Quantitative Measurement of the Choline Acetyltransferase Activity of the Mauthner Cell Axon

Penny L. Eddy, Donald A. Godfrey, Judy A. Parli and C. David Ross
Department of Physiology, Oral Roberts University, Tulsa, OK 74171

To examine the possible cholinergic nature of the Mauthner cell, freeze-dried sections of goldfish spinal cord were prepared, enabling access to the Mauthner axons. Profiles of the Mauthner axons were dissected out, weighed, and quantitatively assayed for activity of choline acetyltransferase. The Mauthner axon was shown to contain significantly less activity than surrounding tissue containing motor neurons, which are likely to be cholinergic. Because of the relatively small amount of choline acetyltransferase activity found in the axons, it is tentatively concluded that acetylcholine probably does not serve a synaptic transmission function in the Mauthner cell, but more study will be necessary before a definite conclusion can be reached.

INTRODUCTION

The Mauthner cells are a pair of giant neurons found in cyclostomes, larval amphibians, and certain fish. The two Mauthner cell bodies are located in the brain stem, and their axons run caudally through the spinal cord. The main function of the Mauthner cells is controlling the reflex response of the locomotion muscles in the tail (1). The Mauthner cell has been studied extensively because of its large size and distinct function, and is one of the best characterized neurons in a vertebrate central nervous system (2). An extensive histological study of the Mauthner cell and surrounding tegmenti was done by Bartelmez (3), using adult and larval teleosts. Goldfish have been used extensively in studies of the Mauthner cell and were chosen for the present study because of their ready availability and low cost.

Acetylcholine is one of the most extensively studied neurotransmitters. Its synthesis is catalyzed by the enzyme choline acetyltransferase (ChAT) and its hydrolysis by acetylcholinesterase (AChE) (4). These enzymes have been preferred to acetylcholine itself as markers to identify neurons as cholinergic (using acetylcholine as transmitter), ChAT being more definitive than AChE (5). Although early work employing histochemical staining for AChE, among other techniques, suggested that the Mauthner cell might be a cholinergic neuron (6), a recent investigation found no distinguishable ChAT immunoreactivity in the Mauthner cell (2). Such a negative result could occur if the rat antibody used in the study is specific for a species of ChAT different from that of the goldfish Mauthner cell (2) or fails to penetrate to its antigenic site on the enzyme molecule (7). Because of these uncertainties, the present study employed a direct quantitative assay for ChAT enzyme activity of the Mauthner cell axon. In a cholinergic neuron, both the soma and axon contain acetylcholine and the enzymes for its metabolism, ChAT and AChE (8,9). The Mauthner axons in the spinal cord were sampled in the present study because of their large diameter and the technical advantage that each transverse section of spinal cord contains both Mauthner axons.

MATERIALS AND METHODS

The goldfish used in this study were purchased locally and maintained in a ten-gallon tank at room temperature. Most fish used were 5 - 6 cm in length. After the fish were anesthetized by immersion in 0.1% ethyl *m*-aminobenzoate, brain and spinal cord tissue were isolated and frozen, within 20 min of death, in Freon cooled to -130 °C with liquid nitrogen (10). Frozen tissue was mounted onto wooden dowels with brain paste and stored at -80 °C for future sectioning. Transverse sections 20 or 40 µm thick were cut at -20 °C with a microtome in a cryostat (American Optical Cryo Cut). Alternate sections were saved in aluminum racks for freeze

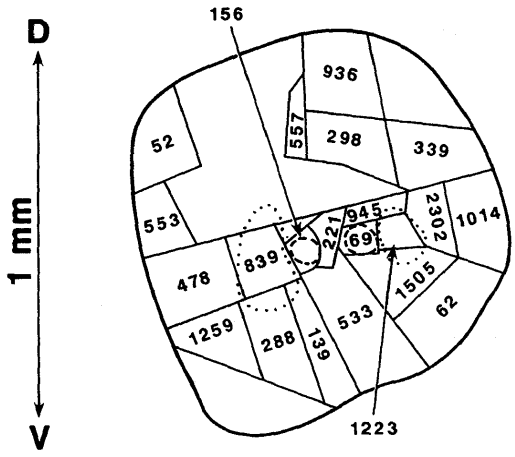

FIGURE 1. Map of microdissected freeze-dried transverse section of goldfish spinal cord. Thicker solid line is outline of section; dashed lines are borders of Mauthner axons seen in freeze-dried section itself; dotted lines are borders of regions containing somata of motoneurons seen in adjacent thionin-stained frozen section. Thinner lines are boundaries of cut samples. Numbers inside sample outlines are choline acetyltransferase (ChAT) activities in μmoles of acetylcholine formed per kilogram dry weight of tissue per minute. Scale shows 1 mm length in map as well as dorsal (D) and ventral (V) directions. The axon samples in this section are representative in terms of the relative proportion of the volume occupied by surrounding tissue. The average amount of surrounding tissue for all the axon samples assayed was 23%.

drying or thawed onto microscope slides for staining with thionin (11) or staining for AChE activity (12). Freeze-dried sections were stored under vacuum at -20 °C in glass vacuum tubes (Ace Glass Company).

