AD-A174 605 CHOLINE UPTAKE BY GLOMERULAR SYNAPSES ISOLATED FROM BOVINE CEREBELLAR VERMIS(U) SCHOOL OF AEROSPACE MEDICINE BROOKS AFB TX D M TERRIAN ET AL. 1986 1/1

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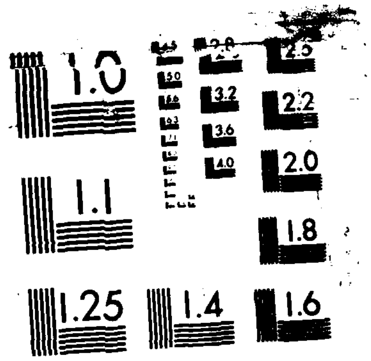

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SECURITY CLASSIFICATION OF THIS PAGE

AD-A174 605

(12)

1a REPORT SECURITY CLASSIFICATION Unclassified	1b. RESTRICTIVE MARKINGS
2a SECURITY CLASSIFICATION AUTHORITY	3 DISTRIBUTION / AVAILABILITY OF REPORT Approved for public release; distribution is unlimited.
2b DECLASSIFICATION / DOWNGRADING SCHEDULE	
4 PERFORMING ORGANIZATION REPORT NUMBER(S) USAFSAM-TR-85-80	5 MONITORING ORGANIZATION REPORT NUMBER(S)
6a NAME OF PERFORMING ORGANIZATION USAF School of Aerospace Medicine 6b OFFICE SYMBOL (If applicable) USAFSAM/NGNS	7a NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code) Aerospace Medical Division (AFSC) Brooks Air Force Base, TX 78235-5301	7b ADDRESS (City, State, and ZIP Code)
8a NAME OF FUNDING / SPONSORING ORGANIZATION Air Force Office of Scientific Research 8b OFFICE SYMBOL (If applicable) AFOSR/NL	9 PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c ADDRESS (City, State, and ZIP Code) Air Force Office of Scientific Research (AFSC) Bolling Air Force Base, DC 20332-6448	10 SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO 61102F; PROJECT NO 2312; TASK NO V4; WORK UNIT ACCESSION NO 01

11 TITLE (Include Security Classification)
CHOLINE UPTAKE BY GLOMERULAR SYNAPSES ISOLATED FROM BOVINE CEREBELLAR VERMIS (U)

12 PERSONAL AUTHOR(S) Terrian, David M.; Noisin, Eduard L.; and Thomas, William, E.

13a TYPE OF REPORT	13b. TIME COVERED	14 DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
FINAL	FROM May 85 TO Aug 85	1986	15

16 SUPPLEMENTARY NOTATION
Reprint of a periodical article appearing in Brain Research 366:401-404 (1986)

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17 COSATI CODES			18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	Bovine; cerebellar cortex; cerebellar glomeruli; choline uptake; hemicholinium-3
06	01		

19 ABSTRACT (Continue on reverse if necessary and identify by block number) Tritium

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20 DISTRIBUTION / AVAILABILITY OF ABSTRACT ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	21 ABSTRACT SECURITY CLASSIFICATION Unclassified
22a NAME OF RESPONSIBLE INDIVIDUAL David M. Terrian, Ph.D.	22b TELEPHONE (Include Area Code) (512) 536-3201 / 3262 22c OFFICE SYMBOL USAFSAM/NGNS

DD FORM 1473, 84 MAR   83 APR edition may be used until exhausted
All other editions are obsolete

SECURITY CLASSIFICATION OF THIS PAGE
UNCLASSIFIED

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Reprinted from:

21587

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ELSEVIER, AMSTERDAM
BIOMEDICAL DIVISION

Brain Research, 366 (1986) 401–404
Elsevier

BRE 21387

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[^{3}H]Choline uptake was investigated in a highly enriched preparation of glomerular particles isolated from bovine cerebellar vermis. Kinetic analysis indicates that a high-affinity choline uptake system with a relatively low maximum uptake velocity is present. At a substrate concentration of 10^{-7} M, [^{3}H]choline uptake was shown to be sodium-dependent, hemicholinium-sensitive and non-reactive to increasing concentrations of unlabeled choline. These findings support the presence of a high-affinity choline uptake system in cerebellar glomeruli and would seem to be consistent with the suggestion that a small proportion of mossy fibers are cholinergic in this brain region.

