Re-evaluation of the Kinetic Mechanism of the Choline Acetyltransferase Reaction*

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Initial velocity patterns for human placental choline acetyltransferase show a series of converging lines for both the forward and reverse reaction; with \( K_{\text{acetyl-CoA}} = 11.9 \mu M \), \( K_{\text{choline}} = 0.41 \text{mM} \), \( K_{\text{CoA}} = 8.8 \mu M \), and \( K_{\text{acetylcholine}} = 1.3 \text{mM} \). The relative rates of acetylcholine synthesis (\( V_{\text{choline}} \)) to acetylcholine breakdown (\( V_{\text{choline}} \)) is 1.5. Product inhibition by acetylcholine is competitive with respect to choline and noncompetitive with respect to acetyl-CoA, while product inhibition by choline is competitive with respect to acetylcholine and noncompetitive with respect to coenzyme A. Chlorocholine, diethyloaminoethanol, and acetylaminocholine were used as dead-end inhibitors and shown to inhibit competitively with respect to acetylcholine, and noncompetitively with respect to choline, acetyl-CoA, and CoA. At high choline concentrations, uncompetitive substrate inhibition is observed, and inhibition by acetylmelanochocholine changes from noncompetitive to competitive. Comparing the reactivity of dimethylaminoethanol to choline, and acetyldimethylaminoethanol to acetylcholine, the maximal velocities obtained with these analogues was ~25% of the natural substrates. These data are not consistent with the previously proposed ordered Theorell-Chance reaction mechanism, and have been interpreted in terms of a random binding mechanism.

Choline acetyltransferase (acetyl-CoA:choline O-acetyltransferase, EC 2.3.1.6) catalyzes the reversible synthesis of acetylcholine from acetyl-coenzyme A and choline. This enzyme has been purified to varying degrees of homogeneity from a variety of sources, including rat brain (1-5), bovine brain (6, 7), squid head ganglia (8-10), human placenta (11, 12), and human brain (13). As many as three different kinetic mechanisms have been proposed for the enzymatic reaction; an ordered bieactant mechanism (6), a double displacement or ping-pong mechanism (12), and a Theorell-Chance mechanism (14, 15). The most recent studies on the kinetic mechanism of this reaction favor the Theorell-Chance mechanism (16-18); however, all of these studies have been limited to initial velocity and product inhibition studies in the direction of acetylcholine synthesis.

The experimental results which were obtained in support of a Theorell-Chance mechanism, namely, initial velocity patterns which intersect below the 1/substrate axis, product inhibition by acetylcholine which is competitive with respect to choline and noncompetitive with respect to acetyl-CoA, and product inhibition by coenzyme A which is competitive with respect to acetyl-coenzyme A and noncompetitive with respect to choline, are in fact not unique to the Theorell-Chance mechanism. Although in agreement with a Theorell-Chance mechanism, Prince and Hide (19) pointed out that a rapid equilibrium random mechanism in which dead-end complexes are formed is also consistent with the kinetic experiments.

In order to more fully elucidate the kinetic mechanism of the choline acetyltransferase reaction, a kinetic analysis of both the forward and reverse reactions was undertaken, as well as kinetic studies employing dead-end inhibitors and alternate substrates. These studies have led us to propose a random mechanism involving the formation of dead-end complexes.

**MATERIALS AND METHODS**

**Enzyme Assays** — Acetylcholine synthesis was measured by coupling this reaction to the ATP-citrate lyase and malic dehydrogenase reaction, and following the disappearance of NADH at 340 nm.

