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THE SUBCELLULAR LOCALIZATION OF HISTAMINE IN GUINEA PIG BRAIN—A RE-EVALUATION*†

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Abstract—We have repeated our studies, ‡ with the *o*-phthalaldehyde (OPT) fluorescence method, § of the subcellular distribution of histamine in guinea pig midbrain, this time purifying the extracts with cation-exchange resin chromatography. Our previous values included spermidine along with histamine. Guinea pig midbrain contains 0·55 nanomole histamine and 300 nanomoles spermidine. Histamine is bound to both a low-speed nuclear precipitate and an intermediate-speed mitochondrial precipitate. Forty-seven per cent of the spermidine is in the nuclear fraction. Attempts to relate histamine to subfractions of the mitochondrial fraction separated by density gradient centrifugation have been unsuccessful.

WITH the *o*-phthalaldehyde (OPT) method,[1] Michaelson and Dowe[2] reported that histamine in subcellular fractions from guinea pig midbrain has a bimodal distribution. They attributed this to the presence of at least two distinct histamine-containing structures with different sedimentation characteristics, namely: (1) mast cells and granules from disrupted mast cells, which sediment with the low-speed nuclear fraction; and (2) other structures that sediment in the "crude mitochondrial" fraction, representing non-mast cell histamine. Carlini and Green[3] reported a different subcellular distribution; they found histamine in the microsomal fraction.

When they used the fluorometric method,[1] Carlini and Green[3] found that guinea pig whole brain contains 2·2 nanomole histamine/g, but when they used bioassay they found only one-quarter this amount. They attributed the higher values for histamine obtained by the fluorometric method as compared with bioassay, in part, to substances other than histamine that become fluorescent after reaction with OPT.

The major interfering substance is spermidine.[4,5] This can be separated from histamine by use of the phosphorylated cellulose weak cation-exchange resin, Cellex-P. We have reinvestigated our earlier findings[2] on brain histamine now by using the ion-exchange purification of tissue extracts.[4,5]

MATERIAL AND METHODS

Male guinea pigs (400–500 g) were obtained from a local supplier. All chemicals were reagent grade; solvents such as *n*-butanol, *n*-heptane, and absolute methanol

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‡ I. A. Michaelson and G. D. Dowe, *Biochem. Pharmac.* **12**, 949 (1963).

§ P. A. Shore, A. Burkhalter and V. H. Cohn, *J. Pharmac. exp. Ther.* **127**, 182 (1959).

were redistilled before use. *o*-Phthalaldehyde (California Biochemical, Los Angeles) was recrystallized before use, and 1% solutions of OPT were stored in the cold. Spermidine was obtained from Nutritional Biochemical (Cleveland, Ohio) and Cellex-P from BioRad Laboratories (Richmond, Calif.). Standard solutions of histamine and spermidine were stored frozen.

All fluorometric assays were performed with the Aminco-Bowman spectrophotofluorometer (American Instrument Co., Silver Springs, Md.). Characteristic excitation and emission spectra were obtained with the aid of an X-Y recorder (American Instrument Co., Silver Springs, Md.).

Preparation of homogenates and extraction. Guinea pigs were killed by cervical dislocation, and the midbrain was excised as described in our earlier paper.[2] Tissue from 4–6 guinea pigs was pooled (1·75–2·5 g). Homogenates of midbrain (10% w/v) and subfractions therefrom, separated by differential and sucrose density gradient centrifugation, were prepared as before, with the following modification: each fraction was adjusted to 0·4 N with respect to perchloric acid and allowed to stand in ice chips for 10 min before additional centrifugation to remove precipitated proteins. The supernatant was subjected to one of the following procedures: (I) *n*-butanol extraction followed by 0·1 HCl extraction, as described by Shore *et al.*,[1] and adjustment of the 0·1 N HCl extract to pH 6·1 as measured with a glass electrode; or (II) neutralization with solid K_2CO_3 and adjustment to pH 6·1. The final neutralized extract from either procedure was diluted with an equal volume of 0·01 M phosphate buffer at pH 6·1, and the whole was transferred to a cellulose phosphate resin column (80 mm × 6 mm) for further purification.

