Ultra structural study of Carboxylester hydrolases activity in the interneuron of the mammalian spinal cord

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Abstract

Background: Neurosciences mainly focused on the enzymatic pattern of the neurons, however, in this study the demonstration of certain carboxylaser hydrolases (Alph naphthyl butyrate esterases) activity in the Intrinsic spinal networks neurons (central pattern generator) was performed.

Objective: Interneurons or Renshow cells assume autoinhibitor functions, which dampens the a-motoneurons through negative feedback circuit, in addition to that they receive an input from higher centers through which it modify reflex responses to peripheral stimuli by facilitating or inhibiting different populations of interneurons ,however, this issue modulates the performence of specific movement performed by a-motoneurons.

Materials and methods: α – naphthyl butyrate used as a broad spectrum substrate in treating minced tissue block of gray matter of the spinal cord in the Rabbit prior to their traetment with the usual way to be examined lateron with the electron micrscopy .

Result: Reactivity was clearly evident in a form of deep stained granules in the nuclear matrex ,mitochondera and ,RER , and in certain preneuclear region ,however, the reactivity was varied in the mitochondrea in different neurons examined.

Conclusion: Rescentely the term, central pattern generators was used, which address the Intrinsic spinal networks of interneurones that control the timing and pattern of the muscle activity underlying locomotion in mammals,however the effect of the higher centers that modulates the type & tone of movement through those neurons elucidate function which was varied from excitatory-inhibitory, flexor-extensor. The reactivity of those enzymes and their different isoformes that migth have an effect on the molecular and genetic pattern of neurotransmitres were discussed in this study .

Keywords: Interneurons,Central pattern generator, Alpha naphthyl butyrate,esterase**,** Spinal cord

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Introduction

Carboxylester hydrolases has been implicated in a wide range of novel dynamic functions which has been modulated according to the neuronal functions, development and regeneration[(1)].SinceThose are widely distributed in mammalian tissues, especially those with higher metabolic activity like brain, and spinal cord[(2)].

They are present as a complex tissue-specific mixture of various components, each of which presumably has a tissue-specific biological role [(3)]. However the metabolic function and the natural substrate of most carboxylester hydrolases in the different tissues are obscure [(4)].

Interneuron's assumes autoinhibitor functions, which dampens the α-motoneurons through negative feedback circuit, in addition to that they receive an input from higher centers through which it modify reflex responses to peripheral stimuli by facilitating or inhibition different populations of interneurons [(5)], however, this issue need profound

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energy that can be served this tusk which translated in different forms of neurotransmitters and their precuersers in the stomata of those neurons.

The term used to highligths the physiological property of certain neurons in the gray matter of the cord ,which is the central pattern generators address the Intrinsic spinal networks that control the timing and pattern of the muscle activity underlying locomotion in mammals, howover, molecular and genetic approaches is a vital issue to elucidate the function of populations of CPG interneurons [6], the Execution of motor behaviors relies on circuitries effectively integrating immediate sensory feedback to efferent pathways controlling muscle activity [7]. It remains unclear how, during neuromuscular circuit assembly, sensory and motor projections become incorporated into tightly coordinated, yet functionally separate pathways. Since the early appearance of discrete intranerve trajectries suggests that trans-axonal interactions migth drive the segregation, within axial nerves, establishment of discrete afferent and efferent pathways depends on coordinate signaling between coextending sensory and motor projections. These heterotypic axon-axon interactions require motor axonal ephrin, guidance molecules which is one of the candidate molecules that performe this issue [8] .EphA3/EphA4 receptor tyrosine kinases activated by cognate sensory axonal ephrin-A ligands.Since genetic elimination of trans-axonal ephrin-A signaling in mice triggers drastic motor-sensory miswiring,culminating in functional efferents within proximal afferent pathways. Effective assembly of a key circuit underlying motor behaviors thus critically depends on trans-axonal signaling interactions resolving motor and sensory projections into discrete pathways.[9]

The α- motoneurons in the gray matter of the cord serive motor activities to the tonic and phasic muscle fibers showes different pattern of reactivites utilizing ANA esterases, and α- 1 and α-2 address the two varity of those neurons, is well established [10]. In the sence of this diversity ,the modulaters neurons must show different pattern in the neurotansmeters and macromolecules,synthsis and degradation which influence there differentation and activities .

