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HISTOLOGICAL STUDY ON NEUROMUSCULAR RECEPTORS OF RAT SKELETAL MUSCLES

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Key words;Neuromuscular,Skeketon,receptors.

ABSTRACT

The present study is to describe the histological characterization of rat neuromuscular receptors and their density in relationship to extrafusal muscle fibres.

Neuromuscular receptors or (muscle spindles) were identified and isolated from fore and hindlimb muscles and examined by the light microscope. The average numbers and densities of muscle spindles were found comparable to some other mammals.

Each muscle spindle was contained 2-4 intrafusal muscle fibres . Three types of intrafusal muscle fibres were distinguished as large (L), medium (M) and small (S).

All muscle spindles innervated by sensory and motor nerve fibres which branching from spindle nerve trunk.

INTRODUCTION

A skeletal muscle is such an obvious example of an effector organ that it generally comes as a surprise to learn that most of the nerve fibres innervating it are sensory, this type of muscles has kinds of sensory receptors called neuromuscular or (muscle spindles) within the substance of the muscle [1,2].

Muscle spindles are mechanoreceptors composed of a bundle of small (intrafusal) muscle fibres that receive both a motor and sensory innervations [3]. The receptors lies in parallel with the ordinary (extrafusal) muscle fibres, and for part of it's length is enclosed within a fluid-filled capsule [4].

Mammalian muscle spindles contain three types of intrafusal muscle fibres which are structurally and functionally distinct [5], these fibres can be separated to nuclear bag fibres or large, medium and small chain fibres [6 , 7].

Most mechanoreceptors lie deep in the belly of the skeletal muscle near branches of the muscle nerve or blood vessels, since they lie in parallel with extrafusal muscle silence it's spindles unless there is a powerful efferent discharge to the intrafusal muscle fibres [8 , 9].

All the information about mammalian neuromuscular receptors morphology has been derived from studies of the receptors in hindlimb muscles of the cat [4 , 10] and other domestic animals [11 ,12]. Muscle spindles of some wild animals were also investigated [7 , 13].

According to this line, the present study gives more histological details on the muscle spindles of rat and compares with other mammals.

MATERIALS AND METHODS

Fore and hindlimb muscles (total 32) were dissected under anaesthesia from both sides of a normal adult rats and processed for light microscopy. Whole muscle silver staining technique of [14] is published elsewhere [7].

After fixation, the muscles were washed and impregnated for a week in silver nitrate. The stained muscles were teased under a dissecting microscope and the muscle spindles were identified and isolated for examination and photography by using an Olympus light microscope.

Some of the fore and hindlimb muscles were prepared and processed (see methods in)[7] for an electron microscopic study. From these muscles, few semithin sections (about 1μm) stained with toulidine blue were used in this study. Muscles weight and spindles content were taken as an average of both left and right sides.

Spindle densities were account as the number of spindles per gram of the average weight of the muscle [4].

RESULTS

1-Spindle densities

The number of spindles per gram of muscles can be calculated and the values for different muscles were compared.

(Table1) showed the number and densities of spindles in each muscle of rat fore and hindlimb muscles. From the forelimb muscles, extensor brevis (288.4), interossei (235.2) and 2 nd lumbrical (115.3) has highest density, while the biceps brachii has lowest density (15.6).

In the hindlimb muscles, popliteus (344.2), peroneus brevis (295) and soleus (266.6) muscles have the highest number, while the pronators (10.9) and gastrocnemius (5.78) show the lowest number.

Table 1: Average muscles weight (wt) and average spindle number (Sp. No.) and spindle density (Sp. D.) in the rat fore and hindlimb muscles.

