Choline Acetyltransferase from Rat Brain*

Lincoln T. Potter and Vivette A. S. Glover

From the Department of Biophysics, University College London, London

Jeffrey K. Saelens

From Geigy Research, Ardsley, New York 10502

Summary

The localization, purification, and some enzymatic properties of choline acetyltransferase from rat brain were studied.

Most assays were performed with a specific radiometric micromethod.

Solubilization of the enzyme was examined after homogenization of cerebral cortices by means which disintegrate nerve endings. In isotonic KCl the enzyme was recovered in solution. In more dilute KCl the enzyme was adsorbed progressively but reversibly to particles, especially at low pH.

The subcellular localizations of the enzyme and of acetylcholine formed from choline-14C in vivo were examined further after gentle homogenization of cortices. Subcellular fractions were prepared in density gradients and were sedimented in isotonic NaCl to desorb transferase from microsomes. From isotonic homogenates, 56% of the total enzyme and 60% of acetylcholine-14C were found in nerve endings. From hypotonic homogenates (in which nerve endings are lysed) the enzyme was recovered in solution, whereas 60% of the acetylcholine-14C was found with microsomes. 3H-Acetylcholine added to the homogenizing medium remained in solution. It is concluded that acetylcholine is synthesized in the cytoplasm and is then incorporated into synaptic vesicles.

The enzyme was purified from whole brains to a specific activity $V_{max}$ of 0.727 pmole of product min$^{-1}$ mg of protein$^{-1}$ at 38°. The final preparation was free of deacylases, acetylcholinesterase, and carnitine acetyltransferase. By gel filtration the molecular weight of the enzyme was about 50,000; other studies indicated that the enzyme possesses essential —SH groups, that the protein is quite cationic, and that it is soluble in 0.1 M buffers.

Fifteen salts appeared to activate the soluble enzyme.

Michaelis constants for the forward reaction were determined; each substrate reduced the affinity for the other substrate.

The enzyme-catalyzed reaction was reversible with an apparent equilibrium constant of 514. It is possible that the amount of acetylcholine synthesized in the cytoplasm of nerve endings is regulated by mass action.

Choline acetyltransferase (acetyl-CoA:choline O-acetyltransferase, EC 2.3.1.6) catalyzes the synthesis of acetylcholine according to the equation

\[
\text{Choline} + \text{acetyl-CoA} \rightleftharpoons \text{acetylcholine} + \text{CoA}
\]

Numerous studies of the distribution of this enzyme in various tissues and species have been carried out, and these indicate that the enzyme is found, in vertebrate tissues, only in cholinergic nerves and in the noninnervated placenta of higher primates (1). A large fraction of the transferase in brain can be recovered, from sucrose homogenates of brain tissue, in isolated nerve endings (2). However, reports on the intracellular localization of the enzyme have been contradictory, as indicated in "Discussion," below. Cell-free preparations of the enzyme were first studied in 1943 (3). Detailed studies have been made of choline acetyltransferase from squid head ganglia (4, 5) and from human placentas (6, 7). Less information is available concerning the enzymic and molecular properties of the enzyme in mammalian nervous tissue, and our knowledge of the molecular weight (8), substrates (9), and kinetics (10) of this enzyme has been obtained with relatively impure preparations.

This report describes a specific assay, the localization, partial purification, apparent salt activation, molecular weight, kinetics, and equilibrium studies of choline acetyltransferase obtained from the brains of rats. Subsequent papers will consider the enzyme substrates, inhibitors, reaction mechanism, and protein properties, and regulation of the amount of acetylcholine synthesis in nerve endings.
EXPERIMENTAL PROCEDURE

Materials

Commercial samples of 4C-acetyl-CoA were found to contain CoA and other substances which affected enzyme activity. This substrate was therefore synthesized (11). CoA (Sigma, corrected for 95% purity), 10 pmol in 1 ml of neutralized 0.1 M KHCO₃, was mixed with 11 pmol of 4C-acetyl anhydride (Radiochemical Centre, Amersham, England) at 4°C. After 10 min, 20 pmol of unlabeled anhydride were added to complete the acetylation of CoA, followed by 100 pmol of HCl 15 min later. The solution was extracted twice with ether and air-dried, and the product was purified by paper electrophoresis (12); acetyl-CoA, CoA, and the labeled product moved together as anions at a rate of 6% of the cationic mobility of potassium. The specific activity of the product was 9.34 C pmol as determined by radiometric assay and with "phosphotranascetylase" (13). Residual -SH groups were 2% of that of the substrate by assay with mM 5,5'-dithiobis(2-nitrobenzoic acid) (MₐEₐ₈mm 13,800 (14)) in 0.1 M KHCO₃ and 10 mM EDTA.