Ion-exchange purification. Cellex-P was purified by the procedure of Kremzner *et al.*[5–7] The buffered extract was allowed to pass into the column followed by eluants of 10 ml each of water, 0·05 N HCl (for histamine), and 0·1 N HCl (for spermidine). One-ml portions of effluent were collected. For the fluorometric assay, each 1-ml portion was made strongly alkaline by the addition of 0·4 ml 1 N NaOH, and then 50 μl OPT was added. After 3 min the fluorophore was stabilized and intensified by addition of 0·2 ml 3·5 M H_3PO_4. The fluorescence at 450 mμ (for histamine) and that at 400 mμ (for spermidine) were measured in the spectrophotofluorometer with an excitation wavelength of 350 mμ (uncorrected) for both compounds.

Standards of histamine (1–2 nanomoles) and spermidine (200–300 nanomoles) were carried through the same procedure as tissue extracted with 0·4 N perchloric acid; with procedure II, recovery from the resin was 87 per cent. Quantification was established by summation of the total fluorescence under the elution curve, after correction for baseline fluorescent readings relative to standard curves plotted in each experiment and for recovery figures cited.

RESULTS

As previously reported,[4,5] an extract of brain tissue prepared as described by Shore *et al.*,[2] when allowed to react with OPT, yields a fluorophore different from that of histamine. By means of ion-exchange purification (procedure I or II), one can separate histamine from a second OPT-reactive substance, spermidine[4–6]. One can circumvent *n*-butanol extraction (procedure II) and still separately measure histamine and spermidine (Fig. 1). Histidine coupled with OPT gives a fluorophore like that of

histamine with 1/30 of its fluorescence intensity. Histidine is not adsorbed onto the resin at pH 6·1, and it is completely washed out by the first water wash of the column. Procedure II was adopted for general use in this study.

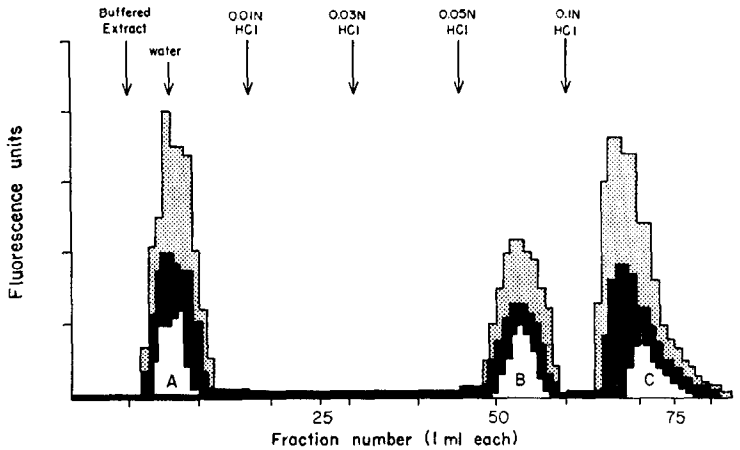

FIG. 1. Composite resinogram showing elution pattern of: (A) histidine, 30 nanomole; (B) histamine, 1 nanomole; and (C) spermidine, 30 nanomoles (white area), compared to perchloric acid extract (procedure II) of 2 g guinea pig midbrain (black areas) and to the same amount of brain plus (A), (B), and (C) (shaded areas).

Total histamine concentration

When whole midbrain was homogenized in 9 vol. of 0·32 M sucrose (+4°), adjusted to 0·4 N perchloric acid, and extracted into *n*-butanol as described by Shore *et al.*[1] without further purification, the total OPT-reactive material in tissue homogenates expressed as histamine was 2·2 nanomoles/g. This is similar to the value attributed to histamine in our earlier report.[2] On separating histamine from spermidine by procedure I or II, total histamine content of midbrain after purification was 0·55 nanomole/g, in agreement with the values found by other workers using similar methods[5,6] and bioassay.[3] In a series of experiments, the fluorescence at 450 mμ (histamine) of each fraction of effluent prepared by procedure I was determined when activated at 350 mμ (Fig. 2). The sum of the total fluorescence of the two components was 615 units, of which 25 per cent corresponded to histamine and the remainder to spermidine. This finding confirms Carlini and Green's[8] suggestion that estimates by Michaelson and Dowe[2] were four times too high.