Muscles	Wt (g)	Sp. No.	Sp.d.
Forelimb:			
Interossei	0.051	12	235.2
Pronator quadratus	0.21	5	23.8
Flexor radialis	0.13	7	53.8
Biceps brachii	0.51	8	15.6
Brachialis	0.133	8	60.1
2nd lumbrical	0.13	15	115.3
Extensor radialis	0.42	19	45.2
Extensor brevis	0.052	15	288.4
Hindlimb:			
Soleus	0.075	20	266.6
Gastrocenemius	1.21	7	5.78
Tibialis anticus	0.091	13	142.8
Tibialis posticus	0.085	10	117.6
Popliteus	0.061	21	344.2
Peroneus longus	0.093	12	129
Peronoeus brevis	0.061	18	295
Pronators	1.11	12	10.9

2- Histological observations:

The muscle spindles were distributed randomly through the rat skeletal muscles, but most of them tend to be more concentrated in the belly of muscles near the nerve trunk entry and the blood vessel's (Fig 1 and 2).

All the isolated neuromuscular spindles usually short show well marked intrafusal single or parallel and sometimes appear from 1-2 spindles at the same region (Fig. 2).

Most of muscle spindles contained 4 intrafusal muscle fibres, the number varied between 2-4 in different skeletal muscles (Fig 3 and 4).

Three types of intrafusal muscle fibres were distinguishable in the spindle according to their diameter and central nucleation, a large nuclear bag1 fibre (b1), a medium nuclear bag2 fibre (b2) and two types of small nuclear chain fibres (c). Therefore the three types were known as large, medium and small intrafusal muscle fibres respectively (Fig 5). The

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b2 fibre is the thickest and have more than one nucleus, the b1 is slightly thinner, the more shorter and thinner was the c fibres which usually end within the limit of the capsule (Fig 2 and 5).

Microscopic examination show a close association between the two C fibres at the equatorial region but the association between b2 and c from rat muscles (Fig 5).

The study was distinguished three regions in each mechanoreceptor, A, B and C between the equator and the origin of a spindle pole. The (A), region was lying between the equator and the equatorial end, (B), that end of the capsule and (C), the extra capsular part of the pole (Fig 6A,B).

Figures also showed that the intrafusal muscle fibres of each spindle form a bundle surrounded by external sheath called the outer capsule . At the mid equatorial region, this capsule enlarged, in addition there was an inner capsule (axial sheath) which ensheaths individual intrafusal fibres or groups of fibres (Fig 7A,B). whereas a periaxial space (indicated by asterisks on all figures) is interposed between the two layers.

Transverse sections in spindle equatorial region also show large number of capillaries often abut the external layer of the outer capsule (Fig 4 and 7B). They exhibiting large luminal and a continuous endothelium.

All muscle spindles isolated from skeletal muscles prepared with silver nitrate were innervated by spindle nerve trunk which constitute from a bundle of nerve fibres of varied diameter (Fig 8). Sensory fibres (afferent axon) were the thickest and branched as spiral or diffuse primary (p) and secondary endings (s) (Fig 8,9). Motor nerve fibres (efferent axon) were the thinner and terminate as motor end plate (p1 and p2) through the polar region of intrafusal muscle fibres (Fig 8 and 10).

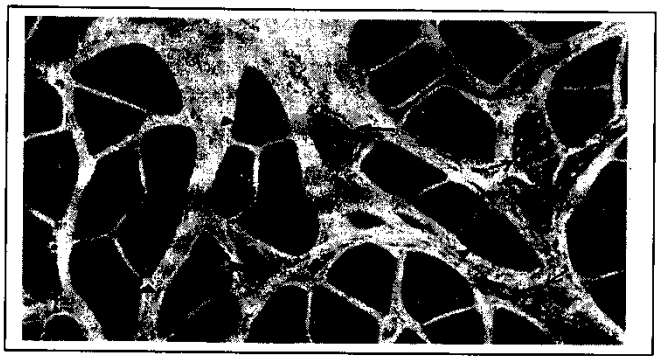

Fig1: Section of rat soleus muscle showing two muscle spindles (Thick arrows) composed of 4 intrafusal muscle fibres. Extrafusal muscle fibres (arrows heads) and branches of nerve trunk (Thin arrows). Toulidine blue stain. (1100).