Identification of the Mauthner axons in freeze-dried sections was mainly by size and location. They are the largest axons in the spinal cord, ranging from 45 to 80 μm in diameter (13), and are located ventrally on either side of the midline. Boundaries within freeze-dried sections were mapped using a Wild dissecting microscope with a drawing tube attachment. Boundaries seen in thionin-stained sections were traced onto each map to aid in identification of Mauthner axons and the somata of other neurons present in the spinal cord. The spinal cord sections were dissected and the exact locations of sample boundaries recorded onto the maps (Fig. 1) (14). Dry weights of samples were measured using quartz fiber balances (10), then samples were placed into 400 μL-capacity microcentrifuge tubes for assay of ChAT activity. The determination of ChAT activity was based on the radiometric method of McCaman and Hunt (15) using the sodium tetraphenylboron procedure of Fonnum (16) for the extraction of [1- ^{14}C] acetylcholine (17).

Contamination of Mauthner axon samples by surrounding tissue, which averaged 23% of total sample volume, was corrected for by measuring the areas of the axons and surrounding tissue in the maps of the freeze-dried sections (Fig. 1). The ChAT activity of the surrounding tissue included in each axon sample was estimated as the average activity of those samples surrounding the axon sample. The formula used to calculate the ChAT activity of the Mauthner axon alone was:

$$\text{Mauthner axon ChAT activity} = [(A_t)(ChAT_t)-(A_s)(ChAT_s)]/(A_a),$$

where A_t = total area of dissected sample containing axon, $ChAT_t$ = ChAT activity of dissected sample containing axon, A_s = area of surrounding tissue included in the axon sample, $ChAT_s$ = estimated ChAT activity of surrounding tissue included in the same sample with the axon, and A_a = area of axon.

For comparison to the ChAT activities measured in freeze-dried tissue, homogenates of brain and spinal cord from two goldfish, prepared as one part of tissue by weight to nine parts of 50 mM potassium phosphate buffer, pH 7.2, were also assayed.

RESULTS

Samples of Mauthner axons were isolated from four transverse sections from each of two fish. Although both axons were found in each section, in some cases only one was successfully dissected and assayed. Average data for axons, motoneuron regions, and homogenates are presented in Fig. 2.

DISCUSSION

A limitation of the present data was that the axons were not cleanly isolated from the

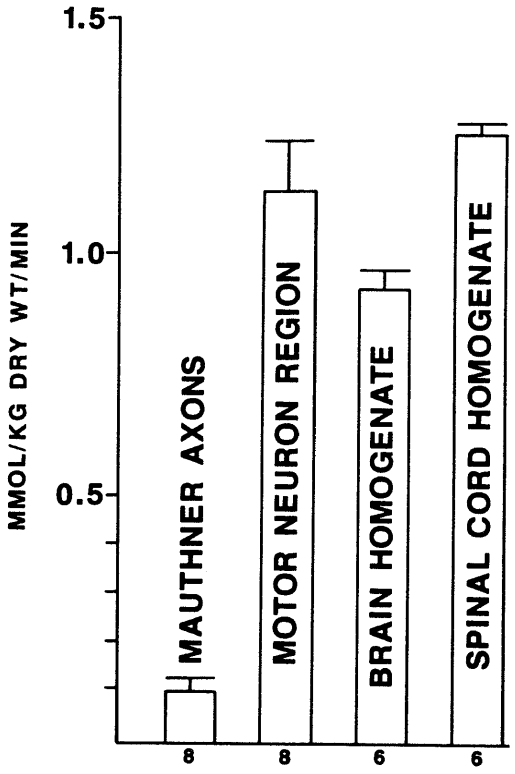

FIGURE 2. Bar graph representing means and standard errors of choline acetyltransferase (ChAT) activities of freeze-dried samples of Mauthner axons and regions containing motoneuron somata as well as of homogenates of whole brain and spinal cord of goldfish. The height of the bar represents the mean and the height of the line above the bar represents the standard error (S.E.). The values for the Mauthner axons were corrected for contamination of surrounding tissue, as described in the methods. The average uncorrected ChAT activity ± S.E. was 212 ± 50 μmol/kg dry wt/min. Homogenate activities were converted to the dry weight basis by multiplying by 4, since wet weight is approximately 4 times dry weight for a given volume of tissue. The number under each bar gives the number of sections included for freeze-dried samples and number of determinations for homogenates.

