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Differential sensitivity of soluble and membrane-bound forms of choline *O*-acetyltransferase to inhibition by coenzyme A

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It is reported that Coenzyme A (CoA) and, to a lesser extent, acetylcholine (ACh) inhibit the formation of ACh by the enzyme acetyl CoA: choline *O*-acetyltransferase (E.C. 2.3.1.6, ChAT) in brain nerve endings [1–4] and the electric organ of Torpedo [5]. The ability of CoA to inhibit ChAT activity at low micromolar concentrations (K_i = 5–20 μM) represents one argument against the possible synthesis of ACh by ChAT closely associated with vesicular membranes, despite the fact that its presence there has been detected using electron microscopic histochemical techniques [6, 7].

Recently, it has been reported that not all nerve ending ChAT activity is soluble. Approximately 10–15 per cent of nerve ending ChAT activity in mouse forebrain is non-ionically associated with vesicular and/or neuronal membranes. The membrane-bound ChAT differs from soluble ChAT in its dependence on Na^+, sensitivity to inhibition by 4-(1-naphthylvinyl) pyridine, ACh and acetylhomocholine, and its ability to acetylate the choline analog homocholine [8, 9], the acetylated product of which forms a calcium-dependent releasable transmitter [10, 11]. The ability of membrane-bound, but not soluble, ChAT to acetylate homocholine tends to support the suggestion of others that membrane-bound ChAT may play an important role in the synthesis of releasable transmitter [12–17]. The objective of this investigation was to determine if CoA would inhibit membrane-bound ChAT less than soluble ChAT.

Soluble and membrane-bound ChAT were prepared as described previously [8]. Briefly, a crude vesicular fraction (P_3) was prepared and washed twice with 100 mM sodium phosphate buffer to solubilize ionically bound ChAT. The *washed* P_3 was suspended in water, and 2 μl aliquots of the pooled washes or the *washed* P_3 fraction were assayed for ChAT activity. Prior to incubation with the buffer substrates, a 1 μl aliquot of stock solution of CoA was added to the enzyme sample so that the final concentration of CoA during incubation was 0, 10, 50, 100, 500 or 1000 μM. ChAT activity was measured according to the method of McCaman and Hunt [18], as modified by Fonnum [19]. Samples were incubated at 39° for 15 min. ChAT activity for each brain and each CoA concentration was assayed in duplicate, and the experiment was repeated once.

In another set of experiments, the *washed* P_3 fraction was layered onto a sucrose density gradient composed of 0.4 and 0.6 M sucrose and centrifuged at 192,000 *g* for 30 min [20, 21]. Approximately 80 per cent of the P_3 ChAT activity was recovered from the "membrane-bound" vesicle or P_4 fraction [9] which also contains occluded ACh, and membrane-associated organelles [22]. The resultant P_4 fraction was suspended in water and the effect of CoA on its ChAT activity was determined according to the procedure described previously.

In a third set of experiments, the effect of CoA on ChAT activity present in non-ruptured synaptosomes was determined. Synaptosomes were prepared by homogenizing minces of mouse forebrain in 5.0 ml of 0.32 M sucrose at 840 rpm and then centrifuging the homogenate at 1000 *g* for 10 min. The S_1 was then spun at 17,000 *g* for 15 min (described in Ref. 9). The P_2 (synaptosomal) pellet was then suspended in 200 mM sodium phosphate buffer (pH 7.4) to prevent rupture of synaptosomes.

In a fourth set of experiments, the ability of the P_4 fraction to acetylate carnitine was measured by replacing choline with carnitine in the incubation medium. Tucek [23] has reported that at a neutral pH carnitine is poorly extracted into tetraphenyl boron/3-heptanone but that it is much more efficiently extracted at pH 4.0 (approximately 70 per cent extraction efficiency). Therefore, following incubation, 2 μl or 1 N HCl was added to each sample, which was sufficient to lower the pH to 4.0. Then each sample was extracted in 75 mg/ml tetraphenyl boron/3-heptanone [19].

The experimental results illustrated in Fig. 1 indicate that CoA inhibited soluble ChAT activity at substantially lower concentrations than it inhibited membrane-bound ChAT activity. The I_{50} for CoA inhibition of soluble ChAT was approximately 10 μM, a value that corresponds closely to K_i values reported by others [2–4]. Conversely, the I_{50} for CoA inhibition of membrane-bound ChAT in the P_3 fraction was approximately 420 μM. The I_{50} obtained for CoA inhibition of membrane-bound ChAT in the P_4 fraction was 410 μM (N = 3, data not shown), a value similar to that determined for the P_3, and well above that determined for the soluble enzyme. The I_{50} obtained for CoA inhibition of a non-ruptured synaptosomal (P_2) fraction was 60 μM (N = 4, data not shown), a value intermediate between the soluble and membrane-bound ChAT, and clearly below that obtained for inhibition of P_3 and P_4 ChAT activity.

