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THE SUBCELLULAR DISTRIBUTION OF HISTAMINE IN BRAIN TISSUE

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Abstract—The subcellular distribution of histamine has been analysed in guinea pig whole brain, dog hypothalamus and dog pituitary gland. In whole brain, histamine is found in both the nuclear and crude mitochondrial fractions. In brain tissue lacking mast cells (hypothalamus) "bound" histamine precipitates with the 17,000 g crude mitochondrial fraction, whereas 94 per cent of the "bound" histamine of the pituitary precipitates with the mast cells in the low speed nuclear fraction. Electron microscopic examination of subfractionated mitochondrial preparations from hypothalamus do not reveal structures comparable to the histamine containing granules of mast cells and a different type of histamine storage granule is suggested.

IT IS known that much of the acetylcholine and other pharmacologically active amines of brain tissue are present in bound form which survives homogenization of the tissue in isotonic media. Subcellular fractionation of sucrose homogenates of brain has revealed that the bound acetylcholine (ACh)[1, 2] and 5-hydroxytryptamine (5-HT)[2, 3] are associated with a specific fraction distinct from mitochondria, nuclei, microsomes and other subcellular organelles, which has been identified as consisting largely of pinched-off nerve endings (NEPs).[4]

The object of the present work was to find out if another brain amine, histamine, is similarly localized. The pituitary has a higher histamine content than any part of the brain.[5] This is attributable to the high concentration of mast cells in this structure. However, the hypothalamus also has a relatively high histamine content, though Adam[6] was unable to demonstrate the presence of mast cells there. White[7, 8] found that brain tissue can form ^{14}C-histamine from ^{14}C-histidine and that histidine decarboxylase activity was highest in the hypothalamus. The cells which make and store histamine have still to be defined.

METHODS

Histamine analysis

Histamine from brain homogenates and from tissue fractions prepared therefrom by differential or density gradient centrifugation was extracted and estimated fluorometrically by the alkaline–butanol method of Shore *et al.*[9] on an Aminco–Bowman spectrofluorometer. Internal standards were prepared by carrying known amounts of

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histamine through the procedure. The fluorescence spectrum at 450 mμ resulting from activation at 350 mμ of a standard histamine solution was similar to those of the internal histamine standard carried through the procedure and the apparent histamine extracted from the various fractions.

Preparation of homogenates

Guinea pigs (350–500 g) were killed by decapitation and the brain rostral to the quadrigemina was removed and immediately placed in cold (0–4°) 0·32 M sucrose. Although the anterior and posterior lobes of the pituitary gland separate from the base of the brain and remain encased within the sella turcica great care was taken to remove the hypophyseal stalk since this region contains considerable numbers of mast cells.[6] After removal of blood and superficial blood vessels the cerebral hemispheres were opened outward in order to expose and remove blood trapped within the ventricles. The tissues were then blotted on filter paper and weighed.

The expected low levels of histamine (relative to other amines such as acetylcholine and 5-hydroxytryptamine) necessitated a modification of the procedure previously reported.[10] Instead of a 10% homogenate a 20% homogenate in 0·32 M sucrose was prepared using a glass and perspex homogeniser.[11] The time taken to homogenize the tissue was 1–2 min. After removal of a suitable aliquot for assay of the histamine content of the whole homogenate, 0·32 M sucrose was added to make the suspension 10% in terms of original tissue. Homogenization was then repeated.

Differential centrifugation

The first step in the fractionation was to separate the homogenate into a number of primary fractions by differential centrifugation.[2] Previous experience had shown that recentrifugation of precipitates washed with fresh 0·32 M sucrose results in poor packing of the particles. The following procedure was therefore adopted.