Methyl-labeled 4C-choline (40 C pmol; Nuclear-Chicago), 4H-acetylcholine (66 C pmol; New England Nuclear), and acetyl-CoA (Sigma) were purified before use by paper electrophoresis (12).

Dialysis tubing was boiled in mM EDTA.

Albino rats weighing 250 to 350 g were used for all experiments.

Methods

Assay of Choline Acetyltransferase

Method 1—Units are micromoles of acetylcholine formed per min. Specific activity is in units per mg of protein.

Enzyme activity was estimated by determining the rate of acetylation of choline with 4C-acetyl-CoA. Equal volumes (usually 20 µl) of an enzyme preparation and a substrate solution (prepared monthly and kept frozen) were mixed in 0.2 ml micro centrifuge tubes held in holes in an aluminum block. Final concentrations were choline iodide, 1 mM; 4C-acetyl-CoA, 0.1 mM; physostigmine sulfate, 0.1 mM; neutralized EDTA, 0.1 mM; potassium phosphate buffer (equimolar mono- and dibasic salts, pH 7 at 38°C), 10 mM; KCl, 300 mM; and butanol-1, 1% by volume. For incubations, the block was transferred to a water bath at 38°C for 5 min and then returned to ice to stop the reaction. The product, acetylcholine-,4C, was isolated by electrophoresis. Sheets of Whatman No. 1 paper were prepared by spotting about 0.1 pmol of butanol in 5 µl of butanol on 2.5-cm lines drawn across the midline of the paper at 2- to 2.5-cm intervals, i.e. parallel to the direction of electrophoresis. Half of each reaction mixture was transferred in disposable capillary microcentrifuge tubes held in holes in an aluminum block. The temperature of the fluids was kept below 4°C. Buffer with KCl was added to samples of the dilute suspension to increase the salt concentration, and buffer alone was used to dilute samples of the homogenate in KCl so as to reach final potassium levels between 10 and 200 mM, and constant protein concentration. Samples (10 ml) were centrifuged at 151,000 g, for 30 min, and the enzyme activity of the pellets was determined for comparison with that of the homogenates (Fig. 1). A similar experiment was performed at pH 5 in mM potassium phosphate buffer after adjustment of the pH of the homogenates with N acetic acid (Fig. 1). At both pH values the transferase which remained associated with particles in 200 mM KCl could be brought into solution with 1% butanol-1.
Addition of mM MgCl₂ or CaCl₂ to the suspension media did not affect enzyme solubility.

The results show that the transferase is soluble in isotonic KCl at pH 7, but that it adsorbs progressively and nearly reversibly to particles in solutions of lower electrolytic strength, especially at pH 5.

Subcellular Localization—Since choline acetyltransferase adsorbs to particles under the low salt conditions used for subcellular fractionation of brain tissues, the localization of the enzyme and its product, acetylcholine, was re-examined. After gentle homogenization of cortical tissue, fractions were isolated in density gradients as previously described (19) and then sedimented in isotonic NaCl to remove any transferase adsorbed to particles.

Each of two rats was given 2.5 μmoles of choline-¹⁴C in an infusion of 5 ml of 150 mM NaCl lasting 30 min. The animals were killed by neck fracture 1 hour after the infusions and their cerebral cortices were finely diced in ice-cold 0.25 mM sucrose containing 0.1 mM physostigmine and mM phosphate buffer, pH 7.2. Half of the tissue was then homogenized with a motor-driven Teflon pestle rotating in a smooth glass tube, in 10 ml of the same solution. The remainder was homogenized in mM buffer (containing physostigmine and 0.1 μC of H-acetylcholine) so as to lyse nerve endings, and sucrose was immediately added to a concentration of 0.25 M. Both homogenates were centrifuged at 500 × g₀ for 10 min to remove capillaries and unbroken cells, and were fractionated in continuous isotonic density gradients of Ficoll (19). Particle layers were obtained (Fig. 2) as previously characterized by biochemical measurements and electron microscopy (19). Twelve 5-ml fractions were collected and analyzed for acetyltransferase and labeled acetylcholine.

