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Relative redistribution of [^{3}H]histamine and [^{14}C]spermidine in homogenates of dog brain*†

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MICHAELSON and Dowe[1] employed *n*-butanol extraction of alkalinized acid extracts of tissue for the *o*-phthaldialdehyde (OPT)[2] fluorometric analysis of brain histamine. Their study suggested that histamine is associated with synaptosomes. Organic solvent extracts[2] contain histamine as well as spermidine, which reacts with OPT to produce a fluorophore with spectral characteristics very nearly like those of histamine.[3,4] A reinvestigation of our earliest findings[1] and chromatographic separation of histamine from spermidine[3,4] in the crude mitochondrial fraction demonstrated that 65 per cent of the recovered spermidine is associated with synaptosomes.[5] Other workers[6] have pointed out that the amount of polyamine found in subcellular fractions does not necessarily reflect the distribution within the intact cell because of the high affinity of these basic substances for cellular polyanions. Redistribution of polyamines in a variety of animal tissues after homogenization has been reviewed by Tabor and Tabor.[7] This communication examines the question of secondary redistribution of histamine and spermidine after mechanical disruption of dog hypothalamus tissue. We have tested this possibility by studying the distribution of radioactive histamine and spermidine added: (1) to the homogenized hypothalamus of the dog before it is fractionated by differential centrifugation, and (2) to the "crude mitochondrial" fraction from the hypothalamus before it is further fractionated on a discontinuous sucrose density gradient.

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[^{14}C]spermidine trihydrochloride [(Aminopropyl)-tetramethylene-1,4-[^{14}C]diamine.3 HCl], specific activity, 10·7 mc/m-mole, and [^{3}H]histamine hydrochloride (generally labeled), specific activity, 10·5 c/m-mole, were obtained from New England Nuclear Corp., Boston, Mass. Determination of the distribution of radiolabel was accomplished by scintillation counting of pooled 25-drop samples from the sucrose gradient suspended in a solution consisting of *p*-dioxane–naphthalene–toluene–butyl-hydroxy toluene–dimethyl-POPOP‡–CAB-O-SIL.[8] Counting efficiency was approximately 70 per cent for [^{14}C]spermidine and 25 per cent for [^{3}H]histamine. All samples were counted for 10 min, or to a limit of 2×10^{-5} counts, in a Packard Tricarb liquid scintillation spectrometer, model No. 3375 or 3320, with automatic external standardization. No attempt was made to correct for quenching.

Preparation of tissue homogenates. Mongrel dogs anesthetized with pentobarbital-sodium (30 mg/kg, i.v.) were bled from a carotid artery. The brain was removed and the hypothalamus was rapidly excised and weighed. The tissue was then homogenized in ice-cold 0·32 M sucrose (0·005 M phosphate buffer, pH 7·1) with Potter–Elvehjem glass and Teflon tissue grinder (Kontes Glass Company, K-886000) with a clearance between pestle and tube of 0·01 in. and rotating at a constant speed of 800 rev/min. The time taken to homogenize the tissue was 1 min or 20 up and down passes. All operations were at 4°.

Separation of fractions. Separation of homogenate into primary fractions of nuclear (P_1), "crude-mitochondrial" (P_2), microsomal (P_3) and high speed supernatant fractions (S_3) via differential centrifugation has been described.[9] The crude mitochondrial fraction resuspended in 0·32 M sucrose was layered on top of a discontinuous density gradient prepared 1–2 hr before use and consisting of 5 ml each of 1·6, 1·2, 1·0, 0·8 and 0·6 M sucrose (0·005 M phosphate buffer, pH 7·1). The whole was then centrifuged at 53,000 *g* for 2 hr in the SW 25 swing-bucket head of the Spinco model L-2 preparative ultracentrifuge.