The first particulate fraction, consisting of nuclei, large myelin fragments, tissue debris and blood cells was obtained in a Servall bench centrifuge by centrifuging the homogenate for 11 min at 1000 × *g*, giving a pellet and supernatant S_1. The pellet was then washed by resuspension in a small volume of fresh 0·32 M sucrose and recentrifuged in the same way to yield a washed pellet and supernatant W_1. The washed pellet, resuspended in sucrose, is referred to as the P_1 fraction. The second particulate fraction, consisting of mitochondria, small myelin fragments, pinched-off nerve endings and some microsomes, was prepared from S_1 by centrifuging at 17,000 × *g* for 1 hr in a Servall Refrigerated Automatic centrifuge yielding a supernatant S_2 and a pellet. The latter was resuspended in W_1 and recentrifuged at 17,000 × *g* for 1 hr. The resulting supernatant W_2 was added to S_2. The washed pellet was resuspended in sucrose to give the P_2 fraction. The combined supernatants (S_2 and W_2) from P_2 were used to prepare the microsomal (P_3) fraction and the high speed soluble fraction (S_3) by centrifuging at 100,000 × *g* for 1 hr in a Spinco Model L preparative ultracentrifuge. The microsomes were washed with fresh 0·32 M sucrose and the washings discarded. A flow diagram is given in Fig. 1.

Subfractionation of P_2 *from dog hypothalamus*

The P_2 fraction (3 ml derived from about 1·5 g tissue) was layered on top of a discontinuous density gradient prepared 1–2 hr before use and consisting of 2 ml 0·8 M

sucrose layered over an equal volume of 1·2 M sucrose. The tube was then centrifuged at 100,000 × *g* for 1 hr in the SW 39 swing bucket head of the Spinco Model L preparative ultracentrifuge. Separation took place into three subfractions as previously observed with whole guinea-pig brain,[4] *A*, consisting of particles lighter than 0·8 M sucrose; *B*, particles floating between 0·8 M and 1·2 M sucrose and *C*, particles denser than 1·2 M sucrose.

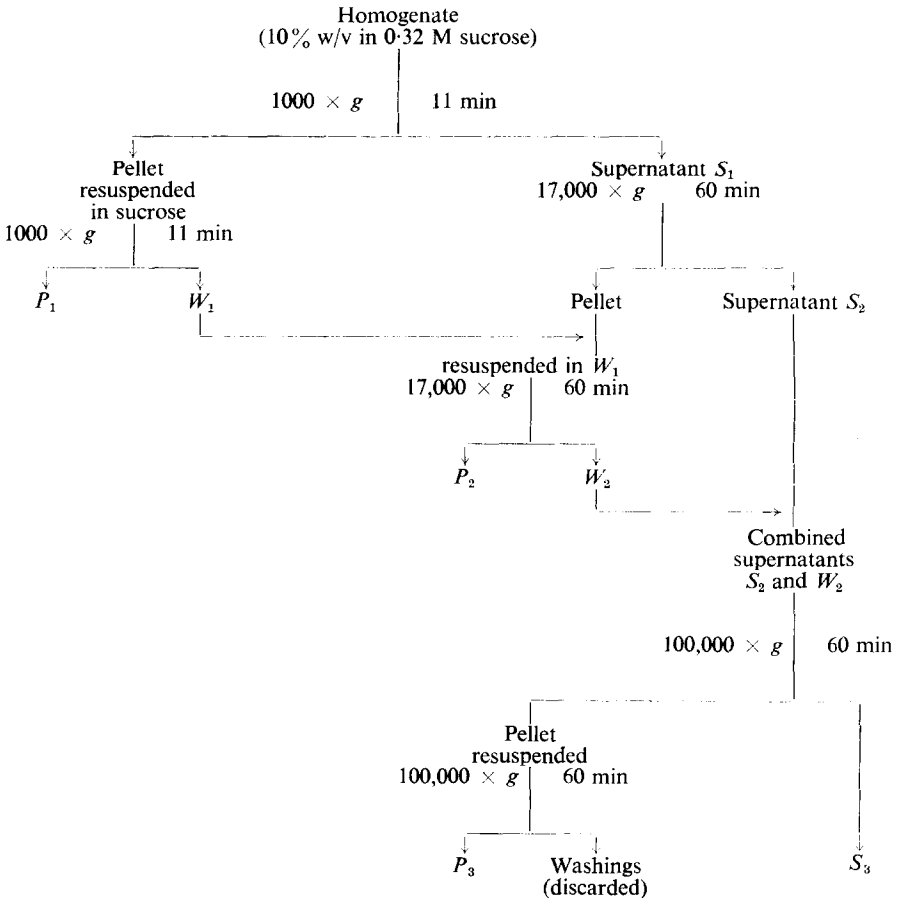

FIG. 1. Scheme showing preparation of fractions.

