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HISTAMINE AND METHYLHISTAMINE IN CAT BRAIN AND OTHER TISSUES

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In his pioneer work on histamine metabolism in mammals Schayer (1959) demonstrated two main pathways for histamine catabolism. One of these leads to the formation of 1,4-methylhistamine [1-methyl-4-(β-aminoethyl)imidazole, hereafter referred to as methylhistamine] and depends on the enzyme histamine *N*-methyl transferase. This enzyme is present in particularly large amounts in brain tissue (Brown, Tomchick & Axelrod, 1959; Axlerod, McLean, Albers & Weissbach, 1961). More recently the occurrence of methylhistamine in brain tissue has been reported (Fram & Green, 1963; Perry, Hansen, Foulks & Ling, 1965). This paper presents a method for the determination of methylhistamine in tissues, and observations on the distribution in cat brain of histamine and methylhistamine under normal conditions and under the influence of drugs.

METHODS

Cats of either sex weighing 2.5 to 4.5 kg were bled to death under brief ether anaesthesia. The brain was removed *in toto* and the various parts dissected and weighed as quickly as possible. "Hypothalamus" extended rostrally to a line joining the anterior commissure and the optic chiasma, and dorsally to the ventral border of the massa intermedia. It included the mammillary bodies and the median eminence. "Mesencephalon" included the corpora quadrigemina and the periaqueductal grey matter, but not the cerebral peduncles or the pons. "Cerebral cortex" denotes grey matter surrounding the cruciate sulcus.

Because of the low biological activity of methylhistamine, chemical assay methods had to be considered for the determination of methylhistamine in tissues. The procedure finally adopted was based on the finding that 2,4-dinitrofluorobenzene can be used for the chemical determination of histamine (McIntyre, White & Sproull, 1950; Lowry, Graham, Harris, Priebat, Marks & Bregman, 1954; Weissbach, Waalkes & Udenfriend, 1958). During the progress of the present investigation, measurements of methylhistamine in human urine, using 2,4-dinitrofluorobenzene, have been published (Green, Fram & Kase, 1964; Fram & Green, 1965).

The essential steps in the measurements of histamine and methylhistamine were: (1) homogenization of the tissue and preparation of a perchloric acid extract; (2) absorption of histamine and methylhistamine on a cation-exchange resin, followed by elution; (3) reaction with 2,4-dinitrofluorobenzene; (4) extraction of dinitrophenylhistamine and dinitrophenylmethylhistamine from reaction mixture; (5) isolation of the dinitrophenyl derivatives by thin-layer chromatography; and (6) spectrophotometric measurements of eluates from chromatogram.

The tissue was homogenized in at least four volumes of 0.1 N-hydrochloric acid in a glass homogenizer. The homogenate was transferred to a centrifuge tube. Perchloric acid was added to give a concentration of 0.5 N. The tube was left for 30 min, after which it was centrifuged at 2,500 *g* for 10 min. The sediment was washed with 1 ml. of 0.5 N-perchloric acid. The combined supernatant fluid and washing were neutralized to *p*H 6.0 to 6.5 (Universal Indicator Solution,

Merck) with solid potassium carbonate. The resulting potassium perchlorate precipitate was spun down (2,500 *g* for 5 min). The sediment was washed with 1 ml. of cold water. The supernatant fluid and washing were combined.

The cation-exchange resin used for purification of the extract was Dowex-50 × 4, 100 to 200 mesh, buffered to *p*H 6.0 as described by Kahlson, Rosengren & Thunberg (1963). A column containing 350 mg (wet weight) was prepared in a glass tube with an inner diameter of 4 mm. After washing the column with 0.1 M-sodium phosphate buffer, *p*H 6.5, the extract was passed through at a rate of approximately 0.4 ml./min. The column was then washed with 2 ml. of water, followed by 3 ml. of 1 N-hydrochloric acid, the effluent fluids being discarded. Histamine and methylhistamine were eluted with 2.5 ml. of 4 N-hydrochloric acid. The eluate was evaporated to dryness under reduced pressure in a water-bath at 45° C. At this stage the procedure was sometimes interrupted, the residue being kept dry in the cold overnight. The residue was then dissolved in 0.5 ml. of water and incubated at 60° C with 1 mg of 2,4-dinitrofluorobenzene, dissolved in 0.1 ml. of ethanol, and 1.0 ml. of 0.1 M-sodium carbonate buffer, pH 9.6, containing 1 mg/ml. of edetic acid. After 15 min the mixture was cooled and shaken with 2.5 ml. of octanone (methyl-*n*-hexylketone). After centrifugation at 2,500 *g* for 2 min 2 ml. of the top (octanone) layer was removed and shaken with 2.5 ml. of 2 N-hydrochloric acid. After centrifugation (2,500 *g* for 2 min) the top layer was sucked off. Of the remaining acid phase 2 ml. was transferred to another tube, and was evaporated to dryness under reduced pressure in a water-bath at 45° C.