For the latter measurements, samples were freeze-dried, extracted with ethanol-20 mM perchloric acid, and subjected to electrophoresis (12); 5.1 μmoles of acetylcholine-¹⁴C were synthesized from choline-¹⁴C in vivo. Details are given in the text.

Subcellular localization of choline acetyltransferase and acetylcholine from the rat cerebral cortex. Recombined gradient fractions (Fig. 2) were sedimented in isotonic salt solutions (see text) to remove adsorbed choline acetyltransferase. Acetylcholine-¹⁴C was synthesized from choline-¹⁴C in vivo. Details are given in the text.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Amount in homogenates prepared in isotonic sucrose</th>
<th>Amount in homogenates prepared in mM buffer + H-acetylcholine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supernatant fluids</td>
<td>38 22 90 31 96</td>
<td></td>
</tr>
<tr>
<td>Microsomes</td>
<td>1 12 2 60</td>
<td></td>
</tr>
<tr>
<td>Nerve endings (or ghosts)</td>
<td>56 60 4 5</td>
<td></td>
</tr>
<tr>
<td>Mitochondria</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Low speed sediment</td>
<td>4 6 1 2 1</td>
<td></td>
</tr>
</tbody>
</table>

Total | 102 100 91 98 91 |

7.2. Half of the tissue was then homogenized with a motor-driven Teflon pestle rotating in a smooth glass tube, in 10 ml of the same solution. The remainder was homogenized in mM buffer (containing physostigmine and 0.1 μC of H-acetylcholine) so as to lyse nerve endings, and sucrose was immediately added to a concentration of 0.25 M. Both homogenates were centrifuged at 500 × g₀ for 10 min to remove capillaries and unbroken cells, and were fractionated in continuous isotonic density gradients of Ficoll (19). Particle layers were obtained (Fig. 2) as previously characterized by biochemical measurements and electron microscopy (19). Twelve 5-ml fractions were collected and analyzed for acetyltransferase and labeled acetylcholine. For the latter measurements, samples were freeze-dried, extracted with ethanol-20 mM perchloric acid, and subjected to electrophoresis (12); 5.1 μmoles of acetylcholine-¹⁴C were recovered from the two homogenates. The gradient fractions were then recombined, on the basis of preliminary experiments, so as to isolate the monomodal distribution peaks of acetylcholine-¹⁴C and the enzyme. The combined fractions were diluted 5-fold in a final solution containing 150 mM NaCl, mM CaCl₂, and mM MgCl₂, and particles were sedimented at 79,000 × g₀ for 30 min. All the supernatant fluids were combined. Assays of the resultant fractions are summarized in Table I.

The adsorption of choline acetyltransferase which occurs at low ionic strength is almost exclusively to microsomes. More than half of the enzyme and acetylcholine-¹⁴C of cortical tissue can be recovered in nerve endings. When these are lysed and the adsorbed enzyme is removed from the remaining particles, the enzyme is soluble whereas the majority of the acetylcholine-¹⁴C is found with microsomes, including synaptic vesicles. The latter localization is not an artifact of homogenization, since H-acetylcholine in the medium remained in solution.

Purification—All operations were performed at 0-4°C. Unless otherwise noted centrifugation was in plastic bottles at 10,000 × g₀ for 30 min. Results are summarized in Table II.

1. Whole rat brains (24 brains, 51.3 g) were collected in glass-distilled water and were homogenized in 400 ml of 0.1 mM...
Table II
Purification of choline acetyltransferase

Details are given in the text. Half of the material from Step 5 was used for Step 6; over-all recovery of the enzyme was 33%. Final specific activity was 0.62. All values were obtained with Method 1.