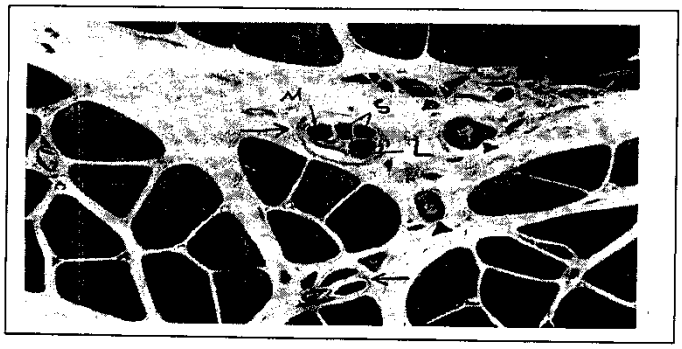

Fig 2: Section (1μm) thick showing two muscle spindles. Lower arrow points to muscle spindle composed of 2 intrafusal muscle fibres. Upper arrow indicates to another muscle spindle composed of 4 intrafusal muscle fibres, one large (L), medium (M) and two small fibres (S). Blood vessel's also identified (arrows heads). Toulidine blue stain. X(1500).

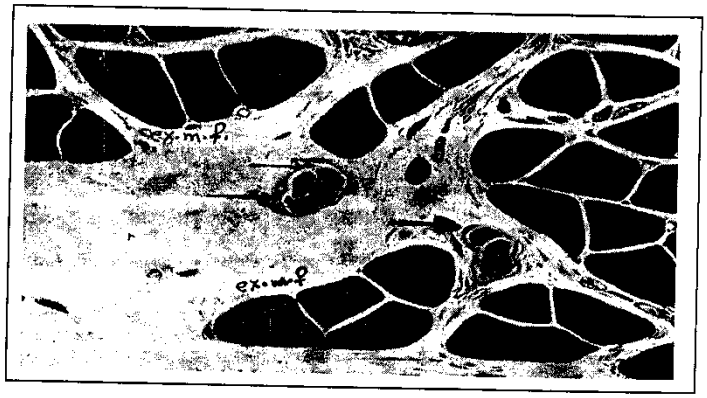

Fig 3: Section showing two muscle spindles occupy the intramus cular space among extrafusal muscle fibres (ex.m.f.).(Thick arrow) indicates to spindle composed of 3 intrafusal muscle fibres. There is a little nerve trunk partly embedded in the spindle capsule (Thin arrows). Toulidine blue. X(1500).

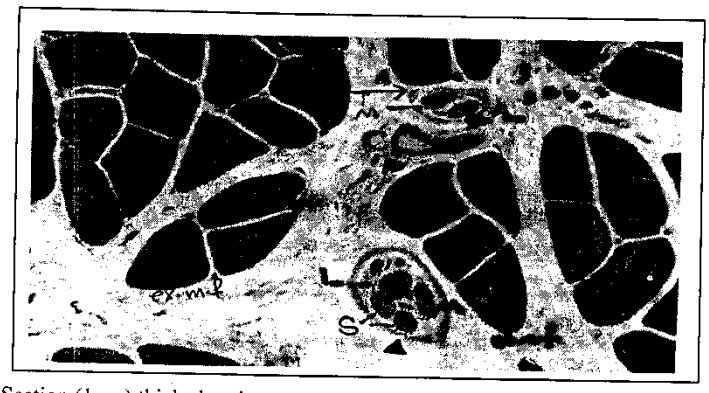

Fig 4: Section (1μm) thick showing two muscle spindles lie among extrafusal muscle fibres (exf. m.). Upper arrow points to spindle composed of 2 intrafusal muscle fibres. One large (L) and one medium (M). Large capillary (C) also identified. Second spindle (arrows heads). Composed of one large (L), one medium (M) and two small (S) fibres. Toulidine blue. X(1500).

Fig 5: Transverse section through the equatorial region of muscle spindle showing features of equatorial nucleation. The intrafusal bundle is composed of large bag2 (L) with three nuclei abrest, medium bsg1 (M) and two small chain (S) surrounded by outer capsule (OC), axial sheath (ax.sh.) and suspended in the periaxial space (*).Toulidine blue . X(1800).