Mossy fibers represent one of the two principal afferent systems projecting to the cerebellar cortex where they terminate in a large glomerular synaptic complex. Over a quarter century has passed since Hebb and Silver[7] suggested that the cerebellar mossy fiber system was at least partially cholinergic; a variety of experimental approaches have generally supported this hypothesis. Histochemical studies have demonstrated that, in the cerebellar cortex of most mammalian species examined, acetylcholinesterase (AChE) is predominantly localized in the granular cell layer[4,18]. When visualized under higher magnification[2], the distribution of this cholinergic enzyme marker appears to correspond with glomerular structures in this cell layer. The exclusive localization of choline acetyltransferase (ChAT) in the mossy fiber terminals of rabbit[9] and human[10] cerebella has also recently been demonstrated using an immunohistochemical approach. In agreement with these studies are the results of biochemical analyses in which it has been shown that AChE and ChAT activities are both significantly reduced by deafferentation[11] and that acetylcholine (ACh)[8] and ChAT[3] are enriched in crude subcellular fractions of cerebellar glomeruli. However, despite the apparent consistency of these observations, a number of considerations render this issue unclear.

Intraspecies differences in the distribution of AChE and ChAT among cell layers[4] and between distinct areas of the cerebellar cortex[11] clearly exist. Any general principles of cholinergic organization which may pertain to the cerebellum have been largely obscured by this variability. It has also been noted that within the granular cell layer a potential contribution by the Golgi cells to histochemical staining cannot be ruled out, since these inhibitory interneurons also make synaptic contact with granule cells in the glomerulus and are known to contain small amounts of ChAT[11]. Finally, a major source of controversy has been a series of iontophoretic experiments in which the sensitivity of cerebellar granule cells to cholinomimetics was tested in the cat with conflicting results[1,16]. Thus, while some evidence suggests that a small proportion of the cerebellar mossy fibers may be cholinergic, a good deal of research remains to be accomplished.

A variety of biochemical parameters has been used to assess the potential cholinergic nature of

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mossy fibers. However, one cholinergic marker that does not appear to have been investigated is high-affinity choline uptake. The presence of a high-affinity uptake system for choline in brain tissue is well established and has come to be considered a relatively specific marker for cholinergic neurons and synaptic terminals[12,19]. This uptake system has an affinity (K_T) in the low micromolar range[22], contributes to the synthesis of ACh[5,6], is dependent on the presence of sodium ions[5,13], and is selectively inhibited by the compound hemicholinium-3 (HC-3)[6]. In the present study, choline uptake was investigated in a highly enriched fraction of isolated cerebellar glomeruli as a means of assaying for the presence of cholinergic terminals.

The bovine cerebellar vermis was used as the tissue source and glomerular particles were obtained as described previously[20]. Glomeruli isolated in this manner have been analyzed under the electron microscope and found to retain their structural integrity and to account for an estimated 92.6 ± 3.9% (mean ± S.E.M.) of the material in this fraction[20]. The final preparation was resuspended in 0.32 M glucose to give a protein concentration of 13.25 ± 1.30 mg/ml (mean ± S.E.M.) and the uptake of [methyl-^{3}H]choline chloride (Amersham, spec. act. 78 Ci/mmol) was assayed by a modification of a procedure which has also been described previously[20]. Briefly, aliquots (50 μl) of the glomerular suspension were diluted with 900 μl of a buffered Krebs–Ringer solution (pH 7.4) which had been saturated with 95% O_2–5% CO_2 prior to use. A final volume of 1.0 ml was obtained by adding varying concentrations of [^{3}H]choline. Preliminary experiments demonstrated that the uptake of 0.1 μM [^{3}H]choline increases in a linear fashion for up to 10 min at 37 °C and that the rate of [^{3}H]choline uptake was also linearly related to the concentrations of glomerular protein used in these studies (data not shown). All subsequent incubations were carried out for 5 min at 37 °C. Uptake was terminated by sedimenting particulate material on a microfuge and rinsing the resultant pellet twice with ice-cold incubation medium[14]. The final pellet was solubilized overnight in 1.0 ml of 1.0 N NaOH and the radioactivity of each sample was measured, following acidification, in an LKB Rackbeta liquid scintillation counter with a counting efficiency of approximately 39%. Controls were treated in an identical manner except that incubations were performed at 0–5 °C. All uptake values presented correspond to the total uptake at 37 °C minus the amount measured in control samples.

The kinetics of the observed uptake were determined by double-reciprocal plots according to the format of Lineweaver and Burk, and the X and Y intercepts were determined by linear regression analysis. The remaining data were analyzed statistically by a t-test. Protein was measured by the method of Lowry et al.[15], following precipitation in trichloroacetic acid (5.0%, w/v) at 0–5 °C for 30 min.

