Choline uptake, choline acetyltransferase and the acetylation of choline in chick and rat brain

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[^{3}H]-Choline accumulated by high affinity uptake is acetylated less efficiently in brain slices from 7 day-old rats (45%) than in samples from adults (90%). The deficiency of a basic choline acetyltransferase (ChA) in immature rat brain may account for this difference (Atterwill & Prince, 1977). Acetylations of 50% in squid optic lobe synaptosomes (Barker, Dowdall & Mittag, 1975) support this view, since squid ChA is exclusively acidic (Polsky & Schuster, 1976). The recent report (Suszkiw & Pilar, 1976) that [^{3}H]-choline, accumulated at low concentrations by high affinity uptake, is also inefficiently acetylated (45%) in chick iris prompted an investigation of ChA and choline uptake in chick brain tissue.

High speed supernatants of brains from 1–3 day-old chicks and adult hens (Light Sussex, 10% w/v homogenates in 50 mM sodium phosphate buffer, pH 7.0, 100,000 g × 30 min) were analysed by isoelectric focusing (Atterwill & Prince, 1977; pH gradients 5 to 8) without further treatment, or after partial purification (40–60% saturation ammonium sulphate, pH 7.0). The ChA was notably more acidic than rat ChA. High speed supernatants of chick brain contained essentially a single, acidic ChA (pI 5.6, 48% recovered activity) with minor components focused at pH 6 to 8. Mature brains yielded several components, pI 5.6 to 7.1 (88% recovered activity, pH 6 to 8). Similar heterogeneity was obtained, however, with ammonium sulphate-treated high speed supernatants from chick brain, and refocusing, or treatment with sodium perchlorate (0.7 M, 8 mins, 30°C) (Houslay & Tipton, 1973) shifted the ChA towards the more acidic focus (pH 5 to 6). These results suggest a single, highly acidic form of ChA in chicks and adults.

The sodium-dependent high affinity uptake of [^{3}H]-choline (0.5 μM) by small slices of frontal cortex from 7–14 day-old chicks, was measured in Krebs-bicarbonate medium (10 min, 37°C). Corrections were made for sodium-independent, low affinity uptake. Radiolabel retained by the tissue was extracted (Toru & Aprison, 1966). [^{3}H]-Choline and ACh were separated by high voltage electrophoresis (Potter & Murphy, 1967). High affinity uptake accounted for approximately 58% of the total uptake and 41% of the choline accumulated by the high affinity process was acetylated.

These results further support the suggestion that where ChA is acidic, the intrasynaptosomal acetylation of choline is less efficient than where ChA is basic. The basic form of rat ChA has an appreciable affinity for synaptosomal membrane fragments (Fonnum & Malthe-Sørenssen, 1973). Its preferential distribution adjacent to the plasma membrane of nerve terminals, resulting in the juxtaposition of the high affinity uptake sites and ChA, may well, therefore, ensure the efficient acetylation of incoming choline. Where this organisation is lacking, in squid and chicks, the intrasynaptosomal acetylation of choline reflects the characteristics of simple, kinetically coupled processes.

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