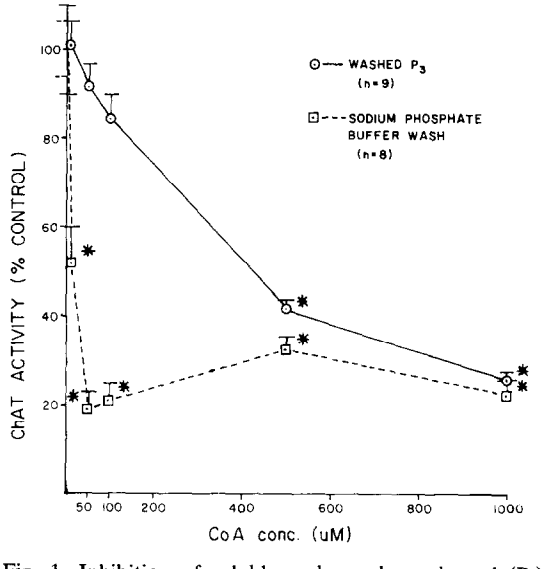

Fig. 1. Inhibition of soluble and membrane-bound (P_3) ChAT by Coenzyme A. ChAT activity was determined in the presence of 4.3 mM choline and 340 μM [^{14}C]acetyl CoA, in the absence or presence of CoA. The effect of various concentrations of CoA with respect to control was determined on the absolute values using one-way analysis of variance. An asterisk (*) indicates that the average value differs from control at $P < 0.05$.

Several investigators have reported that acetyl CoA: carnitine acetyltransferase (EC 2.3.1.7, Carn AT, present in mitochondria) may also acetylate choline (e.g. Ref. 24). Tucek *et al.* [25] found that acetylation of carnitine by nervous tissue was 12 times greater than the acetylation of choline. The results obtained in the present study indicated that Carn AT activity was 13.8 times greater than ChAT activity [choline acetylation in the nerve endings of mouse forebrain equaled 1199.2 ± 119.1 nmoles·(g wet wt)$^{-1}$·hr^{-1} for the sodium phosphate buffer wash (N = 8) and 394.7 ± 3.9 nmoles·(g wet wt)$^{-1}$·hr^{-1} for the P_4 (N = 3) as compared with 21,962.7 ± 948.6 nmoles·(g wet wt)$^{-1}$·hr^{-1} of carnitine acetylated by the P_4 fraction].

We have reported previously that the product ACh is a more potent inhibitor of solubilized ChAT than of membrane-bound ChAT, at concentrations exceeding 100 μM [8, 9]. Now we report that the other product CoA is also a more potent inhibitor of soluble ChAT. This difference in sensitivity to CoA inhibition does not appear to be due to the inability of CoA to cross neuronal membranes, since the I_{50} for CoA inhibition of synaptosomal ChAT activity was well below that for membrane-bound ChAT (60 vs 420 μM). Further, the ability of CoA to inhibit P_4 ChAT was similar to that of the P_3 ChAT (I_{50} equaled 410 and 420 μM respectively) suggesting that the suspension in water and the sucrose density gradient centrifugation did not alter the availability of CoA to the enzyme.

The relative insensitivity of choline acetylation by P_3 or P_4 fractions to inhibition by CoA does not appear to have been due to the activity of Carn AT. Roskoski *et al.* [26] have reported that choline is 1/25,000 times as good a substrate for Carn AT as is carnitine, the natural substrate, and Tucek *et al.* [25] noted that, when the acetylation of carnitine was 12 times greater than choline acetylation, Carn AT could account for 0.05 per cent of ACh formation. Since a similar ratio of carnitine to choline acetylation (13.8) was found in this study, Carn AT would probably account for less than 1 nmole·(g wet wt)$^{-1}$·hr^{-1} of ACh formation by the P_4 fraction.

Several investigators have reported that CoA is a very potent inhibitor of ChAT (K_i approximately 10 μM) [1–4]; it has also been suggested that CoA may serve as a feedback inhibitor in the regulation of ACh synthesis [12]. Our results suggest that membrane-bound ChAT may not be regulated by feedback inhibition. Further, it may not be as dependent as soluble ChAT on a high affinity mechanism (such as a CoA utilizing enzyme) to remove the product CoA from the site of synthesis. For example, acetyl CoA synthetase (EC 6.2.1.1), an enzyme reported to exist in cholinergic nerve endings [27], has a K_m for CoA (50 μM) [5] which is greater than the I_{50} for solubilized ChAT (10 μM) but below that for membrane-bound (420 μM).

In summary, two forms of choline *O*-acetyltransferase, which have been shown previously to differ with respect to substrate specificity, dependence upon Na^+, and sensitivity to inhibition by certain compounds, are now also shown to differ in sensitivity to inhibition by the reaction product Coenzyme A.

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Department of Pharmacology and Toxicology
University of Rhode Island
Kingston, RI 02881, *U.S.A.*

CHRISTINA G. BENISHIN
PAUL T. CARROLL

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