Ascending thin-layer chromatography was performed on silica gel (Silica Gel H; according to Stahl, Merck Darmstadt). Before use the silica gel coated glass plate was washed with a solution of one part acetone and three parts 5 N-hydrochloric acid in order to remove light-absorbing material which would otherwise interfere with the photometric measurements. The evaporated sample was transferred to the chromatography plate by dissolving the residue in one drop of a 9:1 mixture of acetone and 0.2 N-hydrochloric acid. The tube was washed twice with the same mixture, the washings being added to the sample spot. The solvent, benzene:ethanol:concentrated ammonia solution (80:18:2), was allowed to reach a level 10 cm above that of the application of the sample, after which the plate was air dried. Dinitrophenylhistamine and dinitrophenylmethylhistamine, when present in sufficient amounts (corresponding to approximately 0.5 μg of the amines), appeared as yellow spots with R_F values of 0.36 and 0.50 respectively. Areas of 2 cm^2 were scraped off corresponding to dinitrophenylhistamine and dinitrophenylmethylhistamine, and the powder was shaken with 0.4 ml. of a 1:4 mixture of acetone and 0.2 N-hydrochloric acid. After centrifugation (2,500 *g* for 5 min) 0.2 ml. of the supernatant fluid was read in a Beckman DU spectrophotometer at 360 mμ. Readings were corrected for light-absorbing material originating from the silica gel by extracting in parallel areas of silica gel obtained from the levels of the respective dinitrophenyl derivatives.

Standards of 1 and 2 μg of histamine and methylhistamine, dissolved in 5 ml. of 0.5 N-perchloric acid, were routinely carried through the whole procedure, together with the tissue samples. "Internal standards," obtained by adding 1 and 2 μg of the amines to an aliquot of a hydrochloric acid homogenate of brain tissue, were compared to standards prepared as described above. The recoveries calculated from six such experiments varied between 52 and 70%. The adsorption-elution procedure on the resin column caused a loss of 10 to 15% of the amines. If, however, the precipitation of potassium perchlorate, as described above, was omitted, and instead sodium hydroxide was used for the neutralization of the extract, then much greater loss could occur. Reagent blanks, carried through the whole procedure, did not differ significantly from the silica gel blanks described above. The values presented in the tables were not corrected for recovery.

The identity of substances measured was confirmed by their chromatographic behaviour on silica gel in three different solvents. An extract of cat brain stem tissue was carried through the usual procedure to and including chromatography in benzene:ethanol:concentrated ammonia solution (80:18:2). The standard spots of authentic dinitrophenylhistamine and dinitrophenylmethylhistamine, and also the corresponding spots from the tissue extract, were eluted in the usual manner. The eluates were evaporated to dryness. The residues were then rechromatographed in butanol:acetic:water (60:15:25). In this system the R_F values for dinitrophenylhistamine and dinitrophenylmethylhistamine were 0.35 and 0.27, respectively. These values agreed with those for the dinitrophenyl

derivatives from the tissue extract. Again, the spots were eluted and chromatographed in phenol:butanol:1 N-hydrochloric acid (80:15:5). In this system the R_F values for dinitrophenylhistamine and dinitrophenylmethylhistamine were 0.68 and 0.85, respectively. The two spots from the tissue had the same R_F values.

Chromatograms of tissue extracts run in benzene:ethanol:concentrated ammonia solution (80:18:2) usually showed an immobile yellow spot at the point of application, and sometimes also a yellow spot with an R_F value between those of dinitrophenylhistamine and dinitrophenylmethylhistamine, and distinct from both. Apart from this, other material than the substances measured seldom appeared in the chromatograms. Untreated 2,4-dinitrofluorobenzene had an R_F value of approximately 0.10. It was only rarely present in visible amounts.

