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Study of the effect *in vivo* of oxotremorine on the activity of choline acetyltransferase (choline acetylase)

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IT HAS been demonstrated that the administration of oxotremorine (1-(2-pyrrolidono)-4-pyrrolidino-2-butyne, OT) to rats causes a substantial increase in the level of acetylcholine (ACh) in the brain.[1–4] Studies *in vitro* showed that the drug does not cause an increase in the activity of choline acetyltransferase (ChAc, acetyl-CoA:choline *O*-acetyltransferase, EC 2.3.1.6) nor an inhibition of acetylcholinesterase[5] (AChE, EC 3.1.1.7). It has also been observed that there is no effect *in vivo* on AChE.[3] * Therefore the possibility has been raised that there may be an effect *in vivo* on ChAc to account for the ACh increase and indeed such a stimulation of the enzyme in mice has been reported.[6]

In the course of some enzymic studies, the level of ChAc in mouse brain was determined 5, 15 and 30 min after i.p. doses of 0·1 and 0·5 mg/kg of OT. Our data, in contrast to the previously published report, showed that OT given to mice in tremor-producing doses did not cause a significant increase in ChAc activity.

Three groups of white, male mice, three per group, were given i.p. either (a) isotonic saline, (b) 0·1 mg/kg OT or (c) 0·5 mg/kg OT, as the sesquifumarate (Aldrich Chemical Company, Milwaukee, Wisc.). The animals were sacrificed by decapitation after 5, 15 and 30 min in three separate experiments. The onset of tremors in the drug-treated animals was approximately 5 min; the peak tremor effects occurred 15–20 min after drug. The cerebral cortex was removed within 1 min after decapitation and placed into liquid nitrogen. Cortices from the three mice of a given group were pooled; the total weight was approximately 1 g.

The procedures described by Schreier and Shuster[7] for preparation of mouse brain acetone powders, extraction of enzyme and radiometric measurement of ChAc activity utilizing labeled acetyl-Co-A (ACoA) were used. The final reaction mixture was composed of the following solutions:

0·14 *ml phosphate buffer, pH* 6·0, containing Na_2HPO_4, 0·025 M; choline Cl, 0·050 M; neostigmine Br (Sigma Chemical Co., St. Louis, Mo.) 0·0375 mM; and NaCl, 0·15 M.

0·03 *ml ACoA, Acetyl-1-*^{14}*C CoA* (New England Nuclear, Boston, Mass.), 0·247 mc per m-mole, 0·498 μc/ml, in 0·01 N HCl.

0·03 *ml acetone powder extract*. The protein concentration[8] in the extract (25 mg powder/ml) varied from 5 to 6·5 mg/ml so that the total protein in the incubation mixture ranged from 0·15 to 0·20 mg.

Incubations were carried out at 37° for 20 and 40 min; the reaction was initiated by the addition of enzyme solution which was stored at 0°. At the completion of the desired incubation time, the entire solution volume was transferred quickly to an individual 0·5 × 5 cm column of Dowex 1 × 8, Cl form, 200–400 mesh. The column was eluted with 1·2 ml of water, and effluent was collected directly into counting vials. The cpm of radioactive ACh were measured in a Packard liquid scintillation counter after the addition of 10 ml of DAM 611 counting solution.[7]

Blanks were determined by applying a complete incubation mixture to a resin column at zero incubation time; values in the range from 50–70 cpm were routinely recorded.

A typical time course of synthesis of ACh with a control enzyme is shown in Fig. 1. The data obtained with OT are summarized in Table 1. It can be seen that there was no significant increase in enzyme activity from control at either dose of OT, regardless of the incubation time or the time of sacrifice after drug. The only significant change observed was a decrease of activity in the animals which received 0·1 mg/kg and were sacrificed after 15 min. The lack of effect[5] (1×10^{-3} M) of both OT and Tremorine (1,4-dipyrrolidino-2-butyne), the precursor of the active OT, on the activity *in vitro* of the enzyme was also confirmed in separate experiments.

The observed level of enzyme activity is in good agreement with the value found by Holmstedt *et al.*[5] for rat brain. They reported 20·3 mμmoles of ACh formed per mg of acetone powder per hr at 37° while the corresponding value for the average of the saline controls in these experiments is 21·7 when the 20-min incubation values are converted to the same units.

* Unpublished data from this laboratory.

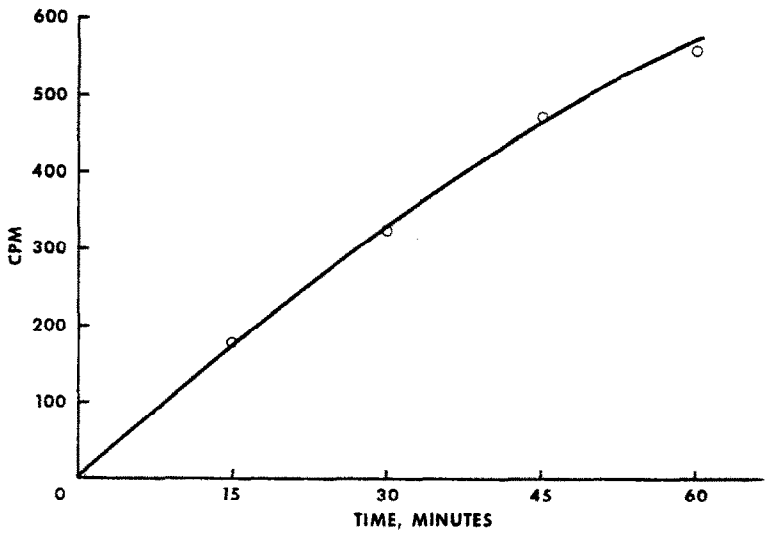

FIG. 1. Time course of incubation-ChAc activity. The cpm values were corrected for zero time background.

