CYTOCHROME OXIDASE ACTIVITY IN THE VENTRAL HORN CELL OF THE SPINAL CORD IN THE RABBIT: ULTRA STRUCTURAL STUDY

Ali .A. A. Al-Taii *MBChB MSc PhD*

Abstract

Background: Dynamic property of the neurons in the ventral horn cells of the spinal cord in the rabbit were partly treated utilizing the activity of cytochrome oxidase, a mitochondrial enzyme which is responsible for electron transport in oxidative phosphorylation needed for vital processes.

Objective: It has been known recently that the vital role of this enzyme was clearly evident in the apoptosis. This study high lightens a point on a part of the metabolic map of the anterior horn cells with the use of cytochrome oxidase activity as a tool.

Methods: Healthy adult New Zealand rabbits in resting condition were used. After Laminectomy slices of cord tissue obtained from the lumbosacral region precisely its gray mater of the anterior horn, then they were treated with a histochemical method based on the oxidative polymerization of diamino-benzidine, then examined under electron microscopy.

Result: Results revealed different intensity of final reaction products at both cellular and subcellular levels.

Conclusion: This reflects that the oxidative metabolism was varied in this area of the CNS, the majority of this activity were due to effect of the higher centers. At subcellular level the initiation of retrograde cell reaction "apoptosis" correlates positively with intensity of the FRP.

Key word: Cytochrome oxidase, Enzyme commission number, Central nervous system, Final reaction product

IRAQI J MED SCI, 2006; VOL. 5 (1): 8-12

Introduction

In order to understand the dynamic properties of neurons, there is a certain fact must outlined from the study of an important energy-deriving oxidative enzyme in the mitochondria. Cytochrome oxidase, **(C.O.)** (Enzyme commission number 1.9.3.1) is a perfect tool for this study. Mammalian Spinal cord precisely its gray matter is composed of a heterogeneous population of neurons whose physiological characteristics often elude morphological identification. The anterior horn cells of the spinal cord contains alpha, gamma motoneurons, Renshaw neurons, and interneuron's[1].

This study high lightens a point on a part of the metabolic map on the anterior horn cells of the mammalian spinal cord with the use of **(C.O.)** activity as a tool. This method is easily to be handled and not coasty in our laboratory, as traditional histochemical methods are still in use for the detection of **(C.O.)** activity because they are rapid, inexpensive and more specific.

The cytochrome oxidase is responsible for electron transport in the oxidative phosphorylation needed for vital processes such as protein synthesis, rapid axoplasmic transport within neurons and maintenance of the resting membrane potential[2]. It is a characteristic enzyme of the mitochondrial membrane which is firmly bound to it. In a highly active cell large amounts of mitochondria can be seen. Thus, the activity of **(C.O.)** is used as an index of the oxidative metabolism in these cells[3]. However morphological, physiological and biochemical properties of neurons, reflects the endogenous system of enzymes.

This system is intimately associated with neuronal metabolic machinery, which is closely related to the level of neuronal activity[4]. This confirmed the finding of many workers, that oxidative metabolic capacity following the ablation of rat

Dept. Human Anatomy, College of Medicine, Al-Nahrain University
Address correspondence to Dr. Ali A..A. Al-Taii
Received 12th November 2005: Accepted 6th March 2006.

sensorimotor cortex on the **(C.O.)** activity which shows that in the injured animals there is a significantly reduced activity throughout the cerebral cortex and in 5 of 11 subcortical structures. This injury-induced depression of oxidative capacity was most pronounced in regions of the hemisphere ipsilateral to the ablation[5].

However, the vital role of **(C.O.)** was evident in neurons of the injured segments of the spinal cord of rats which shows a substantial amount of **(C.O.)** released into the cytoplasm from the mitochondria of the injured neurons suggesting a trigger for apoptosis through the activation of proteases, such as caspase 3[6, 7].

