Choline uptake and the regulation of choline acetyltransferase in relation to neuronal activity

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Preferential distribution of a net positively charged, basic form of rat choline acetyltransferase (ChAT) at the nerve terminal plasma membrane seems to be responsible for the efficient acetylation of choline accumulated by high affinity uptake in rat brain (Atterwill & Prince, 1978). Hydrophobic interactions (Rossier, 1977; Malthe-Sørenssen *et al.*, 1978) with the membrane seem the alternative. ChAT from a variety of species is widely reported to be activated by inorganic salts, but the significance of this remains uncertain. Rat brain ChAT, however, was recently reported five times more sensitive *in vitro* to NaCl than to NaI and specific activation by chloride fluxes *in vivo* was suggested (Rossier, Spantidakis & Benda, 1977). We present here further information about the response of ChAT to inorganic salts.

ChAT activities in whole homogenates (10% w/v in 2 mM sodium phosphate, 1 mM EDTA, pH 7.0, 0°C; treated with Triton X-100, final concentration 0.5% v/v) of rat and guinea pig cerebra and in partially purified (Malthe-Sørenssen & Fonnum, 1972) samples were assayed (37°) radiometrically (Fonnum, 1975) at low and high ionic strengths in homogenisation buffer and in buffer containing NaCl or NaI (150 mM). Preparations containing largely the basic, 'strongly bound' form of rat ChAT and one containing the two more acidic, 'loosely bound' forms were separated from synaptosomal membrane (Fonnum & Malthe-Sørenssen, 1973). Squid ChAT was extracted, purified and assayed as described previously (Prince & Hide, 1971).

V_{max} and $K_{choline}$ were smaller at low than at high ionic strengths (0 or 150 mM NaCl). ChAT activities were increased by NaCl if the choline concentrations exceeded 20–100 μM, but at lower concentrations, activities were decreased. This reversal of the ionic effect occurred at all concentrations of acetyl-CoA tested (>7 μM) with heterogeneous preparations of rat ChAT, the basic and acidic fractions, the single acidic (Malthe-Sørenssen & Fonnum, 1972) ChAT from guinea pig and with the heterogeneous, highly acidic (Polsky & Shuster, 1976) ChAT from squid. It is also implicit in some of the kinetic parameters for highly purified rat ChAT (Rossier *et al.*, 1977) and seems, therefore, an intrinsic property of the enzyme regardless of purity, heterogeneity or charge characteristics. Responses to NaI were similar to those obtained with NaCl, but all preparations were more unstable in the presence of NaI.

Rates of high affinity uptake of choline seem directly related to neuronal activity (Simon, Atweh & Kuhar, 1976). If a critical fraction of nerve terminal ChAT is sited at the plasma membrane, therefore, variations in the local concentration of choline could well determine the nature of the response of this enzyme to the ion fluxes associated with nerve terminal depolarisation.

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References

ATTERWILL, C.K. & PRINCE, A.K. (1978). Choline uptake, choline acetyltransferase and the acetylation of choline in chick and rat brain. *Br. J. Pharmac.*, **62**, 398P.

FONNUM, F. (1975). A rapid radiochemical method for the determination of choline acetyltransferase. *J. Neurochem.*, **24**, 407–409.

FONNUM, F. & MALTHE-SØRENSSEN, D. (1973). Membrane affinities and subcellular distributions of the different molecular forms of choline acetyltransferase from rat. *J. Neurochem.*, **20**, 1351–1359.

MALTHE-SØRENSSEN, D. & FONNUM, F. (1972). Multiple forms of choline acetyltransferase in several species demonstrated by isoelectric focusing. *Biochem. J.*, **127**, 229–236.

MALTHE-SØRENSSEN, D., LEA, T., FONNUM, F. & ESKELAND, T. (1978). Molecular characterisation of choline acetyltransferase from bovine brain caudate nucleus and some immunological properties of the highly purified enzyme. *J. Neurochem.*, **30**, 35–46.

POLSKY, R. & SHUSTER, L. (1976). Preparation and characterisation of two isoenzymes of choline acetyltransferase from squid head ganglia. *Biochim. Biophys. Acta*, **445**, 25–42.

PRINCE, A.K. & HIDE, E.G.J. (1971). Activation of choline acetyltransferase by salts. *Nature New Biology*, **234**, 222–223.

ROSSIER, J. (1977). Acetyl-coenzyme A and coenzyme A analogues. Their effects on rat brain choline acetyltransferase. *Biochem. J.*, **165**, 321–326.

ROSSIER, J., SPANTIDAKIS, Y. & BENDA, P. (1977). The effect of Cl^- on choline acetyltransferase kinetic parameters and a proposed role for Cl^- in the regulation of acetylcholine synthesis. *J. Neurochem.*, **29**, 1007–1012.

SIMON, J.R., ATWEH, S. & KUHAR, M.J. (1976). Sodium-dependent high affinity choline uptake: a regulatory step in the synthesis of acetylcholine. *J. Neurochem.*, **26**, 909–922.