Subcellular distribution

Bound and free histamine. In order to determine what proportion of histamine remained bound to particles after homogenization, the homogenate was immediately centrifuged at 100,000 *g* for 1 hr with the Spinco preparative ultracentrifuge. This estimate is complicated by the variable numbers of cells that escape disruption during the brief period of homogenization. After chromatographic separation, histamine was found solely in the particulate fraction (bound), whereas 88 per cent of the spermidine was in this fraction and the remainder was in the high-speed supernatant fraction (free).

Primary fractions. The homogenate was further separated into primary fractions by differential centrifugation.[2] Each fraction—nuclear (P_1), crude mitochondrial (P_2), microsomal (P_3), and high-speed supernatant (S_3)—was subjected to procedure II. As indicated in Fig. 3, histamine is mainly in the crude mitochondrial fraction, P_2 (60 per cent), and the low-speed nuclear fraction, P_1 (40 per cent), whereas spermidine is in all fractions (P_1 = 47 per cent, P_2 = 31 per cent, P_3 = 10 per cent, and S_3 = 12 per cent).

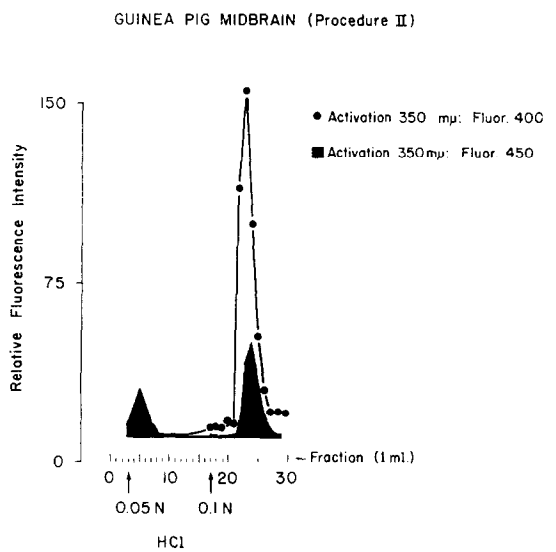

FIG. 2. Cellex-P purification of 2 g guinea pig midbrain, (procedure I, Methods). Each fraction of effluent (1 ml) was allowed to react with OPT, and fluorescence at 450 mμ (black areas) and 400 mμ (white area) was determined when activated at 350 mμ. Histamine is eluted by 0·05 N HCl and spermidine (white area) by 0·1 N HCl. The black area eluted by 0·1 N HCl is spermidine read at 450 instead of 400 mμ.

Subfractions of the crude mitochondrial fraction. The crude mitochondrial fraction (P_2) was fractionated on a discontinuous sucrose density gradient as described earlier.[2] At the end of centrifugation the migration from the top of the tube into the gradient resulted in a number of bands (Fig. 3). There was a pellet at the bottom more dense than 1·2 M sucrose, two bands at the interphase between 0·8 and 1·2 M sucrose, and a band at the interphase between 0·32 and 0·8 M sucrose. Six subfractions were subjected to procedure II and each was assayed for histamine and spermidine. Histamine could not be detected in any of the subfractions, whereas spermidine was mainly in subfraction 3, the region of the highest incidence of intact synaptosomes.[9, 10]

DISCUSSION

On separating histamine from other OPT-reactive substances, we have found the concentrations of histamine and spermidine in guinea pig midbrain to be 0·55 and 300 nanomoles/g respectively. The histamine concentration in guinea pig brain reported earlier[2] is in error, and the present findings are in agreement with those of Carlini and Green[3] and of Kremzner.[5] The spermidine concentration agrees with that found by other workers. [5, 6, 11] The fluorescence per mole of histamine is 30 times that of

spermidine. Since the concentrations of histamine and spermidine in the midbrain are 1:600, the fluorescence due to spermidine is 20 times that contributed by histamine

In spite of exhaustive purification, the subcellular distribution of histamine is like what we previously found[2] and unlike that found by Carlini and Green.[3] Histamine is recovered in the nuclear and mitochondrial fractions. A part of the histamine in the

