The Iraqi J. Vet. Med. 14 (1990)

HISTOCHEMICAL STUDY OF NON-SPECIFIC ESTERASES MOTOR UNIT OF THE RABBIT

A.R.AL- Salihi, H.T. AL-Azzawi and H.M. Al-Khateeb.
Department of Anatomy, College of Medicine, University of Baghdad.

SUMMARY

The distribution of alpha naphthyl asetrase and naphthol AS-D acetate esterase was studied in the motor neurons of the ventral horn of the spinal cord segments L4, L5, and SI, supplying the extensor digitorum longus musscle (EDL) of the rabbit together with the peripheral nerve supplying EDL and the muscle itself.

Strong activity was demonstrated in the motor neurons, in schwann cells and in the axons of peripheral nerve bundlds.
Abundant reaction was located in the motor end-plates of muscle fibres making them easily recognizable. the biological interpretation of esterases was discussed.

INTRODUCTION

Alpha naphthyl acetate esterase and naphthol AS-D acetae esterase belong to the family of carboxylic ester hydrolases (E.C.3.1.1), which include a variety of enzymes that probably possess differant functional roles in vivo, but have the common link of having esters of carboxylic acids as substrates. These esterases include carboxylesterase arylesterase, acetylesterase, often collectively termed non-specific esterases, and cholinesterases. Alpha acetate is cleaved by nearly all non- specific esterases while the acetate of naphthol AS-D is cleaved mainly by arylesterase and acetylesterase(1).

Esterases are widely distributed in mammalian tissues particularly the nervous tissue where cholinesterases have prime importance in cholinergic transmission, and they are most commonly invested of the carboxyl ester

hydrolases. Cholin esterase also demonstrated in the perikarya of motor neurons of spinal cord (2). In many tissues distribuion and level of biochemical activity of esterases indicate a particular functional state. In addition there is also histochemical evidency of a relationship between esterases and function (3).

The present work studies the distribution and localization of alpha naphthyl acetate esterase and naphtho AS-D acetate esterase in the motor units of the extansor digitorum longus (EDL) muscle of the rabbit. This is prformed by investigating the histochemical activity of these esterases in the motor neirons supplying extrafual fibres (alpha motor neurins) of EDL which are arranged in eell defined coumns through 14,15 and SI segments of the spinal cord (4).

The motor nerve to EDL was studied as well by investiga- ting the intramuscular nerve bondles, since the number of alpha motor axon profiles can be up to 40 per cent greater than in sections cut more centrally, the motor nerve fibres supplying EDL appear as relatively large myelinated fibres approximately 2-20Mm in diameter (5). Lastly the EDL is studied with particular attention to the motor end- plates.

MATERIALS AND METHODS

Twelve new zeland white rabbit (Lepus europaeus) weighing 400-900g. were used for investigation, each was anaesthetized by ether then exsanguinated. The EDL of both legs were dissected out. The motor nerve to EDL was studied by investigation the intramuscular nerve nondles since the number of alpha motor axon profiles can be up to 40 per cent greater than in sections cut more centeraly (5). The spinal represantation of EDL according to Sharrard (4) is in cell columns at L4, L5, and Sl segmental levels, noting that to muscles of lower limbs, flexor muscles are supplied by columns situated medial and caudal to those supplying corresponding extensor muscles.

The tissues were rapidly frozen in liquid nitrogen (-196 °C), serial sections, 8u each, were cutout by cryostat (Slee), then fixed in formalin vapour.

The histochemical demonstration of alpha naphthyl acetate esterase and naphthol AS-D acetate esterase were done by the technique of simultaneous azo-coupling method using the naphthol esters (alpha naphthyl acetate and naphthol AS-D acetate) as substrates, and hexazotized pararosaniline and fast blue RR as coupling agents respectively (6).

RESULTS

In the spinal cord, marked activity for non-specific esterase is demonstrated in the perikaryons of neurons of the vantral horn, both in large alpha motor neurons and smaller internumcial neurons (Fig.1), a strong reasction for non- specific esterases is seen in the grey matter (Fig.2).