Material & Methods

Cytochrome oxidase was examined in the ventral horn cell of 10 New Zealand rabbits weighting 3-3.5 Kg. They were killed by sectioning of the great vessels of the neck without anesthesia. Laminectomy was performed, dura and arachnoid opened in the lumbo-sacral region. Cord segments with roots of sciatic nerve of both right and left sides were removed then placed in a small Petri dish containing 30 ml of saline solution at 37ºC. Serial coronal sections with a sharp razor were cut through the ventral horn of the cord and then minced into a small slices. Immediately, those slices were transferred to a small containers that contained a cold fixative containing 2% paraformaldehyde, 1-2% glutaraldehyde in a 0.1 M sodium phosphate buffer at pH 7.3 for 10 min. then washed with phosphate buffer for 10 min prior to incubation.

The histochemical method used was that of Seligman[8] with modification. The method is based on the oxidative polymerization of diamino- benzidine (DAB). It gives very precise localization of the enzyme activity, and the reaction product is permanent and clearly evident.

Tissues slices were incubated in a Columbia jar containing 5 mg Diaminobenzidine (DAB) (Sigma) in 9 ml of 0.05 M, pH 7.4. To this solution, Cytochrome C (BDH) 10mg as substrate and 1 ml Catalase (20µg/ml) (BDH) were used to eliminate the presence of any endogenous H_2O_2. Sucrose (BDH) 750mg was also added. Control sections were treated in the same way without addition of Cytochrome C. Time of incubation was ranged from 30 minutes to 2 hours. The tissue slices were removed when brown reaction product appeared, and then washed overnight in phosphate buffer, treated with 1% osmium tetroxide for 1.5 hours, stained with 1% uranyl acetate, and embedded in Epon. Semi thin sections (0.5–1 µ) were obtained, stained with 1% methylene blue. Those sections were used for selecting the most adequate areas to be examined for the ventral horn neurons. Ultra thin sections (60–90 nm) were taken and examined in Philips CM10 electron microscope operating at 60 kV; some sections were examined without staining.

Results

Good results were visualized within 2 hours of incubation by the DAB reaction as the color of the minced tissues slices became dark - brown in color. Examination of the semi thin sections (0.5–1 µ) treated with 1% methylene blue, delineated different sizes of ventral horn neurons. Electron microscopic examination revealed that those neurons are richly endowed with a variety of organelles. With a higher magnification we overcome the failed of the somata and mitochondria can be easily visualized. Reactive mitochondria were varied in their intensity of the FRP from moderate to dark, although light ones have also been observed (Figures 1 & 2).

In addition to that different intensity of the FRP at cellular level was also observed. With higher magnification single mitochondria was clearly seen with a heavy precipitates of a black dots of the FRP. This activity was localized to the inner mitochondrial membrane and their intracristal spaces (Figure 3).

Examination of tissue sections treated with DAB reaction without the

addition of the substrate shows no reactivity i.e. any black dots in the mitochondria (Figure 4).