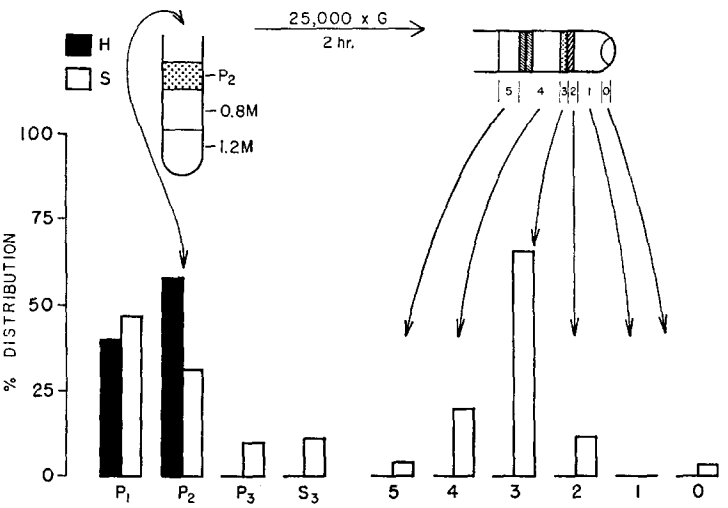

FIG. 3. Subcellular distribution of histamine (H, black columns) and spermidine (S, white columns) as per cent of total content of midbrain homogenate (100 per cent = 0·55 nanomole histamine and 300 nanomoles spermidine/g). Primary fractions at left. Subfractions of P_2 at right. See text for nomenclature of these subcellular fractions.

nuclear fraction may reside in unbroken cells. The histamine in the mitochondrial fraction could arise from granules from disrupted mast cells. Since peritoneal mast cells added to guinea pigs brain enrich the nuclear fraction but not the mitochondrial fraction, however, we suggested that histamine in the mitochondrial precipitate represents non-mast cell histamine.[2] It is also possible that peritoneal mast cells are less easily disrupted by our procedures than the mast cells in the central nervous system.

The abundance of spermidine in the nuclear precipitate (Fig. 3) is consistent with the association of spermidine with nucleic acids.[12] Since the polyamine would be expected to be strongly protonated at the pH of the disrupting medium, its association with the other primary fractions could reflect redistribution during the procedure. On density gradient separation of crude mitochondrial (P_2) fractions from guinea pig midbrain, 65 per cent of the spermidine was associated with the fraction equivalent in density to 1·0 M sucrose at the interphase between 0·8 and 1·2 M sucrose. This is rich in pinched-off nerve endings.[9, 10]

Although as much as 60 per cent of the total tissue histamine is in the crude mitochondrial fraction, we could not find measurable histamine in any of its subfractions. Since whole midbrain contains about 0·55 nanomole histamine/g, the pooling of 4–6 midbrains (1·75–2·5 g tissue) provides 1·0–1·5 nanomoles. The 60 per cent of the histamine in the mitochondrial fraction might be 0·6–0·9 nanomole placed

on the gradient before centrifugation. Dilution in the gradient, extraction into 0·4 N perchloric acid, and the inherent 15 per cent loss of procedure II leave 0·085–0·153 nanomole histamine/ml. This is divided among three to five 1·0-ml fractions during ion-exchange chromatography. The analytical procedure is such that 0·1 nanomole of histamine gives 2·5 times more fluorescence than reagent blank. It is therefore evident that the small concentration of histamine present in guinea pig midbrain renders impractical a study of its subcellular distribution by our method.

The present finding of a biomodal distribution of histamine in guinea pig brain confirms our earlier finding.[2] In addition, the finding of a differential drug effect on hypothalamic histamine relative to that in the pituitary[13] and the uptake of radiolabeled histamine into synaptosomal fractions[17] encourages the continued investigation of the subcellular localization of histamine in regions of the central nervous system that do not contain mast cells. In brains from larger animals one could separate specific brain areas from those areas rich in mast cells such as the pituitary and infundibulum. Adam[15] found that the mean concentration of histamine in dog hypothalamus is about 6 nanomoles/g. This is 12 times the concentration in guinea pig midbrain.

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