Alpha naphthyl acetate esterase produce a strong dotlike reasction in schwann cells of nerve bundles with a lot of diffusion, no reaction is demonstrated in the arons (Fig.3).

Naphthel AS-D acetate esterase produces more localized and multiple granular reaction in nerve bundles with diffuse reasction in the axons (Fig.4).

Both esterases are abundant at the motor end-plates of the muscle fibres making them easily recognizable (Fig.5 & 6).

DISCUSSION

This study has shown that alpha naphthyl acetate esterase and naphthol AS-D acetate esterase are distributed throughout the motor unit, from the neurons of the spinal cord down to the muscle.

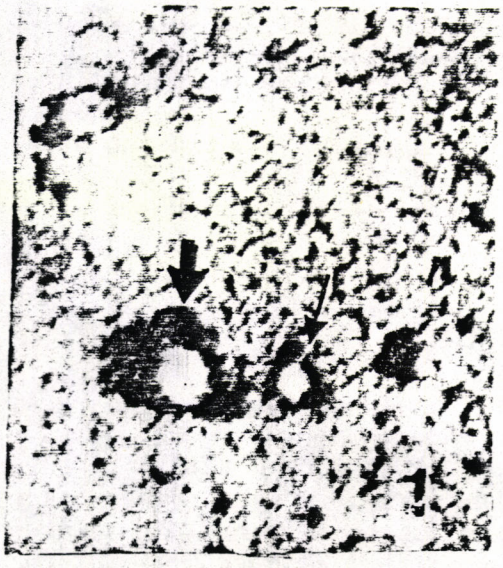

Fig. (1): Alpha naphthyl actate esterase in the alpha motor neuron (thick arrow) and internunial neurin (thin arrow) of the anterior born spinal cord.

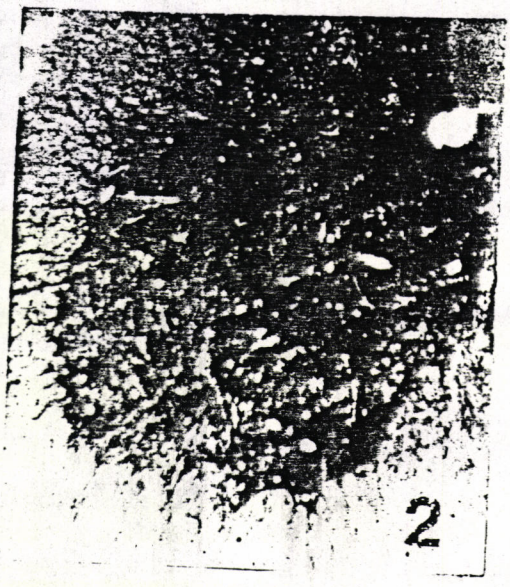

Fig. (2): Non-speoific esterases reaction in the anterior horn of the spinal cord.

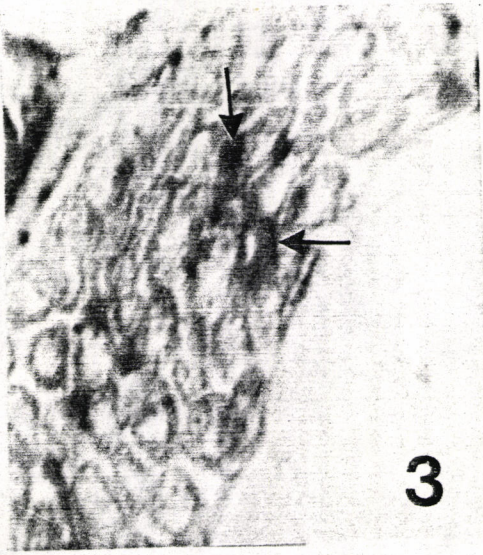

Fig. (3): Peripheral nerve, apha naphthyl acetate esterase reaction hk Schwann cells.