surrounding tissue, which routinely contained relatively high ChAT activity. The ChAT activity of the axons from the mapped sections could, however, be corrected for this contamination. The results support the conclusion of Rhodes et al. (2) that the Mauthner cell probably is not cholinergic, in that the ChAT specific activity in the Mauthner axons was much less than the activity of samples containing the presumably cholinergic motor neuron somata. Expected values for a cholinergic neuron in goldfish may be as high as 50 x 10^3 μmol/kg dry wt./min (18), 500 times the average for Mauthner axons in the present study. On the other hand, compared to a tract considered noncholinergic, namely the goldfish optic tract (18), the average ChAT activity of the Mauthner axon is somewhat higher, about ten times as high. Other than possibly being the result of technical errors, the finding of this small amount of ChAT activity is not readily explained. It is possible that a relatively small amount of ChAT in a neuron may not be related to cholinergic synaptic transmission (8), or it may indicate an accessory role for acetylcholine in support of some other neurotransmitter. However, it is also possible that in an axon as large as the Mauthner axon, the concentration of ChAT is low because a diffuse distribution throughout the abundant cytoplasm (2) is sufficient to provide an adequate supply to the axon terminals. Thus, it is not yet possible to entirely discount a cholinergic character of the Mauthner cell. Additional evidence might be obtained by assaying the ChAT activity in the Mauthner cell body since it is not as much larger, compared to other neurons, as is the axon.

ACKNOWLEDGEMENTS

This study received partial support from NIH grants NS17176 and EY03838 as well as Oral Roberts University intramural funds. Instructional assistance to P.E. received from Michael Biavati, M.D., and additional guidance from Dr. Frank Wilbur are greatly appreciated.

REFERENCES

1. H.B. Sarnat and M.G. Netsky, *Evolution of the Nervous System*, 2nd ed., Oxford Univ. Press, New York, N.Y., 1981, pp. 207-210.

2. K.J. Rhodes, S.J. Zottoli, and E.J. Mufson, Brain Res. 381:215 - 224 (1986).

3. G.W. Bartelmez, J. Comp. Neurol. 25:87-128 (1915).

4. J.R. Cooper, F.E. Bloom, and R.H.

Roth, *The Biochemical Basis of Neuropharmacology,* 5th ed., Oxford Univ. Press, New York, N.Y., 1986, pp. 173-202.

5. D.A. Godfrey, J.L Park, J.D. Dunn, and C.D. Ross, *in*: D.G. Drescher (Ed.), *Auditory Biochemistry,* Charles C. Thomas, Springfield, IL, 1985, pp. 163-183.

6. J.W. Day, D.H. Hall, L.M. Hall, and M.V.L Bennett, J. Neuroscience 3:272-279 (1983).

7. J.M. Polak and S. Van Noorden, *An Introduction to Immunocytochemistry: Current Techniques and Problems,* Oxford Univ. Press, New York, N.Y., 1984, p. 6.

8. C.O. Hebb, Physiol. Rev. 37:196-220 (1957).

9. F. Fonnum, M. Frizell, and J. Sjöstrand, J. Neurochem. 21:1109 - 1120 (1973).

10. O.H. Lowry and J.V. Passonneau, *A Flexible System of Enzymatic Analysis,* Academic Press, New York, N.Y., 1972.

11. O.H. Lowry, J. Histochem. Cytochemistry 1:420-428 (1953).

12. A. El-Badawi and E.A. Schenk, J. Histochem. Cytochem. 15:580-588 (1967).

13. P.G. Funch, S.L. Kinsman, D.S. Faber, E. Koenig and S.J. Zottoli, Neurosci. Lett. 27:159 -164 (1981).

14. D.A. Godfrey and F.M. Matschinsky, J. Histochem. Cytochem. 24:697-712 (1976).

15. R.E. McCaman and J.M. Hunt, J. Neurochem. 12:253 - 259 (1965).

16. F. Fonnum, Biochem. J. 115:465 – 472 (1969).

17. D.A. Godfrey, A.D. Williams, and F.M. Matschinsky, J. Histochem. Cytochemistry 25:397 - 416 (1977).

18. C.D. Ross and D.A. Godfrey, Brain Res. 373:49-56 (1986).