Choline acetyltransferase:

\[
\text{acetyl-CoA + choline} \rightarrow \text{acetylcholine + CoA}
\]

ATP-citrate lyase:

\[
\text{CoA + citrate + ATP} \rightarrow \text{oxalacetate + acetyl-CoA + ADP + P}
\]

Malic dehydrogenase: oxalacetate + NADH → malate + NAD

Reaction mixtures contained 50 mM potassium phosphate buffer (pH 7.4), 0.25 mM sodium chloride, 0.05 mM dithioerythritol, 0.12 mM NADH, 10 mM magnesium chloride, 10 mM citrate, 5.0 mM ATP, 4 units of malic dehydrogenase, 0.5 unit of ATP-citrate lyase, choline, acetyl-CoA, and choline acetyltransferase in a final volume of 1.0 ml.

The reaction was initiated with either choline or choline acetyltransferase and monitored with a Gilford recording spectrophotometer equipped with a thermostated cell chamber which was maintained at 37° and an offset control which enabled full scale deflection on a recorder to equal 0.25 or 0.50 A. The same procedure was used to measure the reaction of dimethyloaminoethanol with acetyl-CoA, when this compound was used in place of choline.

Preliminary experiments showed that the rates obtained with this coupled assay are proportional to choline acetyltransferase and are the same as those obtained when the reaction was measured by following the incorporation of \(^{14}C\text{acetyl-CoA}\) into acetylcholine (20). No effect on the radioactive assay was observed when NADH, citrate, magnesium chloride, or ATP were added individually or in combination.

Acetylcholine cleavage was measured by coupling this reaction to the citrate synthase and malic dehydrogenase reactions and following the appearance of NADH at 340 nm.
Kinetic Mechanism of Choline Acetyltransferase Reaction

Choline acetyltransferase:

\[ \text{acetylcholine} + \text{CoA} \rightarrow \text{acetyl-CoA} + \text{choline} \]

Citrate synthase: \[ \text{acetyl-CoA} + \text{oxalacetate} \rightarrow \text{citrate} + \text{CoA} \]

Malic dehydrogenase: \[ \text{malate} + \text{NAD} \rightarrow \text{oxalacetate} + \text{NADH} \]

Reaction mixtures contained 50 mM potassium phosphate buffer, pH 7.4, 0.25 M sodium chloride, 0.05 mM dithioerythritol, 5.0 mM L-malate, 1.0 mg/ml of NAD, 0.075 mg/ml of NADH, 4.5 units of citrate synthase, acetylcholine, coenzyme A, and choline acetyltransferase in a final volume of 1.0 ml. Assays were conducted at 37°C as described above.

This assay system is similar to that used by Shepherd and Garland (21) to study the kinetics of the citrate synthase reaction, except that, in this case, citrate synthase is used as one of the coupling enzymes. Williamson and Corkey (22) have shown that the inclusion of NADH in the reaction provides a stoichiometric relationship between acetyl-CoA formation and NADH production. Under the assay conditions employed, we observed 0.92 eq of NADH produced/eq of acetyl-CoA consumed. This coupled assay system was found to be proportional to choline acetyltransferase over the entire range of substrate concentrations used in the study and was also employed to study the reaction of acetyltrimethylaminoethanol with coenzyme A.

The validity of this assay has been confirmed by comparing it to the direct spectrophotometric determination of acetyl-CoA formation measured at 232 nm.

During enzyme purification, activity was measured by the procedure of Sehrer and Shuster (20) using 50 mM potassium phosphate buffer (pH 7.4), 0.25 M sodium chloride, 0.05 mM dithioerythritol, 5.0 mM L-malate, 1.0 mg/ml of NAD, 0.075 mg/ml of NADH, 4.5 units of citrate synthase, acetylcholine, coenzyme A, and choline acetyltransferase in a final volume of 1.0 ml. Assays were conducted at 37°C as described above.