Rat peritoneal mast cells

Intact rat peritoneal mast cells were obtained by injecting 12 ml warmed 0·32 M sucrose into the peritoneal cavity of rats (250–400 g). The intestines were gently massaged for 1–2 min in order to dislodge mesenteric mast cells. The rats were then exsanguinated by decapitation, the abdomen was opened and about 10 ml fluid withdrawn. The mast cell suspension was used for either tagging homogenates of brain tissue or studying the density gradient sedimentation characteristics of homogenized mast cells.

Free and bound histamine

In order to obtain an estimate of the proportions of total brain histamine in the "free" and particle-bound forms, homgenates were immediately centrifuged at 100,000 $\times$ *g* for 1 hr in the No. 40 head of the Spinco Model L preparative ultracentrifuge to give total particular (*P*) and high-speed supernatant (*S*) fractions. The latter is taken to represent soluble constituents of the cytoplasm, including free histamine, diluted with sucrose.

Expression of results

The histamine concentration of the tissue fractions is expressed as mμg of base/g fresh tissue from which they are derived. The distribution of the histamine in the various fractions is also expressed as the percentage of the total recovered histamine found in the various fractions. Recoveries are expressed as the percentage of the activity in the original homogenate recovered in the various fractions. The pooled results of a number of similar experiments were expressed as the mean value and the standard deviations from the mean; the number of experiments is given in parentheses.

Electron microscopy

This was carried out by Dr. V. P. Whittaker using the negative staining method as described by Horne and Whittaker.[12] Preparations were fixed at 0° by the addition of equal volumes of 10% w/v formaldehyde in 0·32 M sucrose, previously neutralized to pH 7·2 with M NaOH. The mixture was diluted with 6 times its volume of ice-cold 1% w/v aqueous phosphotungstic acid, previously neutralized to pH 7·2 with 2 N NaOH, then transferred to grids with a micropipette. On removing most of the droplet from the grid with filter paper, a thin film of suspension remained which dried rapidly leaving particles embedded in solid sodium phosphotungstate. In this method particles are seen as a whole and not in thin section.

RESULTS

Whole guinea-pig brain

The initial experiments were done with whole guinea-pig brain (Fig. 2A). The first step was to determine what proportion of histamine remained particle bound after homogenization. Work with 5-hydroxytryptamine[3] has shown that this amine, in contrast to acetylcholine, leaks out during subcellular fractionation. To obtain a true value for the ratio of particle bound to supernatant (free) amine it was necessary to determine the ratio of bound to free amine in separate experiments in which the homogenate was centrifuged immediately after preparation at a high speed (100,000 $\times$ *g* for 1 hr).

As shown in the first block (*S*/*P*) of Fig. 2A, 25 $\pm$ 6 per cent of the total histamine of the guinea-pig brain homogenate was found to be in the free form. This is a similar proportion as was found with 5-hydroxytryptamine.[3] On fractionation 36 $\pm$ 11 per cent of the total recovered histamine was found in the S_3 fraction: this suggests that histamine is more tightly bound on average than 5-hydroxytryptamine where the corresponding value was 54 $\pm$ 12 per cent.[3]

The subcellular distribution in the three primary particulate fractions of whole guinea-pig forebrain is also shown in Fig. 2A. In marked contrast to previous findings

with acetylcholine[2] and hydroxytryptamine,[2, 3] particulate histamine was found to be almost equally distributed between the nuclear (P_1) and crude mitochondrial (P_2) fractions. This suggested that bound histamine is bimodally distributed in brain, one fraction being associated with a large and/or dense type of particle sedimenting with nuclei, the larger myelin fragments, tissue debris and blood cells in P_1; the other with a lighter and/or smaller particle sedimenting with the smaller myelin fragments, pinched-off nerve endings and mitochondria[4] in P_2.