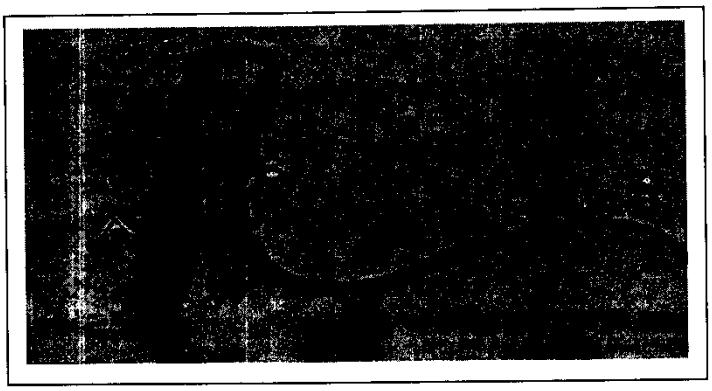

Fig 6.(A): Photomicrograph of isolated muscle spindle illustrate its fusiform shape. The equatorial region or (A), juxta equatorial (B) and polar region (C). Silver stain. X(1300).

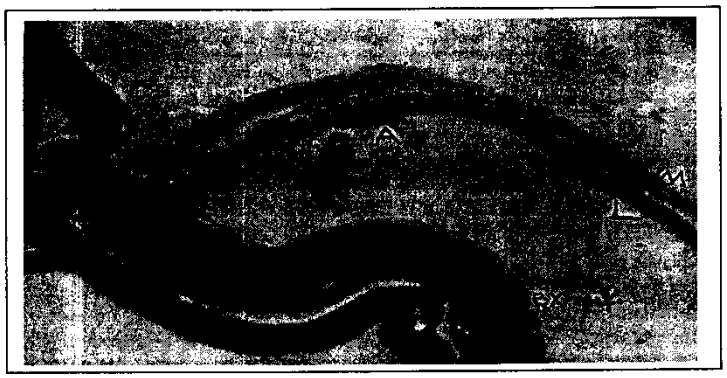

Fig.6. (B): Photograph of muscle spindle showing the muscle spindle parallel to the extrafusal muscle fibres (ex. m. f.). Enlarged equatorial region (A). Limits of capsule extend to the juxtaquatorial region (arrows heads). Large (L) and medium (M) intrafusal fibres extend beyond the capsule. Silver stained. X (1300).

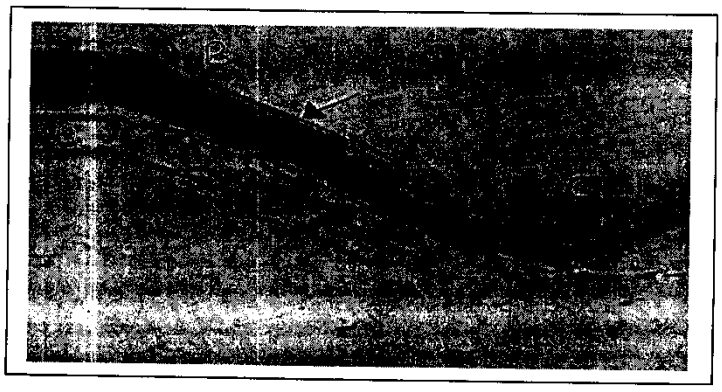

Fig 7 (A): Muscle spindle showing the juxtaequatorial region (B) and polar region (C). Limits of capsule (Thick arrows). Large (L) and medium (M) intrafusal fibres well separated at the polar region. Silver stained. X(1600).