Initial Velocity Studies - Previous kinetic analyses of the choline acetyltransferase reaction, measured in the direction of acetylcholine synthesis, have shown that varying acetyl-CoA concentrations at fixed variable levels of choline, or vice versa, result in a series of lines which intersect below the 1/substrate axis (1, 6, 16-19, 29-34) indicative of a sequential reaction. In agreement with these studies, we also observed the same kinetic pattern for the acetyl-CoA/choline substrate pair and, in addition, have found an intersecting kinetic pattern when CoA and acetylcholine are the varied substrates (Fig. 1). For ease of comparison with the results obtained by other workers, kinetic constants were calculated by fitting the data to the Equation 1, where A = acetyl-CoA or CoA, and B = choline or acetylcholine, and are listed in Table I. The kinetic constants for the forward reaction are considerably lower than those reported by Sastry and Henderson (17) for the human placental enzyme (K_{Acet-CoA} = 113 to 150 μM; K_{choline} = 3 to 5

Data Analysis - Initial velocity patterns were fit to Equation 1

\[ v = \frac{VA}{K_a + KA_A + KB_B + AB} \] (1)

while product inhibition patterns were fit to Equation 2 for competitive inhibition and to Equation 3 for noncompetitive inhibition:

\[ v = \frac{VA}{K_a \left(1 + \frac{I}{K_i} A\right) + A} \] (2)

\[ v = \frac{VA}{K_a \left(1 + \frac{I}{K_i} A\right) + A \left(1 + \frac{I}{K_i} B\right)} \] (3)

In each case, computer programs kindly supplied by Dr. W. W. Cleland were used to fit the data to the appropriate equation, and the lines shown in each figure represent the computer fit of the data, whereas the points shown in the figures are those obtained experimentally.

Synthesis of Substrates - 2-Acetyldimethylaminoethanol was synthesized according to the method of Price et al. (23). We obtained a melting point of 139-141°C which can be compared to the literature value of 139-140°C (23). N,N,N-trimethylchloroethylamine iodide (acetylaminocholine iodide) was synthesized according to the method of Poskoski et al. (11) (corrected to 37°C), and about 2 to 4 times higher than reported by Morris (29). The details of this procedure will be published elsewhere.

FIG. 1. Initial velocity pattern for the reaction of acetylcholine with CoA. Reaction mixtures and assay conditions were as described under "Materials and Methods." Velocity expressed as micromoles of product formed/min/ml of enzyme containing 1.0 mg/ml of protein. Specific activity, 5.8.

Table I

<table>
<thead>
<tr>
<th>Kinetic constants for choline acetyltransferase reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>V_{max} is expressed as micromoles of substrate utilized/min/mg of protein. The kinetic constants have been calculated based on the assumption that acetyl-CoA is the leading substrate. K _j _j of choline or acetylcholine were calculated from the equation K_j = K_j/K_A. The values in parentheses represent standard errors in the determinations. Ac-CoA, acetyl-coenzyme A; Ch, choline; CoA, coenzyme A; and AcCh, acetylcholine.</td>
</tr>
<tr>
<td>V_{max} = 5.2 (0.1)</td>
</tr>
<tr>
<td>K_{Acet-CoA} = 11.9 μM (0.7)</td>
</tr>
<tr>
<td>K_{choline} = 410 μM (28)</td>
</tr>
<tr>
<td>K_{Ac-Ch} = 2.4 μM (0.7)</td>
</tr>
<tr>
<td>K_{Ac-Ch} = 82.4 μM (29)</td>
</tr>
</tbody>
</table>
mm); however, they are in the range observed by most other workers for the enzyme from a variety of other sources (1, 6, 18, 19, 32, 34).

It has also been shown that product inhibition by acetylcholine is competitive with respect to choline and noncompetitive with respect to acetyl-CoA, while product inhibition by coenzyme A is competitive with respect to acetyl-CoA and noncompetitive with respect to choline (16–19, 29–34). Although the assay systems employed in these studies do not permit the assessment of coenzyme A or acetyl-CoA as product inhibitors (see “Materials and Methods”), we have confirmed the finding that acetylcholine inhibition is competitive with respect to choline and noncompetitive with respect to acetyl-CoA. In addition, choline inhibition of the reverse reaction is competitive with respect to acetylcholine and noncompetitive with respect to coenzyme A (Fig. 2). The kinetic constants obtained from these studies are listed in Table II.