Radiolabeling of homogenates and subfractions

[^{3}H]*histamine.* The tissue weighed 1·569 g and the final volume of homogenate was 15 ml. The intention was to achieve approximately 5000 counts/min per 0·5-ml sample, working from a position of uniform distribution of the label in the original homogenate. At 25 per cent counting efficiency, this would necessitate adding approximately 6×10^5 counts/min to the whole homogenate. Accordingly, 10 μl of a 1:30 dilution (5 ng base, equivalent to $\frac{1}{200}$ of the expected concentration) of the stock solution, or no more than twice the amount wanted, was added to the original volume of homogenate; the mixture was then rehomogenized and the whole subjected to differential centrifugation in order to measure binding to the nuclear, crude mitochondrial and microsomal fractions. Each pellet was resuspended to the original volume and 0·5-ml aliquots were used for counting. In the case of the crude mitochondrial fraction, an additional 1 μl (6×10^4 counts/min) was added to this subfraction, and the mixture was rehomogenized with a loose-fitting pestle before commencing with density gradient centrifugation for its separation into myelin, synaptosomal and mitochondrial subfractions.

[^{14}C]*spermidine.* In this instance, the tissue weighed 1·1495 g and the conditions of homogenization and centrifugation were similar to the above. An amount of radiolabeled [^{14}C]spermidine equivalent to $1{\cdot}582 \times 10^5$ counts/min (2·4 μg or $\frac{1}{27}$ of the expected concentration) was added and the solution rehomogenized.

Endogenous histamine and spermidine. The distribution of endogenous histamine and spermidine was estimated by isolating the crude mitochondrial subfractions from within the sucrose gradient and extracting the amines for fluorometric analysis as described earlier.[10]

Table 1 illustrates the distribution of [^{3}H]histamine and [^{14}C]spermidine among the four primary fractions[9] after addition of these substances at 4° to a sucrose homogenate of dog hypothalamus.

TABLE 1. PERCENTAGE DISTRIBUTION OF [^{14}C]SPERMIDINE AND [^{3}H]HISTAMINE ADDED AT 4° TO HOMOGENATES OF DOG HYPOTHALAMUS

Fraction	*g* (min)*	[^{14}C]spermidine			[^{3}H]histamine		
		Expt: 1	2	3	1	2	3
Nuclear	10×10^3	59	58	61	2	9	9
Crude mitochondrial	1×10^5	18	21	16	6	5	5
Microsomal	6×10^5	10	12	9	2	1	1
Supernatant	6×10^5	13	9	14	90	85	85

* Product of the gravitational forces and time of centrifugation.

‡ POPOP = 1,4-bis-2-(4-methyl 5-phenyloxazolyl) benzene.

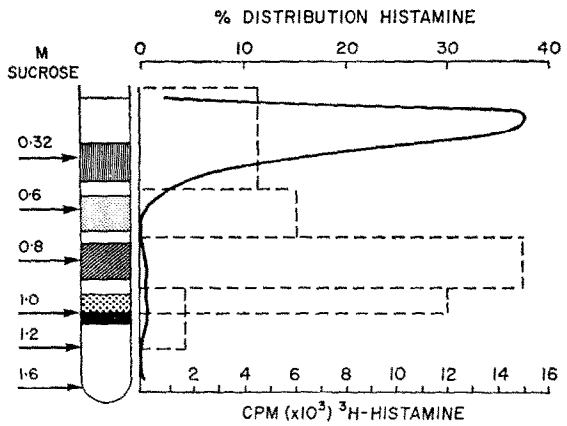

FIG. 1. Distribution within a discontinuous density gradient of: (1) [^{3}H]histamine (continuous curve) added at 4° to a resuspended crude mitochondrial pellet of dog hypothalamus, immediately mixed and applied to the gradient; (2) endogenous histamine (broken line histogram) in subfractions of crude mitochondrial fraction from dog hypothalamus. The data are representative of 3 different experiments.