Histamine and methylhistamine were used as the dihydrochlorides, and the figures are given in terms of the bases. Doses of other drugs refer to the salts used. The drugs were injected intraperitoneally. Reserpine was used in the form of Serpasil solution for injection (Ciba). Bufotenine base (Calbiochem) was dissolved in 30% ethanol. Iproniazid (Marsilid base, Roche), chlorpromazine hydrochloride (Leo, Hälsingborg) and L-histidine hydrochloride (Merck) were dissolved in water. Chlorpromazine was injected twice daily, and the animals were killed 18 hr after the last injection. Iproniazid was injected once daily, and the animals were killed 2 hr after the last injection. Reserpine was given in a single dose 18 hr before death.

RESULTS

Attention was focused on some regions of the brain stem where histamine is concentrated, according to previous investigators (Harris, Jacobsohn & Kahlson, 1952; Adam, 1961; Adam & Hye, 1964). In preliminary experiments also some other tissues were examined (Table 1). The areas routinely examined were hypothalamus, caudate nucleus, thalamus and mesencephalon (Table 2). It can be seen that in most experiments both histamine and methylhistamine were demonstrated. The hypothalamus had a higher concentration of histamine than the other regions of the brain. In each cerebral tissue examined the mean concentrations of the two amines were approximately equal, except in the hypothalamus, which contained less methylhistamine than histamine. Of the extracranial tissues examined (Table 1) all except the kidney contained much less methylhistamine than histamine. In some instances the values obtained were so near the lower limit of the method that minor differences may have escaped detection. Values lower than 0.2 μg/g were usually not considered to be significantly different from zero. The present method for histamine was less sensitive than that used by Adam (1961) and

TABLE 1

CONCENTRATIONS OF HISTAMINE AND METHYLHISTAMINE IN SOME CAT TISSUES
Each value represents the mean of three experiments, except for the hypophysis which represents one determination on pooled tissue from six cats

Tissue	Concentration of Histamine (μg/g)	Concentration of Methylhistamine (μg/g)
Cerebral cortex	0·1	0·3
Cerebellar cortex	0·1	0·1
Hypophysis	1·8	2·2
Lung	12·8	0·7
Kidney	0·1	0·2
Liver	1·2	0·2
Spleen	2·0	0·5
Gastric mucosa	27·7	1·1

TABLE 2

CONCENTRATIONS (μG/G) OF HISTAMINE AND METHYLHISTAMINE IN DIFFERENT REGIONS OF THE BRAIN STEM IN THE CAT

Control values are means and standard errors

	Histamine and methylhistamine (μg/g) for expt. no.						
	1–8 Controls	9, 10 Iproniazid	11, 12 Reserpine	13, 14 Reserpine	15, 16, 17 Chlorpromazine	18 Chlorpromazine	19
Tissue		3×25 mg/kg	0·4 mg/kg	1·0 mg/kg	4×20 mg/kg	8×20 mg/kg	2×50 mg/kg
Histamine							
Hypothalamus	0·93±0·11	0·8, 1·1	0·0, 0·7	0·3, 0·5	1·1, 1·2, 1·3	1·3	1·1
Caudate nucleus	0·34±0·05	0·2, 0·3	0·0, 0·4	0·3, 0·2	0·2, 0·3, 0·3	0·5	0·4
Thalamus	0·34±0·11	0·2, 0·2	0·3, 0·4	0·7, 0·5	0·2, 0·3, 0·3	0·4	0·4
Mesencephalon	0·21±0·07	0·2, 0·1	0·1, 0·3	0·3, 0·1	0·1, —, 0·2	0·2	0·4
Methylhistamine							
Hypothalamus	0·56±0·12	2·8, 2·6	0·3, 1·7	0·1, 0·6	0·7, 0·2, 0·5	0·8	0·3
Caudate nucleus	0·40±0·05	1·7, 1·1	0·3, 0·6	0·2, 0·2	0·5, 0·2, 0·3	0·3	0·5
Thalamus	0·30±0·05	1·7, 1·0	0·1, 0·3	0·1, 0·1	0·1, 0·0, 0·2	0·2	0·3
Mesencephalon	0·27±0·09	0·7, 0·7	0·1, 0·5	0·1, 0·2	0·1, —, 0·2	0·2	0·1

Adam & Hye (1964). However, it offered the advantage of simultaneous determinations of histamine and methylhistamine in one tissue extract.

