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Ontogenesis of the multiple forms of choline acetyltransferase: uptake and acetylation of choline in rat brain

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Rat brain choline acetyltransferase (ChA) exhibits molecular heterogeneity (Malthe-Sørenssen & Fonnum, 1972), but whether the multiple forms are isoenzymes with differing subcellular localizations and functions remains uncertain. There is evidence for the coupling of the high affinity uptake and acetylation of choline in rat brain (Barker, Dowdall & Mittag, 1975; Burgess & Prince, 1977) although the mechanism is unknown. We therefore sought changes in the heterogeneity of ChA and correlated these with changes in uptake and acetylation of choline in developing rat brain.

High speed supernatants and partially purified samples (Malthe-Sørenssen & Fonnum, 1972) from whole brains of 7-day old and adult Wistar rats were analyzed by isoelectric focusing. ChA was assayed radiometrically (Fonnum, 1975). The sodium-dependent high affinity uptake of [^{3}H]-choline (0.5 μM) by small slices of frontal cortex was measured by incubation in Krebs medium (10 min, 37°C). Corrections were made for sodium-independent low affinity uptake. Conversion of labelled choline into acetylcholine was measured (Potter & Murphy, 1967).

Both preparations, from mature and immature brain, possessed the three forms of ChA previously reported (Table 1). The most basic comprised 60% of the total activity recovered after electro-focusing of mature brain. In contrast, in immature brain the most basic form represented only 19% of total recovered activity.

Sodium-dependent high-affinity uptake accounted for approximately 30% of total uptake in brain of 7-day old rats, compared with 55% in the mature brain. 90% of the choline taken up by means of the high affinity mechanism was acetylated in mature brain compared with only 45% in immature brain.

Thus developmental increases in the efficiency of acetylation of choline after high affinity uptake in the

Table 1 Isoelectric focusing of developing rat brain choline acetyltransferase

Preparation of choline acetyltransferase	*Distribution of choline acetyltransferase activity* (%) pH 7.1–7.6	pH 7.6–8.0	pH 8.0–8.7
Adult brain (A)	14.38 ± 4.3	28.30 ± 6.7	52.85 ± 5.4
Adult brain (B)	10.53 ± 3.3	17.48 ± 3.6	64.14 ± 7.2
Immature brain (A)	31.18 ± 3.7	43.81 ± 5.9	18.97 ± 2.4
Immature brain (B)	25.20 ± 2.4	29.29 ± 6.2	19.33 ± 0.4

The enzyme preparations were either: (A) high speed supernatant of brain homogenate, or (B) partially purified as described by Malthe-Sørenssen & Fonnum (1972). Isoelectric focusing was carried out in 110 ml LKB columns (4°C), pH 6–9.5, 400 V for 46 hours. The distribution of ChA activity in the different pH ranges is expressed as a % of the total activity recovered after focusing. Residual activity was largely recovered in pH 6.5–7.1 range. Results are means ± s.e. mean of four focusing experiments in each case.

rat, may be related to the appearance of increased quantities of a basic isoenzyme of ChA. A molecular basis for the efficient coupling of sodium-dependent high affinity uptake and ChA will be discussed in light of these results.

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Activation of high affinity choline uptake in sympathetic ganglia by potassium depolarization

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There is evidence to suggest that high affinity uptake processes for choline are associated with cholinergic nerve terminals. Sympathetic ganglia accumulate choline by both high and low affinity uptake processes but we found that denervation of ganglia 10 to 14 days before their removal had no effect on the high affinity choline uptake process (Bowery & Neal, 1975). In the present study we have examined the effect of potassium depolarization on choline transport in ganglia and have found an association between cholinergic nerve terminals and a high affinity choline uptake process.

Isolated rat superior cervical ganglia were desheathed and given a preliminary incubation at 37°C for 30 min in Krebs bicarbonate Ringer. Then [^{3}H]-choline (1 μ Ci/ml) was added to give a final concentration of 0.1 μM or 100 μM and the incubations were continued for 10 minutes. Finally, the ganglia were dissolved in Soluene (Packard) and the radioactivity was measured by liquid scintillation counting. When ganglia were depolarized, potassium chloride (40 mM) was included in the medium both during the preliminary incubation and incubation period. The results are summarized in Table 1.

Potassium depolarization increased [^{3}H]-choline uptake by the high affinity uptake process but decreased transport by the low affinity process. Chronic denervation, absence of sodium or calcium ions in the medium, and increased magnesium ion concentration (20 mM), not only abolished the potassium induced activation of the high affinity choline uptake process, but changed the response to inhibition.

One explanation for these results is that potassium depolarization causes a calcium dependent release of