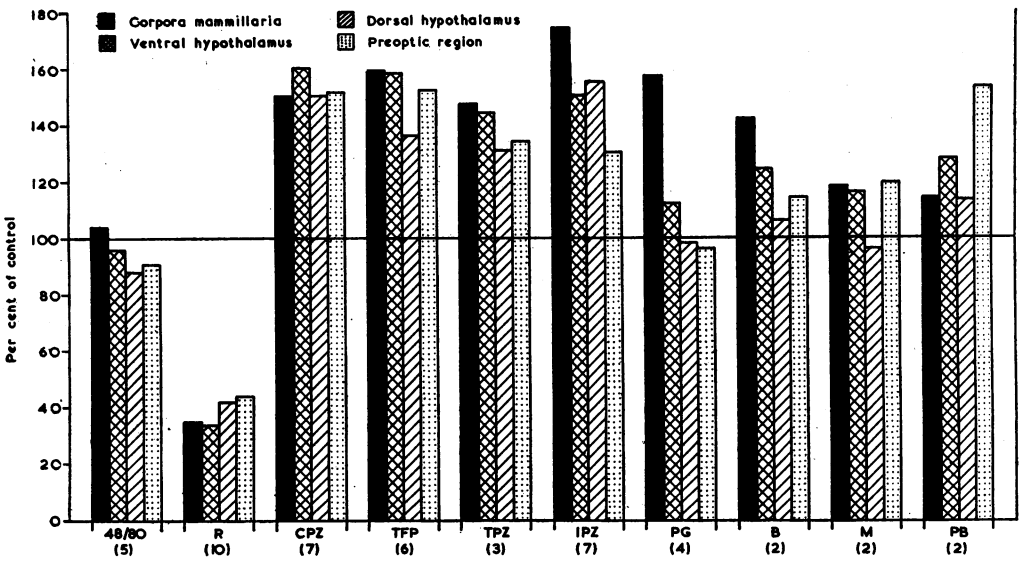

Fig. 3. Effect of various drugs on concentration of histamine in cat's hypothalamus. Horizontal line at 100% represents control values for concentration of histamine (Table 1). Columns represent mean concentration of histamine expressed as percentage of control in four regions of hypothalamus after treatment with various drugs: R=reserpine; CPZ=chlorpromazine; TFP=trifluopromazine; TPZ=thioproperazine; IPZ=iproniazid; PG=pargyline; B=bulbocapnine; M=morphine; PB=pentobarbitone. ( )=number of animals.

Reserpine may have lowered the brain histamine by blocking its storage in intracellular particles, as has been suggested for other amines (Pletscher, Shore & Brodie, 1956). Over a wide range of doses, the concentration fell to only 40% of the control value (mean for whole hypothalamus). This is in contrast with the more extensive fall reported for 5-HT in the dog's hypothalamus (Paasonen & Vogt, 1956) and for noradrenaline (Holzbauer & Vogt, 1956) in the cat's hypothalamus after similar doses of reserpine, when the concentration in each case fell to 10% or less of the original value.

Histamine extractable after treatment with reserpine may therefore represent histamine outside the particles, where its concentration might be expected to depend on a balance between formation and catabolism. A similar view has been advanced for the presence of low concentrations of 5-HT and of noradrenaline in the brain after reserpine (Shore, Pletscher, Tomich, Carlsson, Kuntzman & Brodie, 1957; Brodie & Beavan, 1963). Thus it is conceivable that the difference in concentration of histamine and the monoamines in the brain after reserpine may be related to differences in the turnover rates of the amines.

The rate of recovery of histamine in the hypothalamus after reserpine was similar to that reported for 5-HT and noradrenaline in the rabbit's brain after a much larger dose (5 mg/kg. I.V. (Pletscher *et al.*, 1956 ; Shore & Brodie, 1957). The failure of reserpine to lower the concentration of histamine in the hypophysial stalk and in the posterior lobe can be related to the finding that reserpine does not release histamine from mast cells (Parratt & West, 1957).

The phenothiazines and MAO inhibitors both increased the brain histamine and may have acted by interfering with its catabolism (Schayer, 1959). In brain, imidazole N-methyl transferase converts histamine to 1,4-methylhistamine, which in turn undergoes oxidation by a monoamine oxidase to give 1,4-methylimidazole acetic acid (White, 1959, 1960, 1961 ; Brown *et al.*, 1959).

Since chlorpromazine inhibits the methylation of histamine *in vitro* (Brown *et* al., 1959); Gustaffson & Forshell, 1963) and *in vivo* (White, 1961 ; Snyder & Axelrod, 1964), it may have acted in this way to increase the brain histamine. By contrast, treatment with chlorpromazine has no apparent effect on the concentration of monoamines in brain (Gey & Pletscher, 1961).

Trifluopromazine and thioproperazine (Parkes, 1961) were as effective as chlorpromazine in raising the brain histamine, but their potency as inhibitors of the methylating enzyme does not appear to have been investigated.

The effect of the phenothiazines in raising the brain histamine is not necessarily related to the central actions of the drugs, since the large doses employed could have acted in other ways. Green & Erikson (1964) came to a similar conclusion in their study on the rat. The present results with chlorpromazine agree with those reported by White (1966), who found that although the histamine concentration rose in the hypothalamus, the concentration of 1,4-methylhistamine remained unchanged.