<table>
<thead>
<tr>
<th>Step</th>
<th>Volume</th>
<th>Protein</th>
<th>Enzyme</th>
<th>Recovery %</th>
<th>Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Homogenate</td>
<td>452</td>
<td>5040</td>
<td>4.79</td>
<td>100</td>
<td>60</td>
</tr>
<tr>
<td>2. Supernatant, pH 8</td>
<td>300</td>
<td>1909</td>
<td>4.26</td>
<td>91</td>
<td>50</td>
</tr>
<tr>
<td>3. Extract of pH 5 precipitate</td>
<td>199</td>
<td>324</td>
<td>3.89</td>
<td>79</td>
<td>5.3</td>
</tr>
<tr>
<td>4. Ammonium sulfate</td>
<td>0-50%</td>
<td>21 161</td>
<td>2.88</td>
<td>62</td>
<td>5.5</td>
</tr>
<tr>
<td>5. CM-Sephadex</td>
<td>25 20</td>
<td>2.22</td>
<td>47</td>
<td>50.1</td>
<td>279</td>
</tr>
<tr>
<td>6. Sephadex G-200</td>
<td>8</td>
<td>1.28</td>
<td>0.79</td>
<td>29</td>
<td>93</td>
</tr>
</tbody>
</table>

Table III
Salt activation of soluble choline acetyltransferase

Method 1 was used without KCl or physostigmine, with about 0.3 µg of purified enzyme, and with 25 mM Tris-HCl buffer, pH 7.4. Results are expressed as percentage of the activity with 300 mM KCl. All salts were at pH 7.4.

<table>
<thead>
<tr>
<th>Salt added and concentration</th>
<th>Activity %</th>
<th>Salt added and concentration</th>
<th>Activity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaF</td>
<td>33</td>
<td>100</td>
<td>77</td>
</tr>
<tr>
<td>+ 0.25 M sucrose</td>
<td>31</td>
<td>200</td>
<td>83</td>
</tr>
<tr>
<td>LiCl</td>
<td>300</td>
<td>100</td>
<td>103</td>
</tr>
<tr>
<td>100</td>
<td>90</td>
<td>KCl</td>
<td>117</td>
</tr>
<tr>
<td>200</td>
<td>85</td>
<td>100</td>
<td>111</td>
</tr>
<tr>
<td>300</td>
<td>75</td>
<td>200</td>
<td>78</td>
</tr>
<tr>
<td>NH4Cl</td>
<td>300</td>
<td>101</td>
<td>78</td>
</tr>
<tr>
<td>100</td>
<td>98</td>
<td>KI</td>
<td>112</td>
</tr>
<tr>
<td>200</td>
<td>76</td>
<td>100</td>
<td>83</td>
</tr>
<tr>
<td>300</td>
<td>73</td>
<td>100</td>
<td>60</td>
</tr>
<tr>
<td>500</td>
<td>101</td>
<td>K2SO4</td>
<td>103</td>
</tr>
<tr>
<td>100</td>
<td>81</td>
<td>100</td>
<td>96</td>
</tr>
<tr>
<td>200</td>
<td>35</td>
<td>200</td>
<td>87</td>
</tr>
<tr>
<td>300</td>
<td>11</td>
<td>200</td>
<td>60</td>
</tr>
<tr>
<td>CaCl2</td>
<td>100</td>
<td>78</td>
<td>67</td>
</tr>
<tr>
<td>200</td>
<td>30</td>
<td>200</td>
<td>76</td>
</tr>
<tr>
<td>300</td>
<td>14</td>
<td>300</td>
<td>75</td>
</tr>
<tr>
<td>BaCl2</td>
<td>100</td>
<td>74</td>
<td>60</td>
</tr>
<tr>
<td>200</td>
<td>20</td>
<td>200</td>
<td>60</td>
</tr>
<tr>
<td>300</td>
<td>11</td>
<td>300</td>
<td>60</td>
</tr>
</tbody>
</table>

Most of the protein was eluted. Eight fractions were recombined and kept at 4° with 50 µl of toluene.

Purity and Stability—Decaylases and acetylcholinesterase were nearly absent after Step 5 and were undetectable after Sephadex G-200 by Method 2 of this paper and Method 2 of Reference 20, respectively. No activity was seen with the use of Method 2 and carnitine as a substrate with the final enzyme preparation. After Step 6 the enzyme was kept at 4° for 2 months without loss of activity. Attempts to precipitate the soluble enzyme of Step 6 by dialysis against 0.1 M salt buffers of pH 5 to 8 led to small losses of enzyme activity and some protein precipitation, but the majority of the enzyme remained soluble.