Material & Methods

Carboxylester hydrolases 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 37 Cº . Serial coronal sections with a sharp razor were cut through the ventral horn of the cord and then minced into a small slices of a bout 0.1cm cubes. Immediately, those slices were transferred to a small containers that contained a cold fixative composed of 2.5% glutaraldehyde buffered to pH 6.8 with 0.15M phosphate buffer for 2 hours at 4C, then blocks were further minced into a smaller pieces and washed (2-3) times with the same buffer prior to incubation. However some of blocks were processed without incubation in the substrate to be used as a control. Incubation medium was prepared according to the Modified after Bozdech & Bainton, 1981 [11], α- naphthyl butyrate (Fluka) 10mg (i.e.0.01ml), in

0.5ml ethanol. Phosphate buffer 0.15M pH 6.8. 9ml.and ,Hexazotized pararosanilin, 0.5ml , since this should be freshly prepared by mixing equal volumes of 1 ml 4% sodium nitrite (BDH), newly made, and 1 ml 4% pararosaniline (Fluka) in 2N HCl.

The cloudy mixture of the incubation medium was filtered with No.1 Whatman filter paper, prior to incubation which lasted for 2 hours, .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

Perfect results were obtained within 30 min of incubation in the ANB 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, prior to be examined by electron microscope, delineated different sizes of ventral horn neurons. (Figure1)