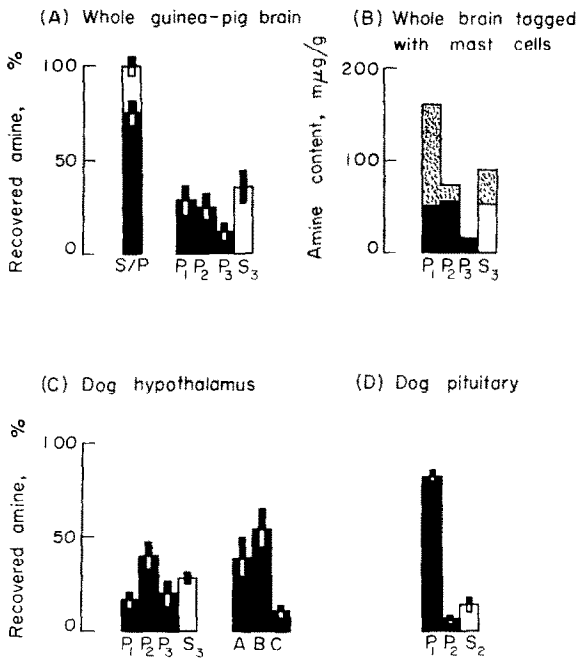

FIG. 2. Distribution of histamine in subcellular fractions of brain tissue. For units and nomenclature of fractions see text. Black blocks relate to mean values of particle-bound brain histamine, open blocks to "free" brain histamine and stippled blocks to histamine added to homogenates in the form of rat peritoneal mast cells. The small bars represent standard deviations. Total histamine content of tissue homogenates (mμg/g): (A) 245 ±10 (18); (B) untagged homogenate, 219; tagged homogenate, 732; (C) 1480 ± 20 (3); (D) 8450 (3 organs pooled). Recoveries (%): (A) (*S/P*) 117 ± 13 (4); P_1 to S_3, 110 ± 25 (7); (B) untagged fractions, 82; tagged fractions, 50; (C) P_1 to S_3 97 ± 17 (3); *A* to *C* 91 ± 23 (3); (D) 95.

Although the mast cell rich pituitary had been removed before homogenization (see Methods), it was felt that the homogenate might well contain mast cells from other parts of the brain (e.g. meninges and blood vessels of the choroid plexus).[13] From the work of Hagen *et al.*,[14] it would be anticipated that mast cells and any mast cell granules derived from them would sediment in the P_1 fraction. This was confirmed in experiments with rat peritoneal mast cells. It was therefore considered that the P_1 fraction of bound histamine might represent mast cell histamine and the P_2 histamine non-mast cell histamine.

This hypothesis was tested in two ways. (1) Mast cells were added to whole brain

homogenates and the homogenates tagged in this way were fractionated to determine which fraction was enriched by the additional histamine added with the mast cells. (2) The subcellular distribution of histamine was compared in two regions of dog brain: the pituitary, known to be rich in mast cells, and hypothalamus, said to be lacking in mast cells.[6]

Effect of tagging brain homogenates with mast cells

The distribution of histamine in the primary fractions prepared from a guinea-pig whole brain homogenate to which mast cells were added is given in Fig. 2B. It will be seen that almost all the bound histamine added in the from of mast cells is recovered in the P_1 fraction.

Subcellular distribution of histamine in dog pituitary and hypothalamus

The distribution of histamine in the primary fractions prepared from homogenates of dog pituitary and hypothalamus is given in the bottom half of Fig. 2. It will be seen that there is a striking similarity between the subcellular distribution of histamine in a tissue rich in mast cells (viz. pituitary, Fig. 2D) and the distribution obtained with rat peritoneal mast cells as shown in the stippled portions of Fig. 2B. By contrast, with a tissue devoid of mast cells (viz. hypothalamus, Fig. 2C) the general distribution pattern is similar to that found for other amines in whole brain, the fraction with the highest histamine content being the P_2 fraction. It was therefore decided to submit this fraction to further fractionation in a density gradient to see whether the resemblance to the distribution of other amines extended to the subfractions of P_2.