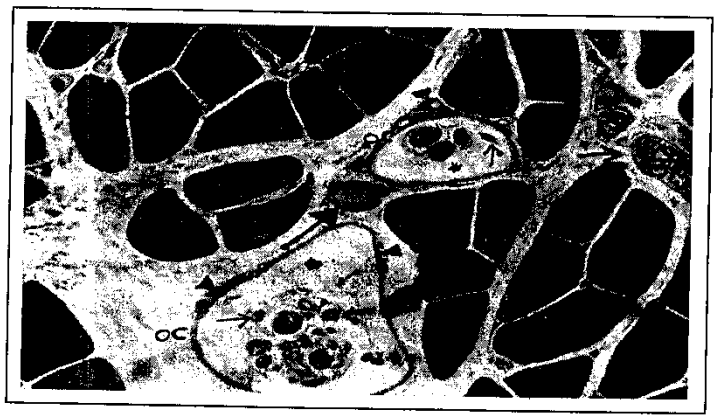

Fig 7 (B): Transverse section of the muscle spindles clarified the cytologic organization of outer capsule (OC) and axial sheath (ax.sh.). Periaxial space (*). Capillaries often abut the outer capsule (arrows heads). Muscle spindle on the tip sectioned near a large capillary (Thick arrow) and branches of sensory nerve trunk (Thin arrows). Toulidine blue . X(1800).

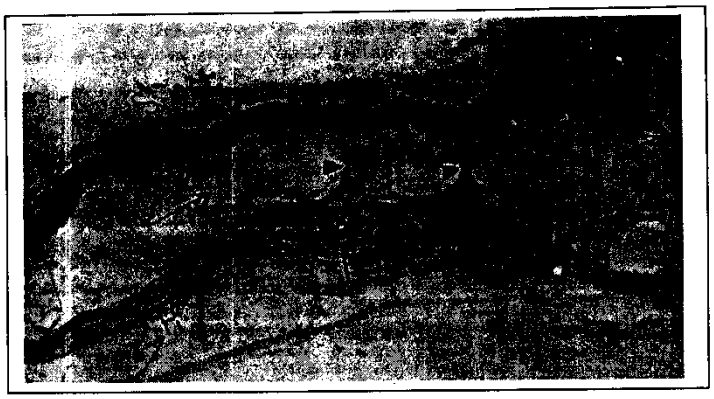

Fig 8: Photograph of teased silver preparation showing the large spindle nerve trunk (s.n.t.) divides into sensory axons (arrows heads) innervate the equatorial region and motor axons (Thin arrows) innervate the polar regions. Primary and secondary sensory endings (p,s) also identified. Motor end plate (p1) at the polar region. X(1300).

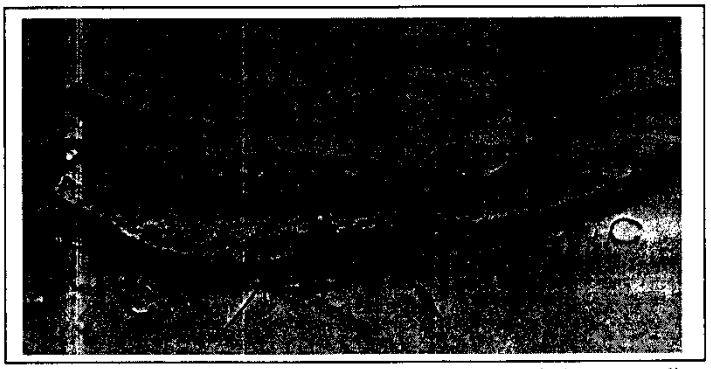

Fig. 9: Micrograph of muscle spindle showing annular or spiral nerve endings (Thick arrow) at the equatorial region (B). Motor axon (arrow head) penetrate the polar region (C) and terminate ase motor end plate (Thin arrow). silver stained. X(1300).

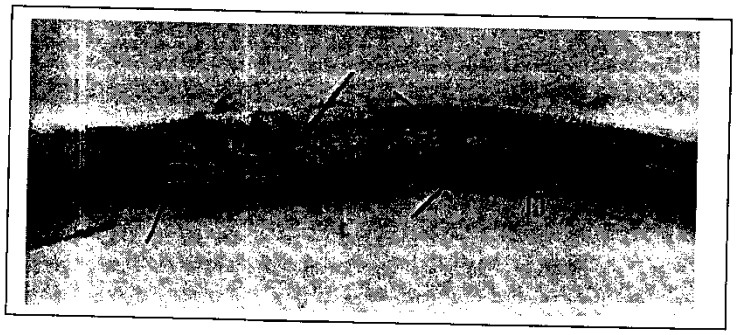

Fig.10: Micrograph of muscle spindle showing the polar region. Motor axon (Thin arrows) extend and terminate as motor end plate (pl). Sole plate was identified with accumulation of nucleus (Thick arrows). silver stained X (1600).