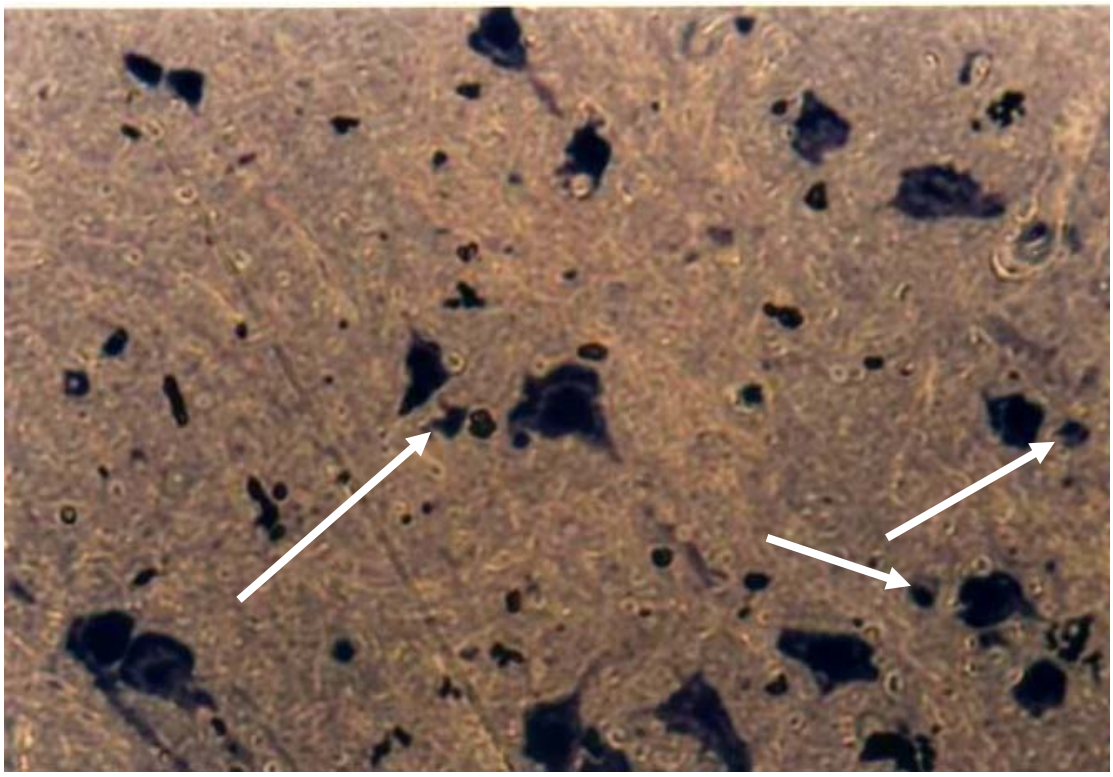

Figure1: Semi thin treated with methylene blue (X .400). Arrows interneurons

Small size neurons were selected for Electron microscopic examination which revealed that, those neurons are richly endowed with a variety of organelles that are loaded with black dots of the final reaction product (FRP). (Figure 2), conversely, sections from control blocks do not show this reactivity.

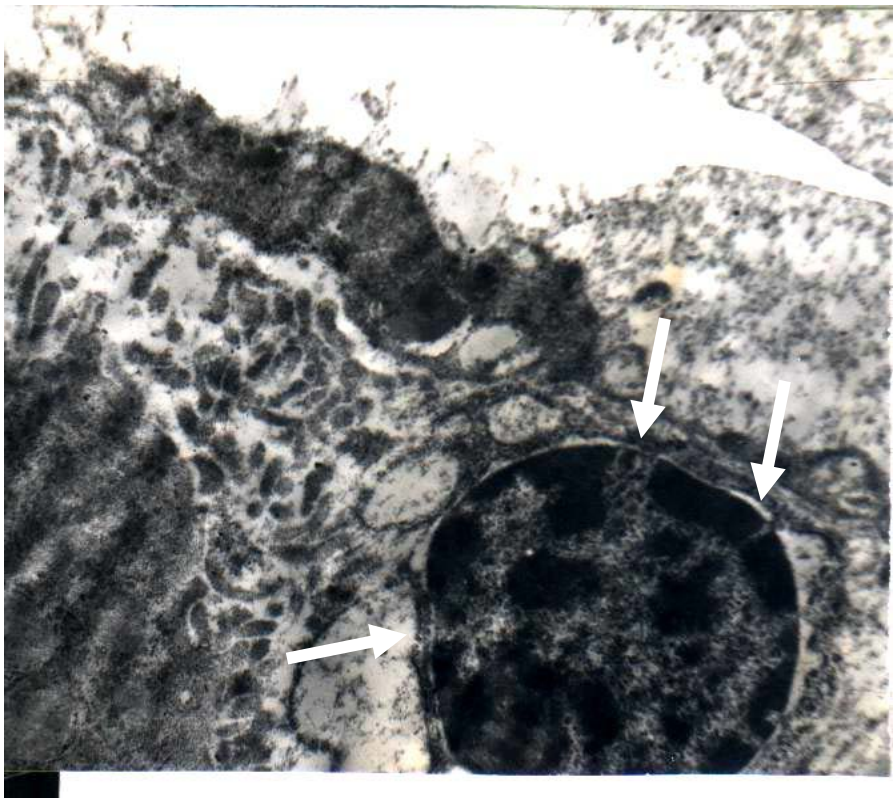

Figure2: Interneuron stained with ANBE. Arrows nuclear pores (X. 12000)

It is clearly evident that the nucleus of those neurons shows intense reactivity in the nuclear matrix with fenestrated nuclear membrane, it is difficult to identify nucleolus, as there are many that show their features regarding the size, since those are heavily stained.

With a higher magnification we overcome the failed of the somata, rough endoplasmic reticulum (RER) and mitochondria can be easily visualized with two forms of distribution in the cytoplasm. In (Figure 3). RER with their extensions to the Polyribosome were abundant, but mitochondria show varied in the intensity of the FRP from moderate to dark, although light ones have also been observed.