Treatment with iproniazid increased the concentration of methylhistamine (Table 2, experiments 9 and 10). The effect was maximal after 2 days' treatment, when the levels in the hypothalamus, thalamus and caudate nucleus were more than three times higher than those of the controls (Fig. 1). No further increase was found after 4 days. This action of iproniazid, a well-known inhibitor of monoamine oxidase, is probably due to inhibition of the oxidation of methylhistamine to methylimidazoleacetic acid (Rothschild & Schayer, 1958). Such inhibition by iproniazid in cat brain has been demonstrated by isotopic technique (White, 1960). In addition to the increase in methylhistamine, iproniazid sometimes, but not consistently, reduced the histamine content of the tissues (Fig. 1).

The effects of reserpine on hypothalamic methylhistamine were variable (Table 2, experiments 11 to 14). The histamine concentration on this region also varied after reserpine, but it was consistently lower than in the controls. A similar reduction was found by Adam & Hye (1964). In the other regions examined no significant deviations from the control values were observed.

The different doses of chlorpromazine (Table 2, experiments 15 to 19) had little or no effect on the amines studied, with the exception of hypothalamic histamine, which was moderately increased. The difference between the mean histamine concentrations in the hypothalamus of chlorpromazine-treated and control cats was statistically significant ($0.02<P<0.05$ by Student's t-test).

In a preliminary experiment bufotenine, reported to inhibit the methylation of histamine *in vivo* (Snyder & Axelrod, 1964), was injected in a dose of 10 mg/kg. The cat was killed 18 hr later. Essentially normal levels of histamine and methylhistamine were found in the hypothalamus, caudate nucleus, thalamus, and mesencephalon. Similar results were obtained in a cat injected with histidine, 200 mg/kg, at 2, 26 and 50 hr before death.

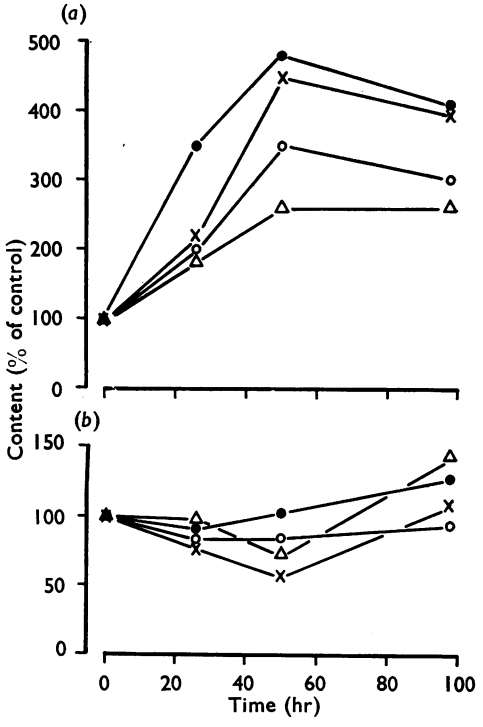

Fig. 1. Methylhistamine (*a*) and histamine (*b*) in cat brain 26, 50, and 98 hr after the beginning of treatment with iproniazid (25 mg/kg, daily). Ordinate: concentrations, as percentages of controls (Table 2). Each point represents the mean of two experiments. ●, Hypothalamus; ○, caudate nucleus; ×, thalamus; and △, mesencephalon.

DISCUSSION

The presence of histamine in brain tissue of various species has been demonstrated by several investigators (Kwiatkowski, 1943; Clouet, Gaitonde & Richter, 1957; Shore, Burkhalter & Cohn, 1959; Adam, 1961; Carlini & Green, 1963; Green & Erickson, 1964). There are, however, few reports on the distribution of histamine in different regions of the brain stem of the cat. Harris *et al.* (1952) found concentrations of less than 0.4 μg/g in the thalamus, and of less than 0.6 μg/g in the caudate nucleus. Much higher values were found in the median eminence. Adam & Hye (1964) found 430 to 1,150 ng/g in different parts of the hypothalamus, and 250 ng/g in the massa intermedia thalami. Similar values were found in the dog (Adam, 1961). The figures quoted were obtained by bioassay. Using a modification of the fluorimetric method of Shore *et al.* (1959), McGeer (1964) found histamine concentrations in cat hypothalamus, thalamus and caudate nucleus that were 30 to 50% higher than those of the present study; the figures for mesencephalon, although not strictly comparable, also seem to be somewhat higher.