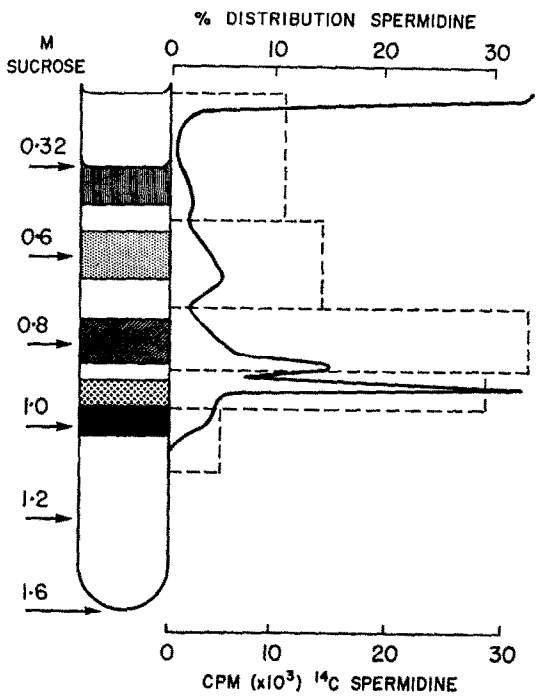

FIG. 2. Distribution within a discontinuous density gradient of: (1) [^{14}C]spermidine (continuous curve) added at 4° to a resuspended crude mitochondrial pellet of dog hypothalamus immediately mixed and applied to the gradient; (2) endogenous spermidine (broken line histogram) in subfractions of crude mitochondrial fraction from dog hypothalamus. The data are representative of three different experiments.

[^{3}H]Histamine was distributed largely (85–90 per cent) in the high speed supernatant of the homogenate; less than 15 per cent was divided among the particulate fractions, the concentration in the crude mitochondrial fraction exceeding the sum of the other two in its content. When more [^{3}H]-histamine was added to the crude mitochondrial fraction and was subfractionated on a sucrose density gradient, the distribution of radioactivity was confined to the uppermost and least dense regions of the gradient. Similar findings have been reported by Snyder *et al.*[11] Penetration of the labeled histamine into the gradient follows the pattern consistent with that of diffusion of a small molecule (Fig. 1). The distribution of endogenous histamine shows it to be bound to particulate material migrating deep within the gradient having sedimentation characteristics similar to synaptosomes. A relatively small amount of [^{3}H]histamine was likewise concentrated in the 1·0 M sucrose fraction. The results suggest that less than 1 per cent of added histamine exchanges with, or is bound to, the particulate matter of these fractions.

In the case of [^{14}C]spermidine, this compound was strongly bound to the nuclear fraction of the homogenized hypothalamus (Table 1). In addition to nuclei, this fraction contains unbroken cells, large membranes, blood cells and other fragments. The localization of spermidine in the other three fractions could, therefore, be of greater significance for the study of its binding to more definable cellular organelles. When the crude mitochondrial fraction, with additional [^{14}C]spermidine added, was further fractionated on a sucrose density gradient, 72 per cent of the radioactivity was in the fractions of density greater than 0·6 M sucrose with three or four peak areas of radioactivity (Fig. 2). The most predominant radiolabeled region was in the 1·0 M fraction wherein 53 per cent of the radioactivity lodged. This fraction contains 65 per cent of the endogenous spermidine found originally in the crude mitochondrial fraction of the dog hypothalamus, as determined fluorometrically without the addition of [^{14}C]spermidine. This is the subfraction richest in synaptosomes.

The results indicate that all three particulate fractions of the homogenized hypothalamus bind added spermidine and that a significant binding site for added spermidine exists on the synaptosomes.

In conclusion, secondary redistribution of histamine does not take place during homogenization, and the original suggestion[1] that histamine is associated with certain nerve-endings in the central nervous system is correct. This view has been substantiated using bioassay analysis of subfractions.[12] While the present study shows that exogenous (and probably endogenous) spermidine redistributes and binds to particulate material after homogenization, it does not negate the possibility of a unique association of spermidine (or other poly- or diamine) with some organelle or macromolecule within the nerve-ending. Indeed Snyder and Russell[13] have shown that the intracisternal injection of [^{3}H]putrescine leads to the rapid formation of [^{3}H]spermidine, which thereafter declines with a ha]f-life of 5 days. It would be interesting to know whether the ornithine decarboxylase (EC 4.1.1.17) activity responsible for this conversion in brain tissue is in the cell body, nerve-ending or some other cellular component of the brain.

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