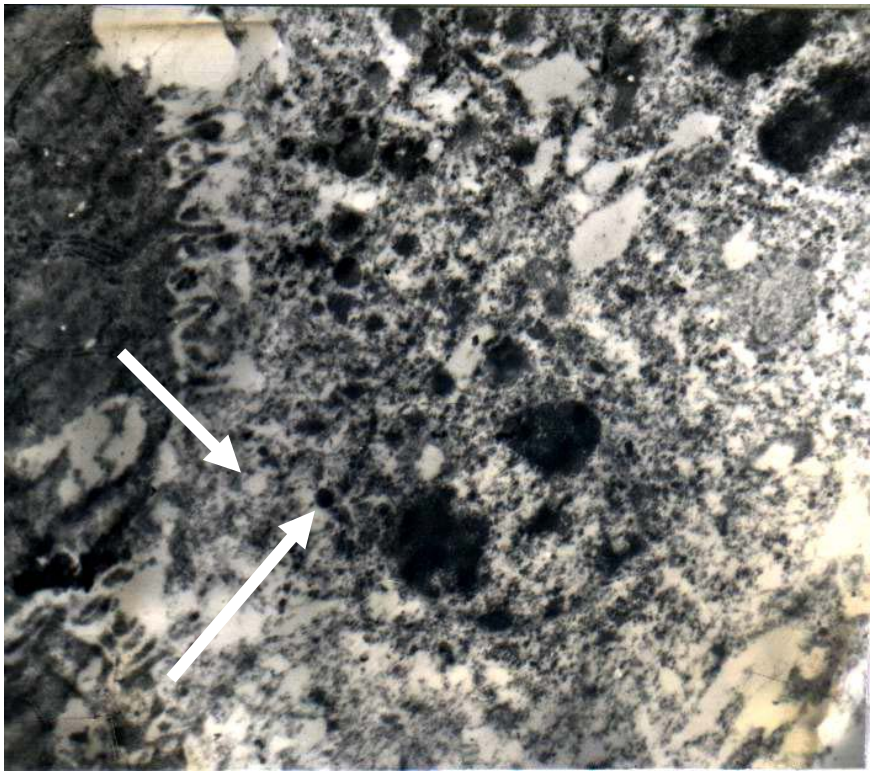

Figure 3: Cytoplasm of interneuron treated with ANBE .Arrows show mitochondria devoid from reactivity (X 24000).

While in other sections (Figure 4), the predominant intense staining was evident. In a form of a uniform heavily intense reactivity in the mitochondria (no light one have been observed) in addition to that the area surrounding the RER.

The main issue in the reactivity is that; the form of distribution of FRP is in the pre nuclear area not the neuropil (dendrites & axon terminals) as in some neurons,

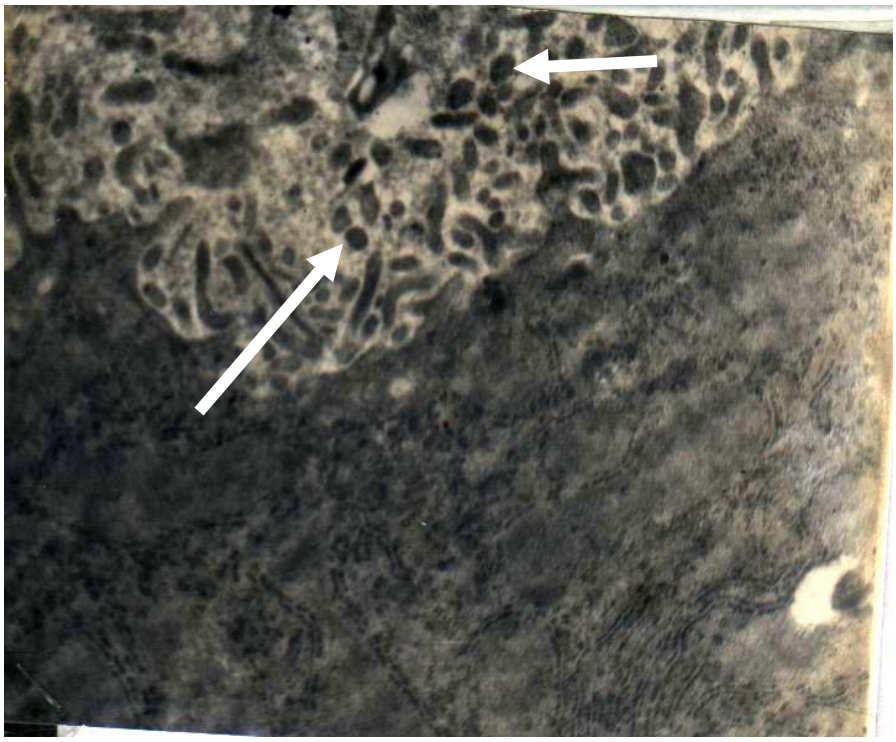

Figure 4: Cytoplasm of anther interneuron treated with ANBE .Arrows show mitochondria (X 24000).