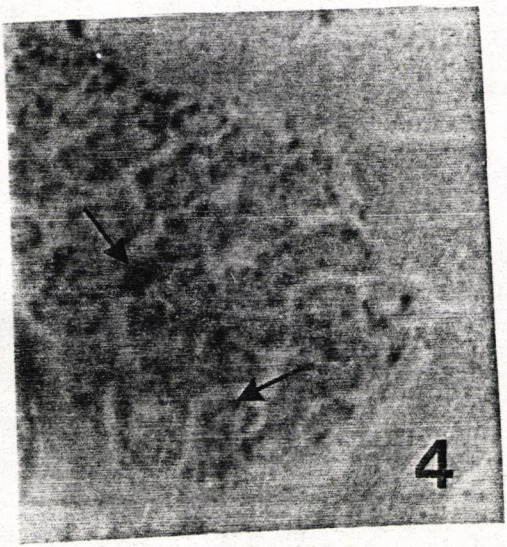

Fig. (4): Perioheral nerve, naththol AS-D acetate esterrase reaction.

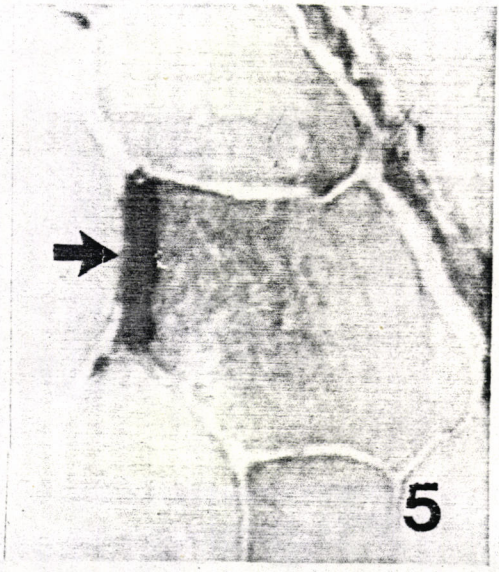

Fig. (5): alpha naphthyl acetate esterase reaction in the motor end-plate.

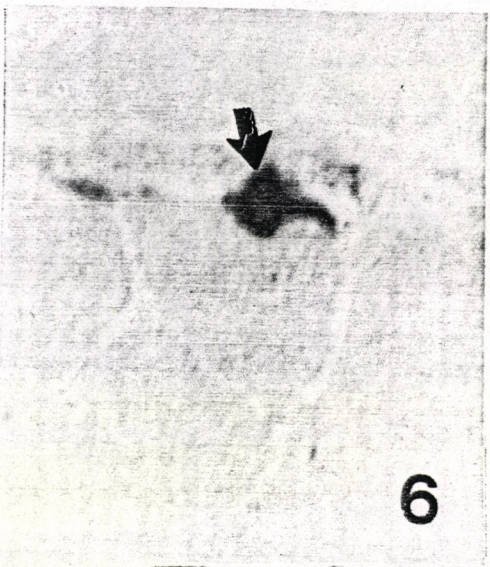

Fig. (6): Naphthol AS-D acetate esterase reaction in the motor and plate of EDL

This distribution is an indicator of a role played by these in the whole unit and not simply at the motor end-plate only.

Many papers have been published about the distribution of non-specific esterases and their variation in activity under endogenouns and exogenous influences such as development, differentiation, nutrition, hormons, and diseases (7, 8 & 9).

At present time the metabolic function and the natural substartes of most non-spcific esterases are still obscure. One can still only speculate about the functional importance. These speculations were made even more difficult by the fact that non-specific esterases are present as a complex tissue- specific mixture of various components, each of which presumbly has a tissue-specific role. There is still a lack of biological interpretation of esterases in the nervous tissue despite the fact that it has been long since they were described in regions of the nervous system as the hypothalamus. neurohypophysis (10), and hippocampal region (1).