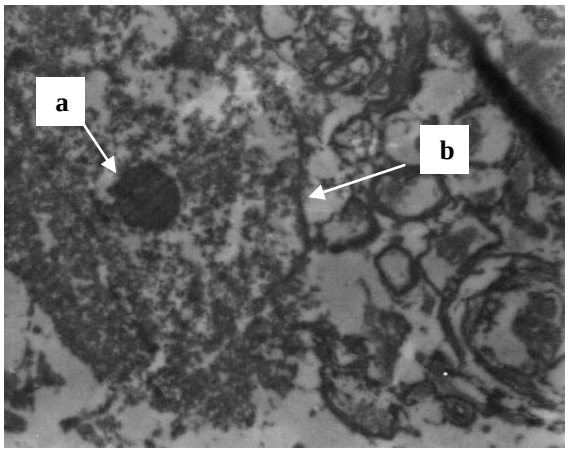

Figure 1: Motoneuron (a: nucleus, b: cell membrane) x 3000

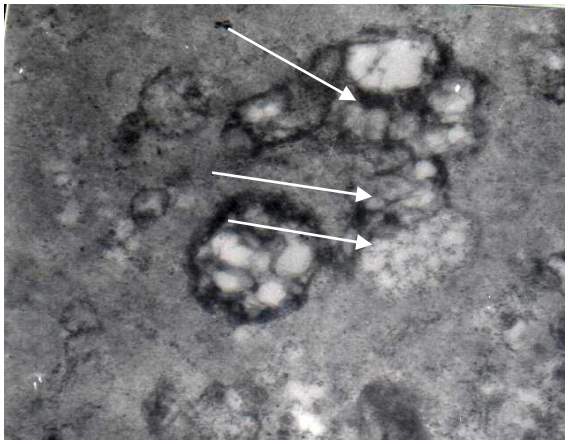

Figure 2: Arrows shows different intensity of the FRP in the mitochondria. x 12000

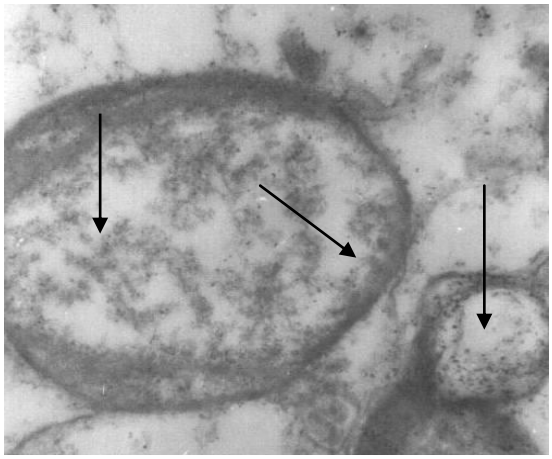

Figure 3: Higher magnification of mitochondria. Arrows show FRP of (C.O.) activity mainly confined to the inner mitochondrial membrane and their intracristal spaces. x 40 000

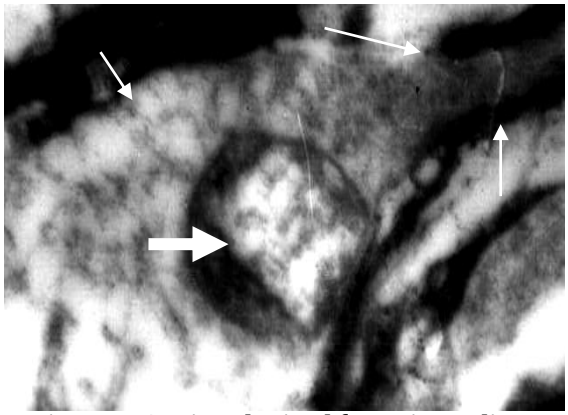

Figure 4: Section obtained from tissue slices without addition of substrate. Thin arrows show boundaries of axonal terminal. Thick arrow shows mitochondria. x 40 000.

The most active area seen in the sections were in the neuropil (dendrites and axon terminals) of the different size somata. However the activity of (**C.O**.) were found nearly in all sections examined within the mitochondria of the somata regardless their size with the exception that, the small size somata exhibit more reactivity in comparison to the large size ones.

Darkly reactive, moderately reactive, and /or lightly reactive mitochondria were evident in dendrites, axonal trunks and axonal terminals of myelinated axons. However the more intense reactivity was observed in the mitochondria of the axonal terminals than those of the dendrites , It is hardly to detect lightly reactive mitochondria in the axon terminals. (Figures 5 & 6).