TABLE 1. ChAc ACTIVITY OF CONTROL AND DRUG-TREATED MICE AT TWO INCUBATION TIMES.

Test drug	mμmoles ACh formed*/mg protein	
	20-min incubation	40-min incubation
	(a) Animals sacrificed 5 min postdrug	
Saline control	29·2 ± 2·3	52·0 ± 3·9
0·1 mg/kg OT	27·8 ± 1·6	50·4 ± 1·5
0·5 mg/kg OT	28·8 ± 1·6	54·4 ± 1·7
	(b) Animals sacrificed 15 min postdrug	
Saline control	34·4 ± 0·4	62·5 ± 2·1
0·1 mg/kg OT	29·8 ± 1·3	53·6 ± 2·2
0·5 mg/kg OT	32·6 ± 1·5	60·7 ± 0·3
	(c) Animals sacrificed 30 min postdrug	
Saline control	32·6 ± 0·1	51·4 ± 1·4
0·1 mg/kg OT	33·3 ± 1·2	56·0 ± 1·2
0·5 mg/kg OT	30·6 ± 0·9	49·1 ± 0·5

* Average of duplicate assays ± standard deviation. The counting efficiency was utilized to convert cpm to mμmoles ACh.

The reason for the discrepancy in the enzymic effect of OT between these data and the prior report is not clear. One possibility, however, might be the mode of assay employed in the latter work, which utilized a coupled enzyme system for the generation of ACoA and a biological detection method for ACh rather than a direct measurement.

It would appear from the studies described here that increases in brain ACh observed after OT administration are not due to a direct stimulation of ChAc activity. An alternative explanation might be that the drug acts by mobilizing a precursor of ACh from some undetected source,[9] or that there are other factors in the situation *in vivo*, such as cofactors or membrane permeabilities, which affect enzymic activity in a way not seen in the cell-free system.

Chemical Pharmacology Department,
Abbott Laboratories,
North Chicago, Ill., 60064, *U.S.A.*

HERMAN H. STEIN
JEROME COHEN

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Studies on drug resistance—II. Kinase patterns in P815 neoplasms sensitive and resistant to 1-β-D-arabinofuranosylcytosine

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RESISTANCE toward 1-β-D-arabinofuranosylcytosine (Ara-C) was attributed by Chu and Fischer,[1] Kessel *et al.*,[2] Schrecker and Urshel,[3] and Uchida and Kreis[4] to a considerable decrease of the enzymatic conversion of Ara-C into 1-β-D-arabinofuranosylcytosine-5′-diphosphate (Ara-CDP) and -triphosphate (Ara-CTP) in the resistant cell line. As reported in our previous study,[4] Ara-C deaminase and cytidine-5′-diphosphate (CDP) reductase seem to be unrelated to the development of resistance in P815 cells. However, the marked difference in respect to phosphorylation of Ara-C is not a general finding in all the reported strains resistant to Ara-C. Momparler *et al.*[5] and Bach[6] failed to find significant differences in the phosphorylation of Ara-C in Ara-C resistant and parental cell lines.

The present experiments were undertaken to better characterize the discrepancy in the occurrence of Ara-CDP and Ara-CTP in sensitive (P815) and resistant (P815/Ara-C) cells. The results indicate that the lack of Ara-CDP and of Ara-CTP in P815/Ara-C cells is due to the almost complete absence of an active Ara-C kinase.

Tritiated 1-β-D-arabinofuranosylcytosine-5′-monophosphate (^{3}H-Ara-CMP) was prepared enzymatically from tritiated 1-β-D-arabinofuranosylcytosine (^{3}H-Ara-C) and purified by paper electrophoresis or paper chromatography. ^{3}H-Ara-C (1 c/m-mole, labeled predominantly in the 5 position) was kindly supplied by the Cancer Chemotherapy National Service Center. Crude enzyme extracts were prepared as reported[4] from BDF_1 mice bearing P815 and P815/Ara-C ascites tumors 6 days after inoculation. The assay mixture for enzymatic phosphorylation of labeled Ara-C and Ara-CMP contained: 3 μmoles adenosine-5′-triphosphate (ATP), 2·8 μmoles $MgCl_2$, 2·3 μmoles 3-phosphoglyceric acid, 3·3 to 10·0 mμmoles of substrate and 100 μl of enzyme extract, all in 250 μl of 100 mM Tris-HCl buffer, pH 8·0. After incubation for 30 min at 37°, the reaction was stopped by chilling the mixture in ice to 0°. An aliquot of the mixture was used directly for paper chromatography, system A of previous publication,[4] or paper electrophoresis in 100 mM citrate–phosphate buffer, pH 3·6, at 20 V/cm for 2 hr. The radioactivity of the spots corresponding to Ara-C, Ara-CMP, Ara-CDP and Ara-CTP was measured directly with liquid scintillation techniques.[7] The evaluation of 5′-nucleotidase activities in the crude enzyme extracts was performed by following the method of Carter,[8] using radioactive ^{3}H-Ara-CMP as substrate and paper electrophoresis for the separation of the reaction products.