Measurement of the rate of [^{3}H]choline uptake over a substrate concentration range of 5 × 10^{-8} to 5 × 10^{-6} M indicated that high-affinity uptake sites were present in cerebellar glomeruli. Kinetic analysis (Fig. 1) estimates the K_T of this choline uptake system to be 1.42 ± 0.34 μM and the maximal uptake velocity (V_{max}) to be 6.67 ± 0.98 pmol·mg protein·min^{-1} (mean ± S.E.M.). The sodium dependence of [^{3}H]choline uptake and its sensitivity to HC-3 were tested at a substrate concentration of 0.1 μM with the results shown in Fig. 2. Equimolar substitution of sodium ions with sucrose in the incubation medium reduced [^{3}H]choline uptake by 75.2 ± 7.7% (mean ± S.E.M.) and HC-3, at a final concentration of 5 μM, consistently inhibited uptake by greater than 90%. Furthermore, the possibility that a homoexchange process[17] contributed to the observed uptake

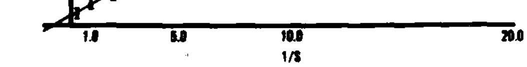

Fig. 1. Double-reciprocal plot of high-affinity [^{3}H]choline uptake by glomerular particles isolated from the vermal cortex of bovine cerebellum. V = pmol·mg protein·min^{-1}; S = choline concentration (10^{-6} M). Kinetic parameters, K_T and V_{max}, were obtained by computer analysis and are respectively as follows: 1.42 ± 0.34 and 6.67 ± 0.98. The data are the means ± S.E.M. of duplicate determinations in 4 separate experiments.

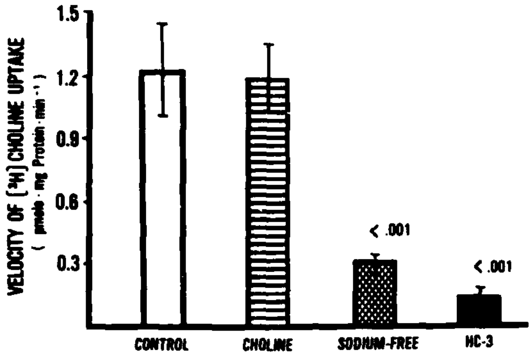

Fig. 2. Inhibition of active [^{3}H]choline uptake into glomerular particles by incubation in the presence of unlabeled choline (125 μM: striped bar), a sodium-free medium (cross-hatched bar), and hemicholinium-3 (5 μM; solid bar). Uptake data are means ± S.E.M. of duplicate determinations from three separate experiments. All samples were preincubated (5 min) before adding [^{3}H]choline (0.1 μM) to the medium and incubating for 5 min at 37 °C. Hemicholinium-3 was present during preincubation; all other uptake procedures were as described in the text.

was ruled out by demonstrating that the amount of radioactivity accumulated by prelabeled (5 min) glomerular particles was not significantly reduced when the concentration of unlabeled choline was increased to 125 μM and glomeruli were incubated an additional 3 min (Fig. 2).

Thus, an active high-affinity process for [^{3}H]choline uptake was observed in glomerular particles isolated from the vermis of bovine cerebellum. This uptake was sodium dependent, almost completely inhibited by low micromolar concentrations of HC-3, and does not appear to be attributable to an exchange of radiolabeled for unlabeled choline. These features of choline uptake in this nerve ending preparation are consistent with those properties reported for high affinity choline uptake in other brain regions of various species[5,6,17,19,22].

The present results are in agreement with the hypothesis that a small proportion of cerebellar mossy fibers are cholinergic in nature. While the assignment of choline uptake to the glomerular synaptic complex cannot be unequivocally established, the high purity of this subcellular fraction is extremely supportive in this regard. Assuming the association of choline uptake with cerebellar glomeruli is valid, the most likely candidate for cholinergic function would seem to be the excitatory mossy fiber terminal. Autoradiographic localization of high affinity choline uptake sites in glomerular particles would provide an important test of this hypothesis. The suitability of the glomerular preparation for such an analysis has been demonstrated previously in studies in which γ-aminobutyric acid uptake sites were shown to be specifically associated with the inhibitory Golgi axon terminals[21]. Yet another critical test which remains to be accomplished is the determination of whether or not glomerular particles release recently synthesized acetylcholine in a calcium-dependent manner following membrane depolarization. Work in this laboratory is being conducted in both of these areas in an attempt to further assess the possibility that cholinergic mossy fibers innervate the cerebellar cortex.