Dead-end Inhibitors—As a further probe of the reaction mechanism, enzymatically inactive analogs of choline and acetylcholine were tested as dead-end inhibitors. Chlorocholine acts as a competitive inhibitor with respect to acetylcholine, and as a noncompetitive inhibitor with respect to coenzyme A, acetyl-CoA and choline (Figs. 3 and 4). Al-

![Fig. 2. Choline as a product inhibitor of the reverse reaction. Reaction mixtures and assay conditions were as described in Fig. 1. Velocity expressed as in Fig. 1. A, choline inhibition with respect to acetylcholine; coenzyme A was held constant at 0.01 mM. B, choline inhibition with respect to coenzyme A; acetylcholine was held constant at 2.5 mM.](image)

![Fig. 3. Chlorocholine as a dead-end inhibitor of the forward reaction. Reaction mixtures and assay conditions were as described under “Materials and Methods.” Velocity expressed as micromoles of product formed/min/ml of enzyme containing 0.4 mg/ml; specific activity, 3.4. A, chlorocholine inhibition with respect to acetyl-CoA; choline held constant at 4.0 mM. B, chlorocholine inhibition with respect to choline; acetyl-CoA held constant at 0.03 mM.](image)

![Fig. 4. Chlorocholine as a dead-end inhibitor of the reverse reaction. Reaction mixtures, assay conditions, and velocity expressed as in Fig. 3. A, chlorocholine inhibition with respect to acetylcholine; coenzyme A held constant at 0.01 mM. B, chlorocholine inhibition with respect to coenzyme A; choline held constant at 2.5 mM.](image)

**Table II**

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Variable substrate</th>
<th>Nonvaried substrate</th>
<th>Type of inhibition</th>
<th>( K_w ) (mM)</th>
<th>( K_h ) (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholine</td>
<td>Choline</td>
<td>Acetyl-CoA (20 μM)</td>
<td>C</td>
<td>2.29 (0.2)</td>
<td></td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>Acetyl-CoA</td>
<td>Choline (500 μM)</td>
<td>C</td>
<td>4.66 (0.6)</td>
<td>7.63 (0.4)</td>
</tr>
<tr>
<td>Choline</td>
<td>Acetylcholine</td>
<td>CoA (10 μM)</td>
<td>C</td>
<td>0.18 (0.01)</td>
<td></td>
</tr>
<tr>
<td>Choline</td>
<td>CoA</td>
<td>Acetylcholine (2.5 mM)</td>
<td>C</td>
<td>0.75 (0.1)</td>
<td>1.87 (0.2)</td>
</tr>
<tr>
<td>Acetylaminocholine</td>
<td>Choline</td>
<td>Acetyl-CoA (30 μM)</td>
<td>N.C.</td>
<td>5.15 (1.0)</td>
<td>6.26 (0.7)</td>
</tr>
<tr>
<td>Acetylaminocholine</td>
<td>Acetyl-CoA</td>
<td>Choline (400 μM)</td>
<td>N.C.</td>
<td>2.80 (0.3)</td>
<td>15.6 (4.0)</td>
</tr>
<tr>
<td>Acetylaminocholine</td>
<td>Acetylcholine</td>
<td>CoA (10 μM)</td>
<td>C</td>
<td>1.93 (0.1)</td>
<td></td>
</tr>
<tr>
<td>Acetylaminocholine</td>
<td>CoA</td>
<td>Acetylcholine (2.5 mM)</td>
<td>C</td>
<td>13.4 (3.2)</td>
<td>5.96 (0.8)</td>
</tr>
<tr>
<td>Chlorocholine</td>
<td>Choline</td>
<td>Acetyl-CoA (30 μM)</td>
<td>N.C.</td>
<td>18.8 (3.0)</td>
<td>28.6 (3.1)</td>
</tr>
<tr>
<td>Chlorocholine</td>
<td>Acetyl-CoA</td>
<td>Choline (400 μM)</td>
<td>N.C.</td>
<td>31.0 (4.4)</td>
<td>22.6 (4.2)</td>
</tr>
<tr>
<td>Chlorocholine</td>
<td>Acetylcholine</td>
<td>CoA (10 μM)</td>
<td>C</td>
<td>14.8 (1.6)</td>
<td></td>
</tr>
<tr>
<td>Chlorocholine</td>
<td>CoA</td>
<td>Acetylcholine (2.5 mM)</td>
<td>N.C.</td>
<td>35.0 (4.0)</td>
<td>50.4 (10.2)</td>
</tr>
<tr>
<td>Diethylaminoethanol</td>
<td>Choline</td>
<td>Acetyl-CoA (30 μM)</td>
<td>N.C.</td>
<td>37.0 (4.2)</td>
<td>155 (20)</td>
</tr>
<tr>
<td>Diethylaminoethanol</td>
<td>Acetylcholine</td>
<td></td>
<td>C</td>
<td>25.0 (2.7)</td>
<td></td>
</tr>
</tbody>
</table>
though not shown, diethylaminoethanol also acted as a non-competitive inhibitor with respect to choline, acetyl-CoA, and CoA, and a competitive inhibitor with respect to acetylcholine. A summary of the kinetic constants are listed in Table II.