Discussion

The spinal motoneurons are embedded within local neuronal; circuitries that determine stereotypic patterns of activities [12]. Presently, the internal organization of the mammalian locomotor central pattern generator (CPG) is unknown due to the difficulty in identifying and localizing interneurones involved in the network. The CPG was initially thought to be composed of half-centers, which set the basic locomotor rhythm by generating alternating excitation of antagonist motoneurone pools (e.gflexors and extensors) via reciprocal inhibition.[7].

Since the intrinsic spinal networks neurons control the timing and pattern of the muscle activity, through the α-motoneurons, however, central pattern generators neurons is a physiological term used to delineate those neurons in the mammalian spinal cord. The histochemical term in this aspect is in a limited in use for identification, as advances in transgenic technologies have greatly facilitated our understanding of the development and function of neural networks, many studies in this filed based on the overall structural role in rhythmic generation, organization of flexor-extensor networks, and the diverse role of commissural interneurons in coordinating left-right movements [6].

In this study we highlighted a histochemical glimpse on those neurons precisely in the lamina IX in the gray mater of the cord where the α-motoneurons existed. It's clearly evident from the shape, size and their locality in the vicinity to the α-motoneurons (Figure1), electron microscopical

reading indicates, tremendous activity of those neurons in the synthesis of transgenes for the different profile of carboxyl ester hydrolysis precursors regarding the ANB esterase, as utilized in this study which is visualized in the nucleus as huge black dots in the nuclear matrix, and the abundant nuclear pores in the nuclear membrane indicating active transportation of these precursors (Figure 2) [(13)].

The observation of Gallarda, et al 2008, about the segregation of axial nerve into motor and sensory fibers was based on the initial interactions between median medial column axon that extened to axial target and dorsal root ganglia neurites eventually resolve into sharply segregated proximal motor-sensory pathways,since the use of many polypaptide like nerve growth factor and neurotrophin-3, to select for nocioeptive and proprioceptive classes of sensory neurons,respectively ,they found that effective segregation of sensory and motor projections occurred irrespective of sensory subtype .Nevertheless motor axon of media medial column more frequently crossed into proprioceptive explants compared with nocioceptive cultures, homotypic (e.g. motor-motor) co-cultures failed to display axon segregation, stressing the heterotypic natuer of the underlying interactions.

On cellular bases the segregation of peripheral nerve fibers in to sensory and motor is well established due to the trans-axonal interactions [(14)].However axon-axon interaction have been implicated in olfactory and retinal axon targeting in Drosophila and mouse [(15)] .

To address the two forms of reactivity in the cytoplasm of interneurons on histochemical bases utilizing ANBE as a substrate (Figure 3 .4),this prompts the question of the two verities of interneurons ,in the cytoplasm precisely in the mitochondria (Figure 3) some of them were devoid from reactivity while in (Figure4) all the mitochondria shows reactivity.

The important issue is that, trans axonal reaction were inducted through the CPG neurons on α-motoneurons with two version in the reactivity of the ANB esterase which is one form of carboxylester hydrolases as, this form of carboxyl ester share in the synthesis and degradation of macromolecules formation in order to segregate motor axons that serve tonic or phasic muscle fibers. In this study it is an easy way in our histochemical practice to donate the trans-axonal interactions on motor nerve fibers with two forms of action modalities via ANB esterase, and CPG1, CPG 2 neurons in the gray matter of the mammalian cord is easily addressed via the activity of this form of carboxyl ester.

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