This research was sponsored by the U.S. Air Force Office of Scientific Research under Task 2312W3 and Contract F49620-82-C-0035. The expert technical assistance of SSgt Jody Stewart and Ms. Marybeth Hofstetter is gratefully acknowledged.

1 Crawford, J.M., Curtis, D.K., Voorhoeve, P.E. and Wilson, V.J., Acetylcholine sensitivity of cerebellar neurones in the cat, *J. Physiol. (London)*, 186 (1966) 139–165.

2 Csillik, B., Joo, F. and Kasa, P., Cholinesterase activity of archicerebellar mossy fibre apparatuses, *J. Histochem. Cytochem.*, 11 (1963) 113–114.

3 Fonnum, F., Application of microchemical analysis and subcellular fractionation techniques to the study of neurotransmitters in discrete areas of mammalian brain, *Adv. Biochem. Pharmacol.*, 6 (1972) 75–88.

4 Friede, R.L. and Fleming, L.M., A comparison of cholinesterase distribution in the cerebellum of several species, *J. Neurochem.*, 11 (1964) 1–7.

5 Haga, T., Synthesis and release of [^{14}C]acetylcholine in synaptosomes, *J. Neurochem.*, 18 (1971) 781–798.

6 Haga, T. and Noda, H., Choline uptake systems of rat brain synaptosomes, *Biochim. Biophys. Acta*, 291 (1973) 564–575.

7 Hebb, C.O. and Silver, A., Choline acetylase in the central nervous system of man and some other mammals, *J. Physiol. (London)*, 134 (1956) 718–728.

8 Israel, M. and Whittaker, V.P., The isolation of mossy fibre endings from the granular layer of the cerebellar cortex, *Experientia*, 21 (1965) 325–326.

9 Kan, K.K.S., Chao, L.P. and Eng, L.F., Immunohistochemical localization of choline acetyltransferase in rabbit spinal cord and cerebellum, *Brain Research*, 146 (1978) 221–229

10 Kan, K.K.S., Chao, L.P. and Forno, L.S., Immunohistochemical localization of choline acetyltransferase in the hu-

man cerebellum, *Brain Research*, 193 (1980) 165–171.

11 Kasa, P. and Silver, A., The correlation between choline acetyltransferase and acetylcholinesterase activity in different areas of the cerebellum of rat and guinea pig, *J. Neurochem.*, 16 (1969) 389–396.

12 Kuhar, M.J., Neurotransmitter uptake: a tool in identifying neurotransmitter-specific pathways, *Life Sci.*, 13 (1973) 1623–1634.

13 Kuhar, M.J. and Murrin, L.C., Sodium-dependent, high affinity choline uptake, *J. Neurochem.*, 10 (1978) 15–21.

14 Levi, G. and Raiteri, M., GABA and glutamate uptake by subcellular fractions enriched in synaptosomes: critical evaluations of some methodological aspects, *Brain Research*, 57 (1973) 165–185.

15 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J., Protein measurement with Folin phenol reagent, *J. Biol. Chem.*, 193 (1951) 265–275.

16 McCance, I. and Phillis, J.W., Cholinergic mechanisms in the cerebellar cortex, *Int. J. Neuropharmacol.*, 7 (1968) 447–462.

17 Simon, J.R., Atweh, S. and Kuhar, M.J., Sodium-dependent high affinity choline uptake: a regulatory step in the synthesis of acetylcholine, *J. Neurochem.*, 26 (1976) 909–922.

18 Shute, C.C.D. and Lewis, P.R., Cholinesterase-containing pathways of the hindbrain: afferent cerebellar and centrifugal cochlear fibers, *Nature (London)*, 205 (1965) 242–246.

19 Sorimachi, M. and Kataoka, K., Choline uptake by nerve terminals: a sensitive and specific marker of cholinergic innervation, *Brain Research*, 72 (1974) 350–353.

20 Terrian, D.M., Butcher, W.I., Wu, P.H. and Armstrong, D.L., Isolation of glomeruli from areas of bovine cerebellum and comparison of [^{3}H]serotonin uptake, *Brain Res. Bull.*, 14 (1985) 469–475.

21 Wilkin, G., Wilson, J.E., Balazs, R., Schon, F. and Kelly, J.S., How selective is high affinity uptake of GABA into inhibitory nerve terminals?, *Nature (London)*, 252 (1974) 397–399.

22 Yamamura, H.I. and Snyder, S.H., Choline: high-affinity uptake by rat brain synaptosomes, *Science*, 178 (1972) 626–628.

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