When acetylaminocholine was tested as a dead-end inhibitor, the inhibition patterns obtained were identical to those observed using chlorocholine and diethylaminoethanol (Figs. 6 and 8). Since acetylaminocholine appeared to be the most potent inhibitor (Table II), it was studied in more detail. When the inhibition pattern was determined by varying choline at a constant concentration of acetyl-CoA, and extending the substrate range to high concentrations (Fig. 7A), three things became evident. First, in the absence of added acetylaminocholine, substrate inhibition was apparent. Second, at high substrate concentrations, in the presence of the inhibitor, all of the curves appear to approach the same maximal velocity, that is, the inhibition pattern appears to change from noncompetitive at low substrate concentrations to competitive at high substrate concentrations. Thirdly, although not shown, at a given choline concentration, inhibition by acetylaminocholine is not linear but instead appears to plateau at a finite rate, that is, plots of 1/velocity versus acetylaminocholine concentrations are hyperbolic rather than linear. When the same experiment was conducted at a fixed choline concentration and valuable acetyl-CoA concentrations, all of the curves were linear (Fig. 7B).

The nature of the substrate inhibition exhibited by choline was examined and, as shown in Fig. 8, the inhibition appears uncompetitive with respect to acetyl-CoA.

**Comparison of Reactivity of Dimethylaminoethanol and Acetyldimethylaminoethanol to Choline and Acetylcholine**—Morris and Grewaal (14) compared the reactivity of dimethylaminoethanol to choline, using an acetyl-CoA regenerating system.
system, and reported less than 15-fold reactivity and a $K_m$ of 20 mM for the choline analog. Using the coupled spectrophotometric assay described under "Materials and Methods," we compared the reactivity of dimethylaminoethanol to choline at 200 $\mu$M acetyl-CoA (Fig. 9) and obtained the following kinetic constants: $K_{choline} = 0.3$ mM; $K_{dimethylaminoethanol} = 13.2$ mM, $V_{choline}/V_{dimethylaminoethanol} = 4.25$. We also compared the reactivity of acetyldimethylaminoethanol to acetylcholine at a coenzyme A concentration of 100 $\mu$M (Fig. 9) and observed the following: $K_{acetylcholine} = 1.25$ mM, $K_{acetyldimethylaminoethanol} = 57.1$ mM, and $V_{acetylcholine}/V_{acetyldimethylaminoethanol} = 4.19$.

