Biochemical Pharmacology, Vol. 25, pp. 1931–1933. Pergamon Press, 1976. Printed in Great Britain.

SYNAPTOSOMAL TRANSPORT AND ACETYLATION OF 3-TRIMETHYLAMINOPROPAN-1-OL

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(Received 26 April 1976; accepted 13 May 1976)

Synaptosomes isolated from homogenates of mammalian brain (1,2) and squid optic lobes (3) accumulate choline by a high affinity and a low affinity transport system. The results of studies on the high affinity transport of choline by synaptosomes suggest that this uptake system is specifically localized at cholinergic nerve terminals in brain tissue (4). Further, only the high affinity uptake of choline appears to provide choline for the synthesis of acetylcholine (ACh) (1,4,5). A direct comparison of the high affinity transport and acetylation of choline and two analogs of choline, monoethylcholine and N-hydroxyethylpyrrolidinium methiodide (pyrrolcholine), showed that all three compounds are equivalent in terms of transport and acetylation (6). This observation was surprising in that the two analogs of choline have apparent affinities for choline acetyltransferase (ChAc, EC 2.3.1.6) much less than that of choline (6) and thus when an equal number of molecules of all three are transported by the high affinity carrier an equivalent acetylation would not be expected. Based on these observations, it was suggested that the high affinity transport and acetylation of choline are in some way coupled (6).

The present communication describes the preliminary results of studies on the synaptosomal transport and acetylation of 3-trimethylaminopropan-1-ol (homocholine), an analog of choline that is not a substrate for ChAc in vitro (7-9).

Methods and Materials. [^{14}C]acetyl-CoA (55 mCi/m-mol) was obtained from New England Nuclear. [N-Me-^{3}H]choline (10.1 Ci/m-mole) and [^{3}H]methyliodide (906 mCi/m-mole) were obtained from Amersham Searle. All other chemicals and reagents used were the best grade available commercially.

[N-Me-^{3}H]homocholine was synthesized by the reaction of [^{3}H]methyliodide with dimethylaminopropanol and purified by ion exchange chromatography (6).

The synaptosomal transport of labeled choline and homocholine was determined on a

crude preparation of synaptosomes by an ultrafiltration technique (6). In separate experiments, it has been shown that the uptake of homocholine by this fraction is due to synaptosomes (B. Collier, S. Lovat, D. Ilson, L. Barker and T. Mittag, in preparation). Briefly, the 'P2' fraction isolated from homogenates of rat brain was suspended in Krebs-Ringer phosphate buffer (pH 7.4) containing labeled choline or homocholine and incubated at 37° for 5 min. Uptake was terminated by transfer to an ice bath and ultrafiltration (Millipore cellulose acetate filters, 0.65 μpore diameter) and a wash with 10 ml of ice cold Krebs-Ringer buffer. In all uptake experiments, synaptosomes from 10 mg tissue were suspended in 0.5 ml buffer and an amount equivalent to 8 mg was taken to determine uptake. In other experiments, where the proportion of accumulated homocholine converted to acetylhomocholine was determined, synaptosomes from 100 mg tissue were suspended in 5 ml buffer and incubated as noted above. The incubation was terminated by transfer to an ice bath and the addition of 5 ml buffer containing physostigmine sulfate (10 μM); the synaptosomes were re-isolated by centrifugation (340,000 g-min). The labeled compounds were extracted from synaptosomes, separated by thin-layer chromatography, and determined by methods previously described (6). Uptake by diffusion and nonspecific binding was corrected for by subtracting values for accumulation measured at 0° from those at 37°. The apparent Michaelis constants, K_T and V_{max}, were determined from plots of V vs V/S (10).

Choline acetyltransferase activity was determined in homogenates of rat brain, rat striatum, and a partially purified preparation of mouse brain ChAc (kindly provided by Dr. J. K. Saelens of Ciba-Geigy Corp., Summit, N.J.) by the method of Schrier and Shuster (11) as modified by Barker and Mittag (6).

Results and Discussion. In agreement with previous studies (7-9) we find that homocholine *in vitro* is not a substrate for ChAc. At all concentrations studied (1-100 mM) the apparent transfer of [^{14}C]acetate from acetyl Co-A to homocholine was less than 5 per cent of that observed for choline at a saturating concentration (5 mM).

The synaptosomal transport of [^{3}H]homocholine and [^{3}H]choline was determined over a range of 1-5 μM. The apparent K_T for the uptake of homocholine was 3.0 ± 0.4 μM (Fig. 1). The K_T for the transport of choline was 2.8 ± 0.7 μM. The two K_T values are not significantly different ($P > 0.1$, two-tailed *t*-test). The observed K_T for the accumulation of choline is similar to that, 2.4 μM, previously reported for the synaptosomal high affinity transport of choline (6). When compared directly, the V_{max} for the uptake of homocholine was found to be only 60 ± 6 percent that of choline. Over the concentration range of 0.5 to 5 μM, 20-30 percent of the accumulated homocholine is converted to acetylhomocholine.

The results of these studies show that homocholine, like choline, is taken up by synaptosomes and converted to an acetyl derivative. Previous studies on the high affinity uptake of choline analogs by synaptosomes have only dealt with compounds that are substrates for

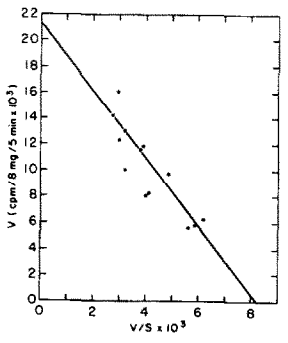

Fig. 1. Hofstee plot (V vs V/S) showing the uptake of homocholine by synaptosomes. Individual data points from one experiment are shown. The line is the least squares line of best fit, $Y = -2.6X + 21.3 \times 10^3$, $r = 0.860$. The Y intercept, V_{max}, corresponds to 69 nmoles/g of tissue/hr and the slope is $-K_T$.

ChAc in vitro. The results of these studies show that a non-substrate for ChAc, homocholine, is accumulated and acetylated by intact synaptosomes. Experiments now in progress are investigating further the transport and acetylation of homocholine by synaptosomes and also are determining whether homocholine, like pyrrolcholine and monoethylcholine (12), can serve as a precursor to a false transmitter at cholinergic terminals in the superior cervical ganglion.

Acknowledgements. The technical assistance of Delia Matriano is greatly appreciated. This research was supported by Research Grants MH24949 to L.A.B. and NS10124 to T.W.M.

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