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PRELIMINARY COMMUNICATION

Is choline acetyltransferase present in synaptic vesicles?

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IT HAS been stated that synaptic vesicles released from rabbit and rat synaptosomes by hypo-osmotic treatment and separated by differential centrifuging contain choline acetyltransferase (ChAc).[1, 2] Since rabbit brain ChAc has a molecular weight of only 67,000,[3] one would expect the enzyme to appear in the soluble fraction of disrupted synaptosomes rather than in a particle-bound form. However, as is well known, some enzymes may be membrane-bound in media of low ionic strength but soluble in the presence of electrolytes.[4, 5] The state of ChAc after release from synaptosomes was therefore studied as a function of ionic strength and pH; parallel studies were carried out on the enzyme lactate dehydrogenase (LDH) which also has a relatively low molecular weight (140,000).

Crude synaptosome preparations[6] from rat, rabbit, guinea pig and pigeon brain were suspended in water (5 ml/wt of pellet derived from 1 g of tissue); samples (2 ml) were transferred to centrifuge tubes containing 2 ml of water or NaCl or Na phosphate buffer solutions of varying concentrations to raise the final ionic strength of the suspension. After mixing, the tubes were centrifuged at 105,000 *g* for 30 min and the distribution of ChAc, LDH and protein between the supernatant and pellet was determined. The Na^+ and K^+ content of the supernatant was also measured by flame photometry. Table 1 shows the amount of ChAc solubilized at various final ionic strengths. It will

TABLE 1. SOLUBILIZATION OF CHAC AS A FUNCTION OF IONIC STRENGTH

Species	Tissue	Amount in supernatant (as a % of the total recovered activity) at the ionic strengths indicated						
		0·001	0·002	0·004	0·006	0·016	0·031	0·076
Rabbit	"Midbrain"*	0(2)	7	14	—	38(2)	31	—
	cortex	6	19(2)	23(2)	59(2)	61(2)	50(2)	—
Rat	cerebra	14(3)	—	—	21	42	68(3)	76
Guinea pig	cortex	23(2)	36	—	41	—	82(2)	—
Pigeon	cerebra	—	72	—	86	—	90	—

The ionic strength due to endogenous electrolytes in the supernatant was estimated to be 0·001; other ionic strengths were achieved by addition of NaCl. ChAc activity was measured radiochemically[9] after release of any occluded enzyme by means of a detergent. Recoveries were 85–100 per cent; the pH was 6·5–7·0; the number of experiments averaged is given in parentheses.

* Included caudate nucleus and diencephalon.

be seen that in all species studied the enzyme was mainly particle-bound at low ionic strengths but became more soluble as the ionic strength was increased. Pigeon and guinea pig ChAc was more readily solubilized than that of rat or rabbit, but rat ChAc was over 75 per cent solubilized at an ionic strength of 0·076. The pH of the solution also markedly affected the degree of solubilization (Table 2), the higher pHs favouring the soluble form.

The amount of LDH released showed less variation with ionic strength; electrophoresis showed that such variation as occurred could be accounted for by binding of the more basic isoenzymes of LDH to particulate material at low ionic strengths. The amount of protein released was approximately constant irrespective of species or ionic strength showing that the variations in ChAc release were not due either to variations in the osmotic sensitivity of the synaptosomes or to varying amounts

of re-entrapment of enzymes resulting from resealing of disrupted synaptosomes when the ionic strength was raised.

On separating hypo-osmotically disrupted synaptosomes from rabbit and rat in a density gradient by the method of Whittaker *et al.*,[6] the soluble ChAc was recovered, as expected, in the top fraction *O* (soluble cytoplasmic constituents of the synaptosome); the particle-bound ChAc was not localized

TABLE 2. SOLUBILIZATION OF RAT CHAC AS A FUNCTION OF PH

	Amount in supernatant (as a % of the total recovered activity) at the pHs indicated						
Ionic Strength	6·1	6·4	6·8	7·1	7·2	7·4	7·6
0·001	—	—	—	14(2)	—	—	—
0·006	4	—	28	—	32	41	48
0·036	—	34	—	—	65	—	—

The ionic strengths are the sum of that due to endogenous $Na^+ + K^+$ and added Na phosphate buffer. Recoveries were 85–100 per cent. The number of experiments averaged is shown in parentheses.

in any one fraction but was distributed through all the particulate fractions with the highest activities in fractions *F*, *G* and *H* (all rich in external synaptosome membranes). The synaptic vesicle fraction (*D*) invariably had the lowest activity, irrespective of species.

Further evidence that the binding of ChAc to membranes is non-specific came from experiments with ether-treated preparations (Table 3). In the absence of added electrolyte, relatively little rat brain ChAc was solubilized, though the guinea pig enzyme again showed good solubilization. However, in the presence of electrolyte, the rat brain enzyme largely went into solution.

TABLE 3. SOLUBILIZATION OF RAT BRAIN CHAC AFTER ETHER TREATMENT

	Amount in supernatant (as % of the total recovered activity)	
Tissue	No electrolyte	30 mM NaCl
Rat cerebra	20(3)	78(2)
Guinea pig cortex	73	97

Synaptosome preparations were shaken with ether at 0° for 10 min in 0·32 M sucrose or 0·30 M sucrose + 0·03 M NaCl, centrifuged at 105,000 *g* for 30 min and the distribution of the enzyme determined between supernatant and pellet. Recoveries were 85–100 per cent. The number of experiments averaged is given in parentheses.

It is concluded that membrane-bound ChAc is an artefact arising from the low ionic strength (0·001) and relatively low pH (6·5–6·8) of dilute water suspensions of synaptosomes. The species and conditions used by the De Robertis group[1] [rat; 9 ml water/wt of pellet derived from 1 g of tissue; contamination of their vesicle fraction (M_2) with larger membrane fragments[6, 7]] favour the deposition of the enzyme and its recovery in the synaptic vesicle fraction whereas those used by Whittaker *et al.*[6] (guinea pig; 2 ml water/g; density gradient separation of vesicles from larger membrane fragments) favour the soluble form of the enzyme and its separation from the synaptic vesicle fraction. Extrapolating to the conditions of ionic strength and pH likely to prevail in the intact cell, the results of Tables 1 and 2 suggest that the enzyme will be largely soluble in the intact ending, irrespective of species.

An account of this work was given at the New York Academy of Sciences Conference on Cholinergic Mechanisms (May 19–21, 1966)[8] and a full report is in course of preparation.

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