In order to determine whether or not a change in kinetic mechanism occurs when dimethylaminoethanol is substituted for choline, initial velocity patterns and product inhibition patterns, using acetyldimethylaminoethanol as a product inhibitor, were determined. As shown in Fig. 10, double reciprocal plots of $1/v$ versus $1$dimethylaminoethanol at varying concentrations of acetyl-CoA yield a family of curves which intersect below the $1/v$ substrate axis. This is the same pattern observed for the choline/acetyl-CoA pair. Acetyldimethylaminoethanol inhibition is competitive with respect to dimethylaminoethanol, and noncompetitive with respect to acetyl-CoA (Fig. 11). Since the concentration of acetyldimethylaminoethanol required to obtain significant inhibition in these studies was as high as 100 mM, the product inhibition patterns were determined at a constant ionic strength of 0.25, maintained by sodium chloride (Fig. 11), or by allowing the ionic strength to vary from $-0.25$ to $-0.45$ (sodium chloride at a concentration of $0.25$ M was included in all experiments; see "Materials and Methods"). The inhibition patterns obtained

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**Fig. 8.** Substrate inhibition at high choline concentrations. Assay conditions are as described in Fig. 7. Acetyl-CoA was varied at the concentrations of choline indicated, with the total chloride concentration held constant at 0.35 M by the addition of sodium chloride. The arrow on the 1/v velocity axis indicates the expected velocity at infinite choline in the absence of substrate inhibition.

**Fig. 9.** Comparison of the reactivity of dimethylaminoethanol to choline, and acetyl dimethylaminoethanol to acetylcholine. Assay conditions are as described under "Materials and Methods." Velocity expressed as micromoles/min/ml of enzyme containing 0.9 mg/ml at a specific activity of 3.8. Top, comparison of choline to dimethylaminoethanol at 0.2 mM acetyl-CoA. Bottom, comparison of acetyldimethylaminoethanol to acetylcholine at 0.1 mM CoA.

**Fig. 10.** Initial velocity studies with dimethylaminoethanol as an alternate substrate. Assay conditions are as described in Fig. 9.

**Fig. 11.** Acetyldimethylaminoethanol as a product inhibitor of the reaction of acetyl-CoA and dimethylaminoethanol. Assay as described in Fig. 9. The total chloride concentration was held constant at 0.45 M by the addition of sodium chloride. A, acetyldimethylaminoethanol inhibition versus dimethylaminoethanol; acetyl-CoA was held fixed at 0.02 mM. B, acetyldimethylaminoethanol inhibition versus acetyl CoA; dimethylaminoethanol was held fixed at 50 mM.

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The experiments reported in this paper are not consistent with a simple Theorell-Chance reaction mechanism. First, the inhibition patterns produced by the substrate analogues, chlorocholine, diethylaminoethanol, and acetylaminocholine (Figs. 3 to 6) are not in accord with a Theorell-Chance mechanism.