Discrepancies between results from chemical and biological methods for brain

histamine can be great, as pointed out by Green (1964). On the other hand, the estimations on cat brain stem tissues by different workers are not grossly at variance, although the chemical methods both of McGeer (1964) and of the present experiments have yielded higher values than the bioassay methods in several instances.

The occurrence of methylhistamine in the brain is not surprising, since the brain is particularly rich in the enzyme which methylates histamine in the ring. The other main pathway for histamine catabolism in mammals is oxidation by histaminase (diamine oxidase) to form imidazoleacetic acid (Schayer, 1959). However, histaminase seems to be absent from mammalian brain tissue (Burkard, Gey & Pletscher, 1963), and significant amounts of radioactive imidazoleacetic acid are not formed from radioactive histamine in cat brain (White, 1960). These observations indicate that in the brain histamine is mainly or exclusively catabolized by the methylation pathway. Blocking of the oxidation of methylhistamine by iproniazid, as in the present experiments, leads to the accumulation of methylhistamine in the tissues. The rate of increase of methylhistamine can be taken to reflect the rate of its formation, and also the turnover rate of histamine. It then follows that there is a higher turnover rate for histamine in the hypothalamus than in the other regions examined. In keeping with this view are the findings that hypothalamic tissue forms histamine at a higher rate than do other parts of the brain (White, 1959), and that histamine *N*-methyl transferase is concentrated in the hypothalamus, lower enzyme activity being present in, among other sites, the caudate nucleus and thalamus (Axelrod *et al.*, 1961).

The finding of an increased histamine concentration in the hypothalamus after chlorpromazine (Table 2) agrees with the results of Adam & Hye (1964) in cat hypothalamus, and of Green & Erickson (1964) in rat whole brain. Chlorpromazine has been reported to inhibit the formation of methylhistamine *in vitro* (Brown *et al.*, 1959 ; Gustafsson & Forshell, 1963) and *in vivo* (White, 1961 ; Snyder & Axelrod, 1964). The effect of chlorpromazine on cerebral histamine concentration may be due to such inhibition (Green & Erickson, 1964). However, in the present experiments chlorpromazine did not lower the concentration of cerebral methylhistamine. It is obvious that further work is needed on the effect of chlorpromazine on formation and tissue content of methylhistamine.

In previous investigations on the metabolism of radioactive histamine more [^{14}C]-histamine than [^{14}C]-methylhistamine, or approximately equal amounts of the two, was found in the brain after administration of [^{14}C]-histidine. On the other hand, much more [^{14}C]-methylhistamine than [^{14}C]-histamine was found in the brain after administration of the latter (White, 1960 ; Jonson & White, 1964). In the present experiments on untreated cats, more histamine than methylhistamine was found in the hypothalamus, and in the other regions the mean values for the two amines did not differ much ; thus the pattern resembled that in the first-mentioned radioactive experiments, where [^{14}C]-histidine was administered. Therefore the present observations support the view that brain histamine is formed locally from histidine. A similar conclusion was reached by Adam, Hye & Waton (1964), who could not demonstrate uptake of histamine from the blood into the hypothalamus in cats. In rats, however, there is an indication that histamine can cross the blood-brain barrier, although the uptake into the brain is less than that into other tissues (Snyder, Axelrod & Bauer, 1964).

SUMMARY

1. A method for simultaneous measurements of histamine and methylhistamine in tissues is described. It is based on the formation of the dinitrophenyl derivatives of the amines.

2. The distribution of histamine and methylhistamine in cat brain, under normal conditions and under the influence of drugs, is described.

3. Iproniazid increased the concentration of methylhistamine in various parts of the brain stem. Reserpine decreased the concentration of histamine in the hypothalamus.

4. The origin and metabolism of brain histamine and methylhistamine are discussed.

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