Molecular Weight—The molecular weight of choline acetyltransferase from rabbit brain has been estimated from centrifuga
tion and diffusion data as 67,000, and that of human placental enzyme as 59,000 (5). By gel filtration on Sephadex G-200, the purified rat brain enzyme appears slightly smaller. A rerun of 0.1 ml of the enzyme from Step 6 with 1 mg of crystalline ovalbumin (Sigma, Type V) and blue dextran gave peak elution volumes: blue dextran, 16; enzyme, 33; and protein, 34 ml.
Fig. 3. Kinetics of acetylcholine synthesis by choline acetyltransferase. The graphic method of Florini and Vestling (22) is used. \( v \), specific activity; \( S_1 \), [choline (m)] \( \times 10^4 \); \( S_2 \), [acetyl-CoA (m)] \( \times 10^3 \). In the upper graphs, double reciprocal plots of \( 1/v \) against \( 1/S \) are shown at four different concentrations of the second substrate. The lines intersect at a point (leftward at \(-1/K_m\)) which indicates the apparent \( K_m \) in the presence of the second substrate alone. The intercepts on the vertical axes of the upper graphs (1/V) are plotted in the lower graphs against the concentrations of the second substrate used above. The horizontal intercepts in the lower graphs give \(-1/K_m\) for the second substrate when the enzyme is saturated with both substrates, and the vertical intercepts are equal at \( 1/V_{max} \). Values are given in the text.

When these data were compared with the extensive results of Andrews (21), ovalbumin (mol wt 45,000) was found at its expected position and the indicated molecular weight of the acetyltransferase was about 50,000.

Salt Activation—Preliminary experiments showed a broad pH optimum for the purified enzyme, between 7 and 7.7, which was unchanged in 25 and 500 mM KCl. The effect of different salts on the activity of the enzyme was examined with the use of Method 1 without KCl or physostigmine, and with 25 mM Tris-HCl buffer, pH 7.4 at 38°, instead of phosphate (Table III). All of the salts tested gave a roughly comparable and several fold activation of the soluble enzyme. Some, such as KCl, caused increasing effects up to 300 mM, although most were inhibitory at this level. Activation increased with monovalent halides from fluoride to iodide, the most effective salt.

Copper sulfate (10 mM) completely inhibited the purified rat enzyme.

Kinetics—The forward reaction was studied in 150 mM KCl and 10 mM potassium phosphate buffer, pH 7, so as to approximate the intracellular environment of the enzyme. Method 1 was used without physostigmine; enzyme activity was constant during the 5-min incubation period at both high and low substrate concentrations. The results were analyzed graphically (22), as shown in Fig. 3, and indicate that each substrate affects the affinity of the enzyme for the other. Four Michaelis constants were determined: \( K_{ch} = 16 \mu M \), \( K'_{ch} = 39 \mu M \), \( K_{Ac-CoA} = 7.7 \mu M \), and \( K'_{Ac-CoA} = 19 \mu M \), where Ch represents choline, and Ac, acetyl. \( V_{forward} = 0.727 \mu mole \) of acetylcholine formed min\(^{-1}\) mg of protein\(^{-1}\).

Substrate inhibition was apparent for both choline and acetylcholine, as evidenced by nonlinear reciprocal plots at substrate concentrations above 100 \( \mu M \). Neither 25 mM acetylcholine nor 50 \( \mu M \) CoA decreased the initial rate of acetylcholine synthesis. The kinetics of the backward reaction has not yet been studied.

Equilibrium Measurements—The equilibrium constant, \( K = (\text{acetylcholine}) \cdot (\text{CoA})/(\text{choline}) \cdot (\text{acetyl-CoA}) \), was estimated by Method 2 in the manner used by Schubert for study of human placental choline acetyltransferase (9). The back reaction was

\[ K \] is the apparent Michaelis constant in the presence of one substrate, and \( K' \) is the constant when the enzyme is saturated with both substrates.
studied with different amounts of CoA; the decrease in CoA
sulfhydryl groups was taken as a measure of the amounts of
acetyl-CoA and choline formed. The acetylcholine used was
purified by electrophoresis, and an initial fixed ratio of acetyl-
choline to choline (10,000:100 \mu M) was used to minimize the
error produced by spontaneous hydrolysis of acetylcholine.

