The distribution of hydroxytryptamine in brain fractions

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IT HAS been previously shown[1, 2], using the rat fundus strip assay method,[3] that bound hydroxytryptamine (HT) is mainly recovered in a distinct fraction of subcellular particles derived from brain by homogenization, differential centrifugation and density gradient separation, which is also characterized by a high content of bound acetylcholine (ACh),[2] and which has been shown to consist largely of pinched off nerve endings.[4, 5, 6] A similar distribution has been found by Baker.[7] The object of the present work was to find out if the previous findings could be confirmed using a fluorometric method[8] of assaying HT and if the ACh- and HT- containing particles could be separated by more elaborate density gradients than used previously.

For the latter purpose, the density gradient procedure previously described[2] was modified as follows. After removal of nuclei and cell debris at 1000 g for 10 min., the particulate (P_2) fraction from 10 g brain containing small myelin and other membrane fragments, nerve endings and mitochondria, was sedimented at 17,000 g for 60 min. It was then suspended in 0·32 M sucrose and layered (6·5 ml) over a continuous density gradient ranging from 0·8 to 1·6 M sucrose (19·5 ml) in each of two tubes of the SW 25 head of the Spinco preparative ultracentrifuge. After 2 hr at 25,000 rev/min. the gradient was separated into several fractions by puncturing the bottom of the lusteroid tubes[9] and samples were withdrawn for ACh and HT assay, nitrogen estimations and electron microscopy.[6] The electron micrographs were evaluated statistically by classifying the particles in each field into "nerve ending particles", "mitochondria" and "membrane fragments". The mitochondria did not include the small mitochondria found inside nerve ending particles.[6] The membrane fragments included myelin fragments and all of the many empty oval profiles of greatly varying size, some of which might have been microsomes and others derived from nerve endings or mitochondria, but did not include very small structureless membrane fragments, which were disregarded. The number of the various particle types was expressed as a percentage of the total number of particles counted in each fraction.

The distribution of HT in the fractions was found to parallel that previously reported. The result of a continuous gradient experiment is summarized in Table 1. Fraction 1 having a relatively low

TABLE 1. DISTRIBUTION OF ACETYLCHOLINE (ACh) AND HYDROXYTRYPTAMINE (HT) IN A SUCROSE DENSITY GRADIENT

Fraction	Sucrose (M)	N %	ACh %	ACh RSA	HT %	HT RSA	% particles identified as membrane fragments	% particles identified as nerve endings	% particles identified as mitochondria
1	0·32–0·8	36	17	0·47	23	0·64	81	17	2
2	0·8 –0·9	12	18	1·50	9	0·75	27	70	3
3	0·9 –1·0	14	28	2·00	21	1·50	40	55	5
4	1·0 –1·1	15	25	1·67	24	1·60	14	76	10
5	1·1 –1·2	13	12	0·97	19	1·46	9	88	3
6	1·2 –1·6	10	0	0	3	0·30	20	26	54
Recovery (as % of P_2 fraction)		107	88		100		—	—	—

The ACh and HT content of each fraction is expressed as the percentage of the total recovered activity (%) appearing in that fraction and as the relative specific activity (RSA), i.e. as the ratio of the % activity recovered in each fraction to the % nitrogen (column 2) recovered in that fraction.

Composition of material fractionated (P_2): total nitrogen, 4·33 mg/g; ACh, 2·05 nmoles/g; HT (free base) 639 ng/g wet weight of guinea pig brain tissue. Animals received iproniazid (100 mg/kg) subcutaneously 12 hr before experiment.

specific ACh and HT activity corresponded to a bulky white layer rich in myelin and microsomal material but containing relatively few nerve endings and almost no mitochondria. Fraction 6 corresponded to a tan layer floating above the clear zone of 1·6 M sucrose at the bottom of the tube. Over half the particles of this fraction were mitochondria; the nerve endings in it were all shrunken and bizarre in shape and the fraction contained little or no HT or ACh. In between, there was a cloudy zone which was divided into four fractions (from 2 to 5) all containing nerve endings and appreciable amounts of ACh and HT. Peak activity for ACh was found in fraction 3 and for HT in fraction 4, suggesting that the ACh particles were on average slightly lighter than those containing HT. It must be emphasized, however, that overlap was complete and the fractionation had not resulted in a sharp separation of particles into two chemically or morphologically distinct types. The low ACh and HT content of fraction 5 and 6 might be taken as evidence for the separation of a third type of nerve ending containing neither ACh nor HT. However, the nerve ending particles of the lower fractions showed to an increasing extent morphological alterations ("black-body formation") attributable to damage by hypertonic sucrose[6] which might have resulted in loss of amines. The present results, therefore, do not support the suggestion[10] that nerve endings can be separated into cholinergic and non-cholinergic types by density gradient separation.

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