It seems, then, that the transport mechanism of choline has a rather low requirement of energy. Further experiments, performed to study the ionic dependence of the transport, have supported the suggestion that the transport of choline is seemingly mediated by a facilitative-diffusion process.


Transport and metabolism of choline in synaptosomes: ionic requirement

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It has been shown that the uptake of choline in synaptosomes through a high-affinity mechanism (Km ≈ 1 μM) is strictly Na+- and energy-dependent (Kuhar, 1979). However, as we have shown in the preceding communication (Wong et al., 1981), the energy of activation of the transport of 1 μM-choline (the concentration used for the study of the high-affinity mechanism) is rather low in the range of physiological temperatures (30kJ/mol). Since choline acetyltransferase (EC 2.3.1.6), the enzyme responsible for the synthesis of acetylcholine, is greatly activated by ions (Rossier, 1977), we wondered whether a part of the decrease observed in choline uptake, after incubation in an Na+-depleted medium, was not due to a decrease in the synthesis of acetylcholine rather than in the transport of choline.

Rat brain crude synaptosomal fractions were obtained using standard procedures. Incubations were performed in Krebs-Ringer phosphate solutions in the presence of 1 μM or 100 μM-[Me-14C]choline (sp. radioactivity 60 Ci/mol). NaCl was substituted by isosmolar concentrations of sucrose or LiCl. After 1 min of incubation at 37°C the reaction was stopped with hemicholinium-3 (0.1 mM, final concentration) and the experimental procedure was as described by Wong et al. (1981).

The uptake of choline (obtained by measuring the radioactive content of the homogenate) was decreased when NaCl was substituted with either sucrose or LiCl (Table 1). However, the decrease in acetylcholine synthesis was more important than the decrease in the transport of choline. An important decrease in the synthesis of the neurotransmitter was also observed after incubation with 100 μM-choline. At this concentration, however, an effect was not observed on the uptake of choline since acetylcholine (which has reached saturation) represents only 5% of the choline pool (Massarelli & Wong, 1980). The transport of choline was also not affected by the absence of Na+ as has been reported previously (Kuhar, 1979).

The depletion of Na+ ions from the incubation medium seems to affect the transport of 1 μM-choline even if it does so to a minor extent compared with that from total uptake measurements. To investigate whether such dependence is due to a co-transport phenomenon or to the requirement of energy derived from the electrochemical Na+ gradient, some experiments were performed with toxins that are known to alter the properties of the Na+ channel. Incubation (1 min) with 0.1 mM-veratridine and 1 μM-[14C]choline decreases the total uptake of choline to 73.2% (P < 0.02). Simultaneous incubation with tetrodotoxin (0.1 μM)

<table>
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<tr>
<th>Na+ (mM)</th>
<th>Uptake</th>
<th>Choline</th>
<th>Acetylcholine</th>
<th>Uptake</th>
<th>Choline</th>
<th>Acetylcholine</th>
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</table>

Table 1. Effect of Na+ substitution on transport of choline and synthesis of acetylcholine

The results are expressed as percentages of the value obtained with 137 mM-NaCl. Na+ ions were substituted with isosmolar sucrose or LiCl.

1981
and veratridine (0.1 mM) brings this value up to 84.7% of the control, whereas treatment with tetrodotoxin (0.1 μM) alone does not affect the uptake (92.3% not significantly different from the control).

Veratridine is known to block the Na⁺ channel, whereas tetrodotoxin closes the Na⁺ channel. The incubation with veratridine leads eventually to the disappearance of the Na⁺ gradient, whereas tetrodotoxin maintains the gradient. These experiments should be considered only as an indication of the dependence of the transport on the Na⁺ gradient since veratridine is known to depolarize the terminals (Narahashi, 1974) and our data concern total uptake of choline, which after long depolarizing conditions is known to be increased (Roskoski, 1978).

In conclusion it appears that the uptake of 1 μM-choline is dependent on the energy furnished by the Na⁺ gradient. A suggestion that might be supported by the low activation energy needed for such an uptake (30kJ/mol; see Wong et al., 1981). The same amount of energy (32.6 kJ/mol) seems to be needed for the uptake of 100 μM-choline, but under these conditions the effects of the external concentrations of Na⁺ ions are less pronounced than after incubation with 1 μM-choline. The reasons underlying this difference might be found in the different metabolic status brought to the cytoplasmic choline pool after incubation with these concentrations (Massarelli & Wong, 1980).

However, it should be pointed out that a drug such as ouabain (which inhibits the Na⁺/K⁺ pump) does not inhibit choline uptake in synaptosomes unless the organelles are not preincubated in the presence of ouabain (0.1 mM, preincubation for 20 min, inhibition 53.4% of the control, P < 0.01), i.e. when the Na⁺ gradient has been presumably decreased.

In conclusion it appears that the uptake of 1 μM-choline is dependent on the energy furnished by the Na⁺ gradient. A suggestion that might be supported by the low activation energy needed for such an uptake (30kJ/mol; see Wong et al., 1981). The same amount of energy (32.6 kJ/mol) seems to be needed for the uptake of 100 μM-choline, but under these conditions the effects of the external concentrations of Na⁺ ions are less pronounced than after incubation with 1 μM-choline. The reasons underlying this difference might be found in the different metabolic status brought to the cytoplasmic choline pool after incubation with these concentrations (Massarelli & Wong, 1980).


Rotational-diffusion and cross-linking studies of cytochrome P-450 in rabbit liver microsomal membranes

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A method for estimating the rotational diffusion of cytochrome P-450 molecules in microsomal membrane has been successfully developed (Richter et al., 1979). Photolysis of bound CO from the haem groups of a population of cytochrome P-450 molecules with a flash of linearly polarized light gives rise to a transient dichroism at 450 nm. Decay of absorption anisotropy over a 1–2 ms time scale is related to the rotational motion of the cytochromes in this population by the expression:

\[ r(t) = A_1 \exp(-D_1 t) + A_2 \exp(-D_2 t) + A_3 \]

where \( r(t) \) is absorption anisotropy at time \( t \) after the flash, \( A_1 \), \( A_2 \), and \( A_3 \) are constants, \( D_1 \) is the rotational diffusion coefficient, and the assumption is made that rotation is about an axis normal to the membrane.

When the same method is applied to the microsomal fraction

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Fig. 1. Time-dependence of absorption anisotropy after flash photolysis of cytochrome P-450–CO in microsomal membranes from β-naphthoflavone-induced rabbits suspended in TCEG buffer [50 mM-Tris/HCl (pH 7.5)/0.01 mM-EDTA/20% (v/v) glycerol] containing 60% (w/w) sucrose

Sample temperatures: ○, 22°C; □, 35.5°C. Microsomal fractions were prepared as described by McIntosh & Freedman (1980) and stored at −20°C before transport from Canterbury to Zürich in solid CO₂.