ORIGINAL PROF-498

ADULT ALBINO RATS ;
STUDY OF MOTOR UNITS INNERVATED BY TIBIAL NERVE

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SUMMARY

The average number and size of motor units were assessed by investigating the components of motor units supplied by Tibial Nerve in adult albino rats. The motor neurons forming the Tibial Nerve localized by HRP technique extended from the caudal part of L3 to S1 segments of the spinal cord. The motoneurons forming the Tibial Nerve (TN) which are distributed mainly in the Posterolateral and Post-Posterolateral groups with few of them are also distributed in the central groups. The average somal diameter of motoneurons ranged between 12 and 63 m. The motor end-plates on skeletal myofibers of muscles supplied by Tibial Nerve were localized by using bromo-indigo and urea-silver technique. The total of 826 motor units with the size of 137 of the muscles innervated by Tibial Nerve have been observed in the present study.

INTRODUCTION

The question of existence of Constant Pattern of relationship between the Nervous system and the Skeletal Muscles of Vertebrates has challenged biologists for more than half a century. Interest in the possibilty of a Nerve-Muscle specificity began with the postulation by Furbringer (1888, cited by Straus JR) of a fundamental, unvarying relationship between each striated muscle and its particular Nerve, So that the same nerve of necessity always innervates the same muscle.

The structural unit of striated muscle is the muscle fibre, while the functional unit is the motor unit. A motor unit begins with a nerve cell (neuron) whose body is in the ventral horn of spinal cord. The nerve cell has a long fine fibre which runs with hundreds of other in a motor nerve, enters the muscle and divides into terminal branches. Each terminal branch of a nerve fiber ends on a muscle fiber. The motor unit includes the nerve cell (body and

process) and a half dozen to many dozen of muscle fibre it supplies[2].

The concept that the basic divisions of the striated musculature of the body in some manner reflected in the arrangement of motoneurons of the Spinal Cord was fully developed by the work of Kaiser (1891, cited by Sprague[3]). Romances studied motoneurons by crushing the Peripheral nerves in the lower limb of cats.

Kristensson and Olsson introduced the use of horseradish peroxidase (HRP) as a neuro-anatomical method for mapping the pathways from axonal terminals to the cell bodies of origin in the peripheral nervous system. Motoneurons in the central and peripheral nervous system has been extensively studied by many investigators using HRP technique.

Lidell and Sherrington (1925, cited by Buchthal and Schmalbruch[5]) introduced the concept of motor units as the basic units of motor activity, i.e.; final common pathway and began a new era in the understanding of movements of limbs. Buchthal and Schmalbruch in their study of motor unit of Mammalian Muscle, commented that is was not possible with certainty to activate a single motor unit by electrical stimuli whether they were applied to the nerve or to the end plate zone.

There is a paucity of data concerning the anatomy of motor unit in human beings and in animals. In this study, an attempt has been made to localize the motor units supplied by the tibial nerve in adult albino rats. Motoneurons forming tibial nerve were localized, their number counted and their size measured. Moreover, the motor end plates of skeletal Myofibers supplied by tibial nerve were localized and their number counted.

MATERIALS AND METHODS

Ten (10) adult albino rats were used in this study, carried out in the research laboratory, Department of Anatomy, Bolan Medical College Quetta. These animals were anesthetized with intrapertioneal injection of pento-barbitone sodium at a dose of 15 mg/kg body weight.

The Tibial Nerve (TN) was exposed along with the common personal Nerve at the termination of Sciatic nerve in the popliteal fossa. Common Peroneal Nerve (CPN) was ligated before the application of HRP crystals to the Tibial Nerve (TN). The Tibial Nerve was cut tangentially to obtain maximum "Tracer Nerve" contact area. HRP crystals (Sigma Type-VI) were applied with fine probe to the proximal cut end of nerve at frequent intervals of 3 - 5 minutes for 2 - 3 hours. At the end of HRP application, the wound was closed in layers. After this procedure, the animals were allowed to survive for a period, varying between 48 - 72 hours.

For perfusion and fixation, the animals were re-anaesthetized after a suitable survival period. The fixation of animals was done by the method of Rosene and Mesulam.[6]

REMOVAL AND CUTTING OF LUMBAR SPINAL SEGMENTS

The lumbar portion of Spinal Cord was removed from the animals immediately after refusion. It was transferred to labelled bottles, containing 30% sucrose in 0.1 M Phosphate Buffer, Ph 7.4[7].

The tissue removed was stored at 4°C for 12 - 48 hours. Each Spinal Cord was cut in a serial order rostro caudally at the thickness of 40u. The cut sections of the Spinal Cord Segments were collected in a compartmentalized plastic tray. The tray was then placed in an incubating vessel containing 200 ML of 0.1 M Phosphate buffer at PH 7.4. The cut sections were kept at 4°C for 6 - 12 hