Subcellular localization of 5-hydroxytryptamine in rat brain*

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KNOWLEDGE of the subcellular distribution of 5-hydroxytryptamine (5-HT) may be of considerable importance to ascertain the exact role of this amine in the function of the CNS.

Walaszek and Abood[1] found 5-HT concentrated in the so-called mitochondrial (Mit) fraction of the brain which by electron microscopy is heterogeneous and contains, besides free mitochondria, considerable amounts of myelin fragments and isolated nerve endings[2, 3]. Whittaker[4] subfractionated Mit in a gradient and observed 5-HT concentrated in the so-called vesicular fraction, later shown to be composed of nerve endings.[3] In the above paper[4] the total amounts of 5-HT were considerably higher than those reported in the literature for other species. This was interpreted by Inouye *et al.*[5] as due to the extraction technique used,[4] which apparently does not eliminate the presence of substance P and other active polypeptides. In this last paper[5] the main 5-HT activity was found in the supernatant or soluble cytoplasmic fraction. More recently Michaelson and Whittaker[6] found 5-HT in three subfractions of Mit which contain nerve terminals.

In this work we have used the fractionation technique previously described that permits the isolation of cholinergic (containing acetylcholine (Ach), acetylcholinesterase (AchE) and choline acetylase (ChAc)),[8] from non-cholinergic nerve endings[7] and also the subfractionation of Mit after osmotic shock,[8, 9] which allows the separation of synaptic vesicles.

The initial homogenate was done in 0·32 M sucrose containing 10^{-5}M Ca^{2+}/l. In order to inhibit monoamine oxidase, *trans*-2-phenylcyclopropylamine (SKF trans-385B) 3×10^{-4}M/l. was added. Temperature was found to be very critical in order to avoid the release of bound 5-HT and all the procedures were carried at 0–2 °C.

The acetone extraction was according to Costa[10] and included incubation with chymotrypsin to inactivate substance P and other polypeptides and treatment with petroleum ether. The assay was done on the rat fundus[11] perfused with Del Jalon solution containing 0·1 mg hyoscine/l.

TABLE 1. PROTEIN (P) AND 5-HYDROXYTRYPTAMINE (5-HT) CONTENT IN PERCENTAGE AND RELATIVE SPECIFIC ACTIVITY $\left(\text{RSA} = \frac{\text{per cent recovered 5-HT}}{\text{per cent recovered P}}\right)$ IN THE PRIMARY FRACTIONS OF THE RAT BRAIN. N, nuclear; Mit, mitochondria; Mic, microsomal and Sup., supernatant fractions. Absolute values per g wet weight were: P, 90·0 mg and 5-HT (base) 280 mg. Percent recoveries were: P, 92 and 5-HT, 108.

Fraction	Conditions	Ultrastructure	P %	5-HT %	5-HT RSA
N	900 g × 10′ 2 washings	Nuclei, myelin cellular debris	14·0	6·9	0·49
Mit	11,500 g × 30′ 1 washing	Myelin, membranes mitochondria nerve endings	49·0	49·0	1·00
Mic	100,000 g × 30′	Microsomes*	12·0	40·0	3·33
Sup	Supernatant	Soluble	25·0	4·1	0·16

* This particular fraction was not investigated with the electron microscope.

Protein estimations were done with the biuret reaction, after removal of lipids with alcohol–ether and ether–alcohol according to Palladin.[12]

RESULTS AND DISCUSSIONS

Table 1 shows that only 4 per cent of 5-HT is free. Bound 5-HT is mainly distributed between the mitochondrial (Mit) and microsomal (Mic) fractions. Because of the low protein content of the latter,

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5-HT appears to be highly concentrated in Mic, with a RSA of 3·33. This fact was not previously reported in the literature.

Table 2 demonstrates that the 5-HT of fraction Mit is mainly concentrated in subfraction C of cholinergic nerve endings[7] and is practically absent in free mitochondria (Subfraction E).

TABLE 2. PROTEIN (P) CONTENT IN PERCENTAGE, 5-HT AND CHOLINACETYLASE (ChAc) IN PERCENTAGE AND RSA IN THE SUBFRACTIONS OF MIT OBTAINED BY ULTRACENTRIFUGATION IN A DENSITY GRADIENT. TO CHARACTERIZE CHOLINERGIC NERVE ENDINGS, ChAc VALUES WERE USED.[8]

Absolute values per g wet weight in Mit fraction were: P, 33·0 mg; 5-HT, 144 mg; ChAc, 140 U. Percent recoveries were: P, 90; 5-HT, 65 and ChAc, 102.