Density gradient separation of P_2 *from dog hypothalamus*

The first density gradient fraction (*A*), accounting for 37 per cent of the recovered histamine, consisted of particles less dense than 0·8 M sucrose; the second (*B*), containing 53 per cent of recovered histamine, consisted of particles intermediate in density between 0·8 M and 1·2 M sucrose, and the third (*C*) of particles denser than 1·2 M sucrose. It can be seen that, although the *B* fraction had the highest activity, the subfractionation of the P_2 preparation by a method which gave, morphologically, relatively homogeneous subfractions with whole guinea-pig brain,[4] failed to concentrate the amine as sharply into one particular subfraction as was the case with acetylcholine[2] or 5-hydroxytryptamine.[3]

Electron microscopic finding

Samples for electron microscopic examination (see Methods) were removed from each subfraction before chemical analysis. The electron microscopic examination of the P_2 subfraction showed that the fractions were not as homogeneous as the corresponding fractions from guinea-pig brain. Pinched-off nerve endings were identified in large numbers in fraction *B*, but there were also appreciable numbers in *A*. There were, in addition, unidentified dense membrane bound particles with no visible internal structure and many membrane fragments of greatly varying size in both fractions. In the *C* fraction, most particles present appeared to be mitochondria, but there were some which could not be identified as such owing to the absence of cristae. It would appear therefore that the inability to concentrate the amine into one particular band

as with earlier studies on guinea pig whole brain[2, 3] is related to some particulate storage sites less dense than 0·8 M sucrose.

DISCUSSION

The uneven distribution of pharmacologically active substance in the brain strongly suggests that the agent has a role to play in the specialized function of those regions where the concentration is high.[15] A physiological role for histamine in the brain can therefore be suggested[6] and is further indicated by the similarly uneven distribution of the enzymatic capacity to form histamine.[7, 8] Erspamer[16] suggests that histamine should be reconsidered and re-evaluated as a possible chemical mediator in the transmission of nervous impulses within and outside the central nervous system. However, it is not even known to what tissue, vascular, neural or other, the occurrence and functions of the brain histamine is primarily related.[17] The subcellular fractionation of sucrose homogenate of guinea pig brain by differential and density gradient centrifugation has revealed that bound acetylcholine[1, 2] and 5-hydroxytryptamine[2, 3] are associated with a specific fraction consisting largely of pinched-off nerve endings.[4] The present study analyzes the subcellular distribution of brain histamine in order to ascertain whether histamine is similarly localized.

It was found that particulate histamine in subfractions from guinea-pig brain had a bimodal distribution and that this was due to the presence of at least two distinct structures containing histamine with different sedimentation characteristics. One of these, to be identified with mast cells and/or granules from disrupted mast cells, sediments in the low speed, nuclear (P_1) fraction; the other sediments in the "crude mitochondrial" (P_2) fraction and probably represents non-mast cell histamine.

The subfractionation of the P_2 preparation from dog hypothalammus by a method which gave, morphologically, relatively homogeneous subfractions with whole guinea-pig brain failed to concentrate the amine so sharply into one particular subfraction as was found for acetylcholine[2] and 5-hydroxytryptamine[2, 3] in whole guinea-pig brain. Electron microscopic examination of these subfractions revealed that the (A) band, containing 37 per cent of the recovered amine contained an appreciable number of pinched-off nerve endings, whereas the (B) fraction, accounting for 53 per cent of the recovered histamine, contained large numbers of pinched-off nerve endings. The area lowest in amine was mostly free from pinched-off nerve endings. It is possible that the histamine is located in the pinched-off nerve endings, the considerable number of these in the A fraction accounting for the relatively large amount of the recovered histamine found there. However, in view of the numbers of unidentified particles in both fractions, a location of histamine in some other type of storage particle cannot be excluded. The not negligible amount of histamine in the P_3 fraction also merits further investigation. Definitive characterization of the histamine storage particles in the C.N.S. solely by the methods used in the present investigation is complicated by the fact that the morphology of the intact hypothalamus remains largely unexplored by the electron microscope. Until such parallel studies are completed, it would be premature to ascribe a physiological role to non-mast cell histamine in the central nervous system. The nature of the non-mast cell histamine in brain tissue thus remains to be defined.

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