PN 1242

Intracellular distribution of brain noradrenalin and De Robertis' non-cholinergic nerve endings

With their very fine electron micrographs of nerve endings isolated by centrifugation in a density gradient, DE ROBERTIS and his co-workers[1] revealed recently that the cholinergic and non-cholinergic nerve endings could be separated from each other in brain homogenates, the former being lighter than the latter and characterized by the presence of acetylcholine. In our laboratory, studies on the subcellular units of the rabbit brain and on the intracellular distribution of noradrenalin in the brain provide indirect support for the views of the De Robertis' school.

Homogenation and subcellular fractionation of rabbit brain were usually made as described previously[2] in the presence of iproniazid. The crude mitochondria (P_2 fraction) were further subfractionated by centrifugation in a density gradient of sucrose (0.32–1.7 M). The centrifugation was carried out in a Hitachi 40 P ultracentrifuge. A swing-bucket rotor similar to the Spinco SW39 was used for fractionation in the sucrose gradient. The 5–7 subcellular fractions were cut out by a tube

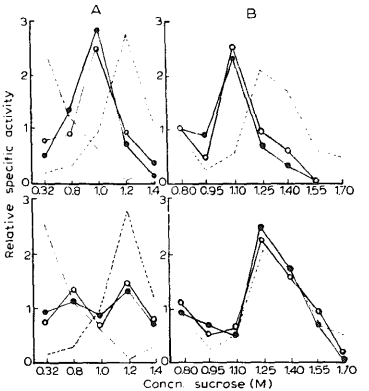

Fig. 1. Subcellular distribution of acetylcholine, Substance P, noradrenalin, 5-hydroxytryptamine, succinate dehydrogenase (dashed line) and phosphatase (broken line). A, subfractionation by a stepwise density-gradient centrifugation; B, subfractionation by a continuous density-gradient centrifugation. Upper figure: acetylcholine (●), Substance P (○); lower figure, noradrenalin (●), 5-hydroxytryptamine (○).

slicer. After washing with 0.15 M sucrose, acetylcholine[3], 5-hydroxytryptamine[2], Substance P[4], noradrenalin[5], succinate dehydrogenase (EC 1.3.99.1)[6], acid phosphatase (EC 3.1.3.2)[7] and protein[8] were determined.

Our results obtained on the P_2 fraction by the procedure similar to that of DE ROBERTIS and his co-workers[1] are presented in Fig. 1A. (average of 3 experiments), in which 0.32, 0.8, 1.0, 1.2 and 1.4 M sucrose solutions were layered from the top to the bottom of the centrifuge tube. A continuous density gradient was also used.

With an apparatus similar to that reported by BRITTEN and his co-worker[9], 0.8 M and 1.7 M sucrose were mixed under continuous stirring. 14ml of P_2 fraction suspended in 0.8 M sucrose was layered on the top of this density gradient. The data obtained by this method after centrifugation at 122000 × *g* for 30 min is presented in Fig. 1B (average of 2 experiments), in which 7 portions were cut out by a tube slicer, their density being given by that of the bottom of the portion cut out. The ordinates in these presentations were, for simplicity, expressed in relative specific activity as employed by DE ROBERTIS[1]; the relative specific activity of X fraction equals (% activity recovered in fraction X)/(% protein recovered in fraction X); it is 1.0 for the original P_2 fraction.

These results confirm the intracellular distribution of acetylcholine reported by WHITTAKER[3] and DE ROBERTIS[1] as well as that of Substance P by us[10]. The intracellular distribution of noradrenalin and 5-hydroxytryptamine run parallel to each other. With the stepwise density gradient, however, the relative specific activities of the fractions did not differ much from 1.0, and a specific particulate fraction rich in both amines was not obtained. When the washing with 0.15 M sucrose was omitted, the 1.4 M subfraction was found to have the highest concentrations of noradrenalin and 5-hydroxytryptamin, a fact which is in agreement with results previously reported[2]. When the continuous density gradient method was used, the highest concentration of these amines was found in the 1.25 M fraction, while the peak shifted to 1.4 M fraction when washing with 0.15 M sucrose was omitted. The distribution of succinate dehydrogenase and acid phosphatase activity was not affected by the washing procedure. Electron microscopically, vesicular elements similar to the pinched-off nerve endings reported by DE ROBERTIS were found in the 0.8–1.2 M fraction, but in the 1.4 M fraction aggregation of vesicular elements was marked, making their identification difficult. The effect of washing the subcellular fractions on their content of the active substances can be attributed to the redispersion of the aggregated vesicles. Since the typical mitochondria or microsomal microvesicles show hardly any tendency to aggregate no effect of washing was observed.

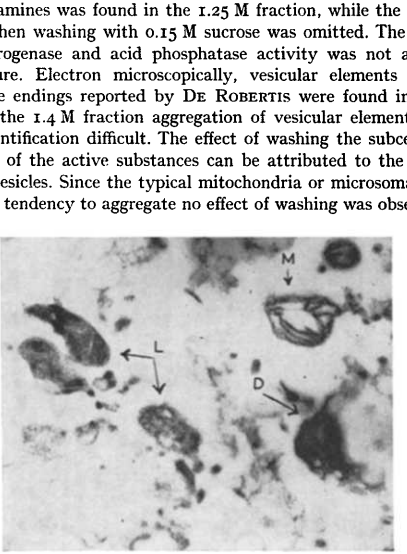

Fig. 2. Electron micrograph of dense (D) and less-dense (L) nerve ending obtained from 1.0 M subfraction embedded in Epon resin and stained with OsO_4. A mitochondrial structure (M) is also seen (16000 ×).

The nerve ending-like elements appear to be of two types, one densely stained with OsO_4, the other less densely stained (Fig. 2).

Table I shows that the dense type were more abundant in the heavier fractions. Though thin-sectioned specimens for electron microscopy can not always be considered as providing a sample of average composition, there appears to be a general parallelism between the ratios dense type: less type and noradrenalin:acetylcholine. The considerable discrepancy found in 0.8 M fraction could be explained by the presence

TABLE I

RELATIONSHIP BETWEEN RATIO OF DENSE AND LESS DENSE TYPES OF NERVE ENDINGS AND RATIO OF NOR-ADRENALIN: ACETYLCHOLINE

Subfraction (M)	*Dense type:less dense type*	*Noradrenalin:acetylcholine*
0.8	0.28 (total count 82)	0.85
1.0	0.30 (total count 74)	0.31
1.2	1.73 (total count 66)	1.70

in this fraction of synaptic vesicles disrupted from the ending, as suggested by DE ROBERTIS[1]. It might be concluded tentatively, therefore, that the dense nerve ending-like vesicular elements correspond to the non-cholinergic ending of DE ROBERTIS which would contain noradrenalin or 5-hydroxytryptamine. At any rate, it seems noteworthy that acetylcholine and Substance P were mainly contained in the lighter particles and noradrenalin and 5-hydroxytryptamine in the heavier ones, suggesting that the subcellular particles containing substances which have been suggested to be central transmitters are of two types.

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