In a Theorell-Chance Mechanism, a dead-end inhibitor can combine with one or more of the stable enzyme forms: \( E, EA, \) or \( EQ \), as shown in Scheme 1. Assuming acetyl-CoA and CoA are the leading substrates and that acetylaminocholine acts as an analog of acetylcholine (\( A = \) acetyl-CoA, \( B = \) choline, \( P = \) acetylcholine, \( Q = \) CoA, \( I = \) acetylaminocholine), inhibition by acetylaminocholine should lead to the formation of the \( EQI \) complex only and should be competitive with respect to acetylcholine and uncompetitive with respect to choline, acetyl-CoA, and CoA. Likewise, if chlorocholine or diethylaminoethanol act as choline analogs (\( A = \) CoA, \( B = \) acetylcholine, \( P = \) choline, and \( Q = \) acetyl-CoA), inhibition by these compounds should also be competitive with respect to choline and uncompetitive with respect to the three other substrates. If one assumes that all three substrate analogs bind to two enzyme forms, then one would obtain the dead-end complex \( EAI \) in addition to the \( EQI \) complex. In this case, all three analogs should produce inhibition noncompetitive with respect to both choline and acetylcholine and uncompetitive with respect to both acetyl-CoA and CoA. If one were to assume the situation where the inhibitors bound to all three enzyme forms, yielding the dead-end complexes, \( EI, EA, \) and \( EQI \), inhibition by all three analogs would be noncompetitive with respect to acetyl-CoA, choline, CoA, and acetylcholine, in the forward and reverse directions. The fourth possible inhibition pattern in a Theorell-Chance mechanism is obtained if one assumes choline and acetylcholine to be the leading substrates, \( A = \) choline or acetylcholine, \( B = \) CoA or acetyl-CoA, and in this case all three analogs would yield an \( EI \) complex, resulting in inhibition competitive with respect to both choline and acetylcholine and noncompetitive with respect to both acetyl-CoA and CoA. Clearly, the inhibition patterns obtained in these experiments do not fit any of these mechanisms.

Secondly, the observations that different maximal velocities are obtained when comparing choline to dimethylaminoethanol as substrates and when comparing acetylcholine to ace-

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**Scheme 1**

**Scheme 2**
E-CoA-I complex to an E-CoA-choline complex, and the inhibition will become competitive with respect to choline (Fig. 7). The formation of an E-CoA-choline complex in which the rate of dissociation of CoA is slower than from the E-CoA complex results in noncompetitive substrate inhibition at high choline concentrations (Fig. 9). Likewise, the formation of an E-CoA-I complex which slows down, but does not completely prevent, the dissociation of CoA will result in inhibition which reaches a finite value when all of the E-CoA is converted into the E-CoA-I complex. The observed noncompetitive inhibition between chlorocholine or diethylaminoethanol and choline, at relatively low choline concentrations, are also consistent with the formation of E-CoA-inhibitor complexes which decrease the rate of CoA release. The observation that acetylaminocholine inhibition is competitive with respect to acetylcholine can be due to either the fact that the dissociation of acetyl-CoA is not rate-limiting in the reverse direction or the fact that the formation of E-acetyl-CoA-acetylaminocholine complex does not affect the release of acetyl-CoA. Competitive inhibition by chlorocholine and diethylaminoethanol are also consistent with either of these explanations.

The fact that choline and acetylcholine are 4 times more reactive in terms of \( V_{\text{max}} \) when compared to dimethylaminoethanol and acetyldimethylaminoethanol is, as previously discussed, not in accord with a simple Theorell-Chance reaction mechanism. Although these data do not distinguish mechanisms, they are consistent with the proposed random mechanism in which the rate-determining step of the reaction is at least partially dependent on the rate of interconversion of the ternary complex.

Currier and Mautner (35) demonstrated a deuterium isotope effect of ~1.8 for the reaction catalyzed by the enzyme from squid head ganglia and interpreted this finding as evidence for general base catalysis in the reaction. If the rate-determining step of the reaction was solely the dissociation of coenzyme A from the enzyme-coenzyme A binary complex, as required in a Theorell-Chance mechanism, a deuterium isotope effect reflecting reaction of the ternary complex should not be observed. Thus, these data further support the contention that the rate-determining step of the reaction is dependent on the reactions involved in ternary complex interconversion.

In summary, the initial velocity and product inhibition patterns obtained in these studies are essentially the same as those observed by others; however, an extension of these studies to the kinetics of dead-end inhibitors, and a comparison of alternate substrates, has led us to conclude that the choline acetyltransferase reaction involves the random addition of substrates rather than the previously suggested sequential addition of substrates. Although it seems unlikely that species differences reflect differences in reaction mechanism, further studies on the kinetics of choline acetyltransferase from other sources are needed to clarify this point.

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REFERENCES

Re-evaluation of the kinetic mechanism of the choline acetyltransferase reaction.
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