DISCUSSION

The fore and hindlimb muscles have varied numbers of spindles and different densities. These variations are common in many other studied animals and related to differences in muscle structure and function [4].

Barker [1] divided the number of spindles in muscles into groups, a value of 15 spindles or more per gram being high, 8 to 12 medium and 7 or less low. This puts most of the hand and foot muscles into the high growth, while the big muscles are poorly supplied .

Small muscles like soleus and popliteus has many more spindles per gram than other muscles [4]. Flexor muscles tend to be phasic in function like flexor ulnaris. Other muscles which show rich spindles have a marked tonic function in maintaining the posture of fore and hindlimb [10].

Muscles controlling fine movements, body posture and antigravity have higher muscle spindles than those which controlling coarse movements [15 , 16]. For comparison with other mammals the maximum and minimum spindle densities in the rat limb muscles were found in popliteus (344.2) and gastrocnemius (5.78) respectively. These are considerably higher than that in the cat [1]. In contrast, they are lower than in hedgehog and bat [7 , 13].

Results from serial cross sections showed that both nuclear bag and nuclear chain intrafusal muscle fibres were found, the number of intrafusal muscle fibres was 4. Similar results were reported in the rabbit [17] hedgehog [13]. And those of the bat [18]. Most of human muscle spindles contain at least 9-12 intrafusal muscle fibres [2].

The inttafusal muscle fibres were classified to (Large, medium and small). These types correspond to nuclear b2, b1 and chain intrafusal muscle fibres of mammalian muscle spindles [9].

Previos studies also designated intrafusal muscle fibres types as large, medium and small [19 , 18]. The two nuclear bag fibres are themselves of two distinct types termed b1 and b2 fibres because of the histochemical, structural and physiological differences between them [20 , 21 , 2].

In this present study close association between chain fibres was clear but the associstion between b2 and c fibres not confirmed. The association between b2 and c fibres was reported to be a common feature of the equatorial region of cat spindles [22].

Transverse sections also show the cytologic organization of outer capsule and inner capsule, whereas a periaxial space lies between the two layers. Capillaries also found near the outer capsule of the muscle spindles. These findings resemble those in rabbit spindles [23 , 24].

The outer capsule is continuous with the perineurium of the nerves which supply the muscle spindles [9].

This study also showed that the capsule enlarged in the equatorial region. This was related to embryological development of muscle spindles which it is start from the mid-equatorial region and extend towards the polar region [25].

Muscle spindles which isolated from rat skeletal muscle and stained with silver nitrate show that each muscle spindle receive both sensory and motor nerve fibres branch from the spindle nerve trunk. Moreover each nerve fibres ending either as primary sensory endings in the equatorial and juxta-equatorial region or as motor end plate at the polar regions.

These results agreed with the study of the sensory and motor innervation of cat hindlimb spindles published by [26 , 27].

دراسة نسيجية في المستلمات العضلية العصبية في العضلات الهيكلية

مها خليل الملاك
قسم علوم الحياة ، كلية العلوم ، جامعة البصرة ، البصرة ، العراق

الخلاصـــة

أوضحت الدراسة الحالية بعض الخصائص النسيجية للمستلمات العضلية العصبية وكثافة هذه المستلمات بالنسبة إلى الألياف العضلية الاعتيادية.

عزلت المستلمات العضلية العصبية (المغازل العضلية) من عضلات الأطراف الأمامية والخلفية وفحصت بواسطة المجهر الضوئي.

حسب معدل وكثافة المغازل العضلية وقورنت مع غيرها من اللبائن. كل مغزل عضلي يتكون من ٢-٤ ألياف عضلية مغزلية صنفت إلى ألياف كبيرة (L)، متوسطة (M) وألياف صغيرة (S).

جميع المغازل العضلية تستلم ألياف عصبية حسية وحركية تتوزع عن الجذع العصبي المغزلي.

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