Preliminary experiments indicated apparent equilibrium after
5 to 25 min with high and low concentrations of CoA and 10
\mu g of purified enzyme; subsequent measurements were for 30
min.

Twenty-six estimates in 5 to 100 \mu M initial CoA gave a mean
K value (~ ± 207). \textsuperscript{3}H-Acetylcholine was included in
eight incubation mixtures and the amounts of \textsuperscript{3}H-acetylcholine
remaining and \textsuperscript{3}H-acetyl-CoA formed were directly determined
after paper electrophoresis. This method gave a mean K value
of 529 ± 113.

**DISCUSSION**

Reports on the intracellular site of choline acetyltransferase
have been contradictory, e.g. Whittaker (2) and Whittaker,
Michaelson, and Kirkland (23) have found the enzyme in
supernatant fractions after centrifugal analysis of lysed nerve
endings, whereas de Robertis et al. (24) have demonstrated that
much of the enzyme can be isolated in association with micro-
somal particles similar to the vesicles which hold acetylcholine.
The difference in results obtained in different laboratories has
been partially explained on the basis of considerable species
differences in the solubility of the enzyme (25). In this regard
it may be noted that the transferase has been partially purified
from guinea pig brains by recovering it in solution at pH 4.5 in
20 to 30 \textsuperscript{mM} total salts (28), i.e. under conditions in which the
rat enzyme is almost fully adsorbed to microsomes. Fonnum
(18) found that salts markedly increased solubilization of the
enzyme, and suggested that its association with microsomes was
an artifact produced experimentally by dilution of tissue salts
with sucrose solutions. The results in this paper directly show
reversible adsorption of the enzyme to microsomes in dilute
electrolytes, especially at low pH, and this fact was used to
advantage for enzyme purification. The enzyme is soluble
(after nerve ending lysis) under conditions which approximate
the intracellular levels of potassium and pH.

A re-examination of the localization of the transferase and
acetylcholine in nerve endings, under conditions minimizing
adsorption of the enzyme, confirmed previous results (2) that the
major part of both substances can be recovered, from isotonic
homogenates, in isolated nerve endings. Some of the enzyme
and acetylcholine found in the supernatant fluids presumably
comes from cell bodies and axons, and some from nerve endings
damaged during homogenization. The results further show that
at least 60% of newly synthesized acetylcholine in cortical
tissue can be recovered from hypotonic homogenates in associa-
tion with microsomes, including vesicles.

Previous studies provide adequate reason to believe that acetylcholine is held in
synaptic vesicles (23, 24); about four-fifths of acetylcholine\textsuperscript{14}C
in nearly pure samples of isolated nerve endings can be recovered in
"vesicle" preparations (19). It is concluded that acetyl-
choline is synthesized in the cytoplasm of nerve endings and is
concentrated in some manner by synaptic vesicles. The
mechanism for this accumulation is under study.

The tendency of choline acetyltransferase to adsorb to micro-
somes, many of which are negatively charged, and its tight
adsorption to CM-Sephadex at pH 5.9 indicate that the enzyme
is more cationic than most proteins. The effect of salts on
enzyme solubilization may well be due to disruption of ionic
linkages rather than to changes in the enzyme itself, which, when
purified, remained soluble at very low ionic strength.

The specific activity of the enzyme from Step 6 is about 14
times higher than previously achieved from vertebrate nervous

tissue (26). We have, however, completed a large scale purifi-
cation of the enzyme from cow caudate nuclei,\textsuperscript{2} the most active
plentiful source of the enzyme in mammalian nervous tissue
(1); this enzyme will be used for further studies. Highly active
enzymes from non-neural and invertebrate sources have received
considerable study. These include the housefly brain, from
which the enzyme has been purified 21-fold to a specific activity
of 0.73 (27); human placenta, purified 80- to 100-fold to 2.34
(7); and squid head ganglia, purified 10-fold to about 1.8 (4, 5).
Comparisons between these enzymes and that from rat brain
are noted below.