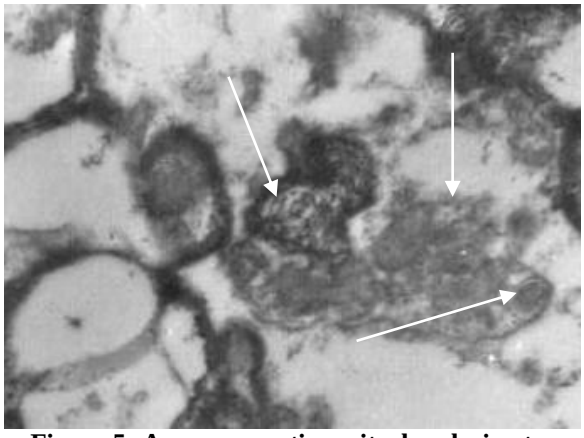

Figure 5: Arrows reactive mitochonderia at dendritic terminals.Several of these synapses show different intensities of C.O. activity. x 12000

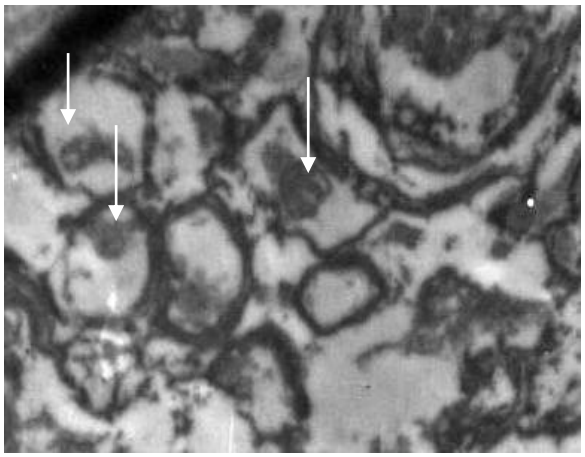

Figure 6: Arrows show highly reactive mitochondria in the axonal terminal. x 12000

Discussion

The results of this study show that the DAB method used, specifically demonstrated the **(C.O.)** activities. Among other things this assumption is supported by ultrahistochemistry showing FRP only in the mitochondria and the reaction was completely absent with omission of the substrate (Figure 4) .In addition, mitochondria show slightly swelling due to the method of the incubation used in this work (Figure 3) ,since this shows perfect visualization of the FRP. With the use of a computer image processing system inhibition of enzyme activity by chemical fixation was variable in single motoneurons of the rat spinal cord[9]. However, those workers concluded that quantification of enzymatic activity in chemically fixed tissue provides an imprecise estimate of enzyme activities found in fresh-frozen tissues, this idea was omitted in our study.

Different intensities of the FRP .in the mitochondria in the somata of the ventral horn neurons have been observed (Figure 2). This indicates that the entire neuron is often not metabolically homogeneous. This is in agreement with finding of Wong-Riley in 1989[10].

The production and requirements of energy reflect the differences in cellular activity in this part of the central nervous system, as small size neurons i.e. interneuron's and Renshaw neurons shows more reactivity than those of large size one i.e. motoneurones, since the large metabolic demands of the ventral horn neurons are clearly evident as their known involvements in the majority of synaptic interactions to keep muscular tone and movements[1].

The subcellular distribution of (**C.O.)** activity in the motoneurons of the ventral horn cells primarily to profiles within the neuropil rather than somata, as the highly reactive mitochondria were mainly localized in the axonal terminal rather than that of dendrite ,as the later region contain different forms of reactivity (Figures 5 & 6). This observation differs from that observed by Wong-Riley and Kageyama in 1986 that the reactive mitochondria in the ventral horn neurons were found in the neuropil, mainly in dendritic profiles and some axon terminals. However the high metabolic activity required by dendrites is consistent with the notion that, as the principal postsynaptic targets in most **CNS** regions. Their high energy consumption is required for membrane repolarization, since there are differences in the in the metabolic activity between regions of the ventral horn cells neuropil where in the large sizes cells the axonal terminals gain more reactivity which elude the higher metabolic effects of the higher centers on those neurons.

Many tools have been used for metabolic mapping of nervous tissue. These are 2-deoxy-glucose autoradiography, cytochrome oxidase histochemistry and positron emission tomography. However these methods have been used to map the brain during normal or resting conditions, and during conditions of particular interest such as sensory stimulation, development, ageing and disease[11].