The hypophysis also methylates histamine (Axelrod *et al.*, 1961) but the concentration in the gland did not rise after chlorpromazine. Since this region appears to be devoid of a " blood-brain barrier " (Davson, 1956), histamine may have been lost by diffusion into the blood. Alternatively, it could be that the turnover rate for histamine in the hypophysis is much slower than in the hypothalamus (Adam, Hye & Waton, 1964). This view gains support from the fact that in the cat, part of the hypophysial histamine resides in mast cells, where the turnover of the amine is known to be slow (Schayer, 1952).

Our results with MAO inhibitors differ from those obtained by White (1966), who found that treatment with iproniazid raised the concentration of 1,4-methylhistamine, but not that of histamine, in different parts of the cat's brain. The difference between our results and White's might be attributable to the larger total dose and the longer period of treatment in the present work (25 mg/kg daily for five days, as compared with 25 mg/kg for three days in White's experiments) ; or possibly to methaemoglobinaemia and haemolysis which were a complication in some of our experiments. The effect of iproniazid was confirmed by that of pargyline (Everett, Wiegand & Rinaldi, 1963), but the histamine concentration rose only in the corpora mammillaria.

It is possible that treatment with MAO inhibitors also increased the concentration of 5-HT in the brain (Udenfriend, Weissbach & Bogdanski, 1957 ; Brodie, Spector & Shore,

1959; Everett *et al.*, 1963). Further, there is evidence that both 1,4-methylhistamine (Brown *et al.*, 1959; Lindahl, 1960) and 5-HT (Brown *et al.*, 1959) inhibit imidazole N-methyl transferase *in vitro*. It might therefore be argued tentatively that under the conditions of our experiments the MAO inhibitors acted indirectly, through the accumulation of 1,4-methyl-histamine and 5-HT, to raise the brain histamine. They may, however, have acted in other ways (Pletscher, Gey & Burkhard, 1966).

Owing to the small number of experiments with the remaining drugs, it is not possible to draw definite conclusions from the results. Bulbocapnine has been reported to raise the brain histamine in the rat (Walaszek & Chapman, 1963) and it has been suggested on indirect evidence (Chapman & Walaszek, 1962) that it acts by inhibiting diamine oxidase in the brain. There is, however, no clear evidence for the presence of this enzyme in mammalian brain (Burkard, Gey & Pletscher, 1963). Pentobarbitone has been reported to raise 5-HT in rat brain (Bonnycastle, Bonnycastle & Anderson, 1962). In the present experiments, and with a smaller dose, the effect of pentobarbitone on brain histamine was confined to the preoptic region. In the cat, morphine is known to release histamine from the skin (Feldberg & Paton, 1951), and noradrenaline from the hypothalamus (Vogt, 1954). There was, however, no detectable change in the concentration of histamine in the hypothalamus or in the hypophysis after morphine.

The present results provide pharmacological evidence that the storage of histamine in brain differs from that in the hypophysis. It has been shown that drugs which are known to raise or lower the concentration of monoamines in the brain, also altered the concentration of histamine in the same direction. The results do not give information on how these drugs acted. Nevertheless, they strengthen the view that, in the brain, histamine and the monoamines are closely related in their storage and metabolism.

SUMMARY

1. Histamine extractable from the brain and hypophysis was estimated by a method which depended on purification of the amine by ion-exchange chromatography and on biological assay. It was shown by paper chromatography that the activity contained in the solutions for assay was probably due only to histamine and not to a mixture with β-N-methyl and/or β-N-dimethylhistamine.

2. A detailed map is given of the distribution of histamine in the cat's brain and hypophysis. The highest values were found in the hypophysis where the histamine derives partly from mast cells. About 90% of the mast cells were found in the hypophysial stalk and in the posterior lobe. Estimates for the different parts of the gland were as follows: anterior lobe, 2,400 ng/g; posterior lobe, 1,700 ng/g; hypophysial stalk, 5,200 ng/g (mean values).

3. In the brain, the highest values were obtained in the corpora mammillaria (1,150 ng/g) and in the ventral part of the hypothalamus (800 ng/g), where the histamine was not associated with mast cells. Thereafter the concentration fell progressively in the remainder of the brain stem and was lowest in the bulb ($<$100 ng). None was detected in the cerebellum or in the white matter.

4. The effect of drugs was studied in the hypophysis, the hypothalamus and thalamus. Compound 48/80, in doses which depleted the skin histamine, reduced the concentration

of histamine in the hypophysis but not in the brain. In the hypophysis, the effect was seen mainly in the stalk and posterior lobe.

5. Reserpine, in a single dose, lowered the concentration of histamine in the brain but not in the hypophysial stalk or posterior lobe. The effect was independent of dose in the range 0.5–10.0 mg/kg and maximal at 18 hr. Recovery of the histamine concentration in the hypothalamus began at 24 hr but was still incomplete at 120 hr.

6. Chlorpromazine and other phenothiazines, when given in large doses (3×50 mg/kg in 24 hr), increased the concentration of histamine in the hypothalamus and the brain; the concentration in the hypothalamus also rose after treatment with monoamine oxidase inhibitors. No change was seen after morphine. None of these drugs appeared to alter the concentration of histamine in the hypophysis.

7. The results are discussed with reference to storage and metabolism of histamine in brain.

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