It seems that this particular distribution of esterases in the motor unit presents a tissue a tissue-specific phenomenon, related to function, the intimate relations and influences of the motor neuron on the muscle fibres may be madiated through these non-specifice esterases have a wide spectrum of catalytic activites, overlapping in their specificity for substrates particularly with some proteolytic enzymes especially endopeptidases.

Biologcical interpretation awaits a comprehehension of the quantitive distribution of individual easter hydrolysing enzzymes. Therrfors, the results of this study open the way for further evaluation of different nonspecific esterases (carnoxylesterase, arylesterase, acetylesterase... etc.) as markers for histochemical and physiological status of cells, thssues and organs, in particular the differentiation between various motor

neurons of the spinal cord and the influence of motor neurons on muscle fibres development and differentition both in normal and diseased conditions.

REFERENCES

1. Luppa, H. and andra, J. (1983). The histochemistry of carboxyl ester hydrolases: Proplems and possibilities. Histochemical J. 15:111-137.

2. Kasa, P. and Csillika, B. (1966). Electron microscopic localization of cholinesterase by a copper-Leas-thiicholine techique. J. Neurochem., 13: 1345-1349.

3. Deimling O.V. and Bocling. A. (1976). Esterases in histochemistry and ultrahistochemistry. Histochemical J., 8: 215-252.

4. Sharrad, W.J.W. (1955). The distribution of the permanant paralysis in the lower limb in poliomylitis. J. Bone and Joint Surgery, 388:540-558.

5. wary, S.H. (19660. Quoted by Gilliatt, r.W. in: Axon branching in motor nevers, In : Andrew, B.L. (Editor) Contraol and innervation of skeletal mucsle: pp. 53-63/ The Universtity of St. andrews.

6. pearse, A.G.E. (1972). Carboxylic ester hydrolases. In: Histochemistry, theoretical and applied Vol. 2, 3rd. ed., pp. 761-807. churchill Livingstone, Edinburgh, London.

7. Sohiff, R. (1975). Eaterase isoenzymes as markers in normal and disease processes, In: Markert, C. (Editor), Isoanzymes, pp. 775-797. academic Press, New Hork.

8. Coates, P.M: Mestriner, M.A. and Hopkinson, D.A. (1976). A preliminary genetic interpretation of esterase isoenzymes of human tissues, Ann. Genet., 39: 1-20.

9. Kass, L. (1979). Cytochemistry of esterases. SRO Crit. Rev. clin. lab.Sci. 10: 295-223.

10. Pearse, A.G.E. (1958). Fsterases of the hypothalamus and their fincthonal sigficance. In: pathiysiologica Diencephalica, pp. 329-335.Springer, Vienna.

المجلة الطبية البيطرية العراقية المجلد الرابع عشر سنة (١٩٩٠)

دراسة كيميا نسجية لانظيم الاستراز غير المحدد في الوصف الحركة للارنب

انعم رشيد الصالحي، هاني طه العزاوي، هدى مهدي الخطيب
قسم التشريح - كلية الطب - جامعة بغداد

الخلاصة

تم دراسة انتشار فعالية انزيم استرازاسيتات ارليد حامض النفثول الدالي في العمبونات المحركة في الشدف القطنيه الاربع السفلية ثم الشدفة العجزية الاولى للنخاع الشوكي في الأرنب والتي يفترض انها قد تشترك في تزويد الباسطة الطويلة لاباخس الطرف الخلفي في هذا الحيوان. كما تمت وفي عين الوقت الدراسة نفسها على العصب المزود لهذه العضلة والمغائح الانتهائية المحركة في الياقها العضلية.

كانت الفعالية الاسترازية قوية في العمبونات المحركة في النخاع الشوكي وفي خلايا شوان ومحاور الحزم العصبية المحيطية والمغائح الانتهائية المحركة في الالياف العضلية لهذه العضلة. مما يسهل التعرف على هذه المغائح.

تم مناقشة الاهمية البايولوجية لهذا الانزيم بصورة عامة وفي المواقع المذكورة.