Fraction	Ultrastructure	P	ChAc		5-HT	
		%	%	RSA	%	RSA
A	Myelin fragments	20·0	7·4	0·40	12·2	0·61
B	Membranes Synaptic debris	9·6	16·0	1·70	7·5	0·78
C	Cholinergic nerve endings	23·6	47·0	2·00	51·2	2·17
D	Non-cholinergic nerve endings	32·0	22·6	0·70	23·5	0·76
E	Free mitochondria	15·4	7·0	0·40	7·5	0·48

TABLE 3. PROTEIN (P), 5-HT, ACETYLCHOLINE (Ach), AND CHOLINACETYLASE (ChAc) CONTENT, IN PERCENTAGES, IN SUBFRACTIONS OF MIT OBTAINED BY OSMOTIC SHOCK IN DISTILLED WATER AND FURTHER CENTRIFUGATION.

Absolute values per g wet weight were: P, 33·0 mg; 5-HT, 144 mg; Ach, 0·75 μg and ChAc, 140 U. Percent recoveries were: P, 90; 5-HT 95; Ach, 100 and ChAc, 63.

Fraction	Conditions	Ultrastructure	P	Ach	ChAc	5-HT
M_1	11·500 g × 30′	Myelin, mitochondria, torn nerve endings	64·0	35·3	33·5	60·0
M_2	100·000 g × 30′	Synaptic vesicles, membranes	14·5	37·0	52·0	14·5
M_3	Supernatant	Soluble	23·0	27·7	14·5	25·1

Table 3 shows that after the osmotic shock of Mit,[8] at variance with Ach and ChAc, about 60 per cent of 5-HT remains with subfraction M_1 which contains mainly free mitochondria, myelin fragments and rests of torn nerve endings.

The above results show that 5-HT is mainly bound to the structure and found in both the "mitochondrial" and "microsomal" primary fractions. The low content of free 5-HT found in our assays

could be due to the Ca^{2+} added to the medium. The high concentration in the microsomal fraction should be further investigated to show if 5-HT is present in small endings that might not have sedimented in Mit or in some other particle. Further studies should also be carried out to try to separate Ach from 5-HT containing nerve endings.

The results shown in Table 2 demonstrate that the largest proportion of 5-HT of Mit is in the subfraction C of cholinergic nerve endings which contain Ach, AchE[7] and ChAc.[8] Several interpretations may be advanced to explain this finding. It may be that both Ach and 5-HT are in the same nerve terminal, but it is also possible that 5-HT and Ach are in two different structural entities that sediment at the same level in the gradient so far used. In favor of this last hypothesis are the reports of the literature on a different anatomical distribution in certain regions of the brain, for Ach and 5-HT. Furthermore the distribution of Ach,[7] ChAc and 5-HT in subfraction B of fragmented nerve endings (Table 2) is not strictly parallel.

Present observations do not contradict the previous findings on the isolation of cholinergic and non-cholinergic nerve endings.[7] The lack of a good isolation of cholinergic and non-cholinergic terminals reported by Michaelson and Whittaker[6] may be due to the gradient used and the lack of Ca^{2+} in the sucrose. This cation seems to be important to achieve this separation.[8]

At present it is much more difficult to interpret the results of the osmotic shock (Table 3). In this case the behavior of Ach and 5-HT is different. While Ach and ChAc[8] is concentrated in subfraction M_2 containing the synaptic vesicles, the largest proportion of 5-HT remains in M_1. This could be interpreted as an indication that the 5-HT of nerve endings is not in the synaptic vesicles, but another alternative explanation could be a different sensitivity of nerve endings containing Ach and 5-HT toward the osmotic shock. Further experiments are required to clarify these points.

To interpret the possible role of 5-HT in nervous function the demonstration that the largest proportion of this amine is in the cholinergic fraction of nerve endings, could be of considerable interest. However the presence of a high concentration in microsomes should also be taken in account. For the moment it is impossible to elaborate further about the fine localization of 5-HT within the nerve ending proper. In this connection it is of interest that monoamine oxidase, the enzyme more probably involved in the inactivation of 5-HT in brain, is exclusively located in mitochondria.[13]

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