In this study we examined **(C.O.),** a mitochondrial enzyme which is a marker for neuronal functional activity. However the enzyme was distributed in a characteristic patterns and amounts that differed among various neuronal pathways in this region of the **CNS**. Mapping was done quantitatively by enzyme histochemistry on resting"

animals**.** However this issue needs further study using optical densitometry of stained sections and biochemical assays of spinal cord tissue homogenates. The metabolic map of cytochrome oxidase activity reveals patterns of normal spinal cord function, and may be used as a marker for comparison in studies of spinal cord development and plasticity.

Intrasomatic densities of enzyme histochemical reaction product were employed as indicators of relative mitochondrial activity (**C.O.**)[12].The present results revealed different intensities of **(C.O.)** in different site of the ventral horn neurons and neuropil. This reflects different responses to injury as it creates a depression of oxidative metabolism capacity. However, retrograde cell reaction in the ventral horn cell of spinal cord regarded as apoptosis structurally[13]. Thus, the site initiation of this phenomenon in the ventral horn neurons was positively correlated with the **(C.O.)** activity, since this point needs further investigation.

References

1. Bannister LH, Berry MM, Collins P, Dyson M, Dussek JE, and Ferguson MWJ: Nervous System. In: Gray's anatomy, 38th edition. 1999; p.p. 739-771, p.p. 957-74.
2. Gautheron DC: Mitochondrial oxidative phosphorylation and respiratory chain: review. J Inherited Metab Dis, 1984; 7 (suppl.1): 57- 61.
3. Kandel ER, Schwartz JH, and Jessell TM: In Principles of Neural Science 4th edition. McGraw Hill: In. The cytology of neurons. 2000; p.p. 76-87.
4. Wong-Riley MTT, and Kageyama GH: Localization of cytochrome oxidase in the mammalian spinal cord and dorsal rot ganglia, with quantitative analysis of ventral horn cells in monkeys. J Comp Neurol, 1986; 245(1): 41-61.
5. Sutton RL, Hovda DA, Chen MJ, and Feeney DM: Alleviation of brain injury-induced cerebral metabolic depression by amphetamine: a cytochrome oxidase histochemistry study. Neural Plast, 2000; 7 (1-2): 109-25.
6. Wingrave JM, Schaecher KE, Sribnick EA, Wilford GG, Ray SK, Hazen-Martin DJ et al: Early induction of secondary injury factors causing activation of calpain and mitochondria-mediated neuronal apoptosis following spinal cord injury in rats. J Neurosci Res, 2003; 73 (1): 95-104.
7. Mattson MP: Excitotoxic and excitoprotective mechanisms: abundant targets for the prevention and treatment of neurodegenerative disorders. Neuromolecular Med, 2003; 3 (2): 65-94.
8. Seligman AM, Karnovsky MJ, Wasserkrug HL, and Hanker JS: Nondroplet ultrastructural demonstration of cytochrome oxidase activity with a polymerizing osmiophilic reagent, Diaminobenzidine (DAB). J Cell Biol, 1968; 38: 1-14.
9. Chalmers GR, and Edgerton VR: Marked and variable inhibition by chemical fixation of cytochrome oxidase and succinate dehydrogenase in single motoneurons. J Histochem Cytochem, 1989; 37 (6): 899-901.
10. Wong-Riley MT: Cytochrome oxidase: an endogenous metabolic marker for neuronal activity. Trends Neurosci, 1989; 12 (3): 94-101.
11. Hevner RF. Liu S, and Wong-Riley MT: A metabolic map of cytochrome oxidase in the rat brain: histochemical, densitometric and biochemical studies. Neuroscience, 1995; 65 (2): 313-42
12. Carr PA, Liu M, and Zaruba RA: Enzyme histochemical profile of immuno-histochemically identified Renshaw cells in rat lumbar spinal cord. Brain Res Bull, 2001; 54 (6): 669-74.
13. Martin LJ, Kaiser A, and Price AC: Motor neuron degeneration after sciatic nerve avulsion in adult rat evolves with oxidative stress and is apoptosis. J Neurobiol, 1999; 40 (2): 185-201.