Fifteen different salts appeared to activate the soluble enzyme.
Soluble transferases from the rabbit brain (4), squid ganglia (5),
and placenta (6) are also activated by some salts. In the latter
two studies the inactivating effects of the larger halides were
emphasized, whereas the present results show that there is
increasing activation from fluoride to iodide at optimal concen-
trations. A previous conclusion that only chlorides activate
choline acetyltransferase (6) appears to be based on inadequate
data. NaCl raises the pH optimum of the placental enzyme (6).
The mechanism of the observed salt effects is not clear, especially
since dialyzed preparations of the caudate enzyme are almost
unaffected by salts\textsuperscript{3}.

The kinetic data show that choline and acetyl-CoA combine
with the rat brain enzyme before any products are released.
Previous studies of the neural enzyme have not been done at
several different substrate concentrations; published K\textsubscript{m}
values have varied, from figures near those reported here (10) to values	
many times higher. The substrate affinities are sufficiently high,
and Method 1 and the enzyme are sufficiently specific, so that we
have found it convenient to micromassays either acetyl-CoA or
choline by using the other labeled substrate and the enzyme in
excess (29). The data of Schuberth (6) suggest that choline
and acetyl-CoA combine independently with the placental
enzyme. It will be interesting to see whether there is a compar-
able difference between the enzymes of vertebrate and in-
vertebrate nerves.

The inhibitory effects of copper ions and the sulfhydryl reagent
5,5'-dithiobis(2-nitrobenzoic acid) on the rat brain enzyme are
in accord with previous studies of the transferases from squid
(4), placenta (6), Torpedo, and mammalian tissues (30). Each of
these enzymes appears to have —SH groups which are essential
for activity. Curiously, however, the fly brain enzyme is only
30% inhibited by 0.1 \textsuperscript{mM} iodoacetate (31).

Choline acetyltransferase catalyzes both the synthesis of
acetylcholine and the back reaction producing acetyl-CoA.
The difference in free energies of hydrolysis of the acetylated
substrates has been estimated at 4000 to 5000 cal (32). Since
\Delta F = RT \ln K, the equilibrium constant at 38° could be
expected to be in the range, 700 to 3550. The apparent K value
for the purified rat enzyme appears low and requires further

\textsuperscript{2} V. A. S. Glover and L. T. Potter, unpublished data.
\textsuperscript{3} L. T. Potter, V. A. S. Glover, and J. K. Saelens, unpublished
data.
verification from data on the kinetics of the back reaction. A $K$ value of 145 was found for the placental enzyme in 0.3 mM NaCl, and 5100 in dilute solution (6).

Where the apparent maximum rates of acetylcholine synthesis and release have been compared, as in the rat diaphragm (33), the potential activity of choline acetyltransferase appears far more than adequate for transmitter requirements, but the tissue content of acetylcholine remains relatively constant, even if neuronal esterases are inhibited (34). We assume, therefore, that acetylcholine synthesis is in some way regulated in response to use of the chemical transmitter. Any of three mechanisms could explain such regulation: product inhibition of the enzyme, limiting substrates, or mass action. Product inhibition was not notable in the present experiments at acetylcholine or CoA levels of about 50 $\mu$M (35), and its level near nerve endings is probably higher when acetylcholine is released and hydrolyzed by acetylcholinesterase. Since choline enters nerve endings by a facilitated uptake mechanism with a $K_m$ of about 50 $\mu$M (19), its concentration in neuroplasm is probably at least as high as its $K_m$ value with the transferase under normal conditions. The free level of acetyl-CoA in nerve endings is not known and could be limiting under some circumstances. It appears more plausible, however, that the normal amount of synthesis depends upon the equilibrium position of the transferase with all four substrates. If it is assumed that cytoplasmic choline is roughly 50 $\mu$M, and the cytoplasmic ratio of CoA to acetyl-CoA is roughly 20 as is the ratio of the total levels of these substances in whole rat brains and the liver (36), then levels of free acetylcholine of the order of 1 to 10 nM would be sufficient to reach equilibrium at the $K$ value observed. This level could reasonably be in equilibrium with acetylcholine in vesicles, where the concentration has been estimated from direct, quantal, and theoretical considerations to be in the range, 100 to 1000 nM (2).

REFERENCES


Choline Acetyltransferase from Rat Brain
Lincoln T. Potter, Vivette A. S. Glover and Jeffrey K. Saelens


Access the most updated version of this article at http://www.jbc.org/content/243/14/3864

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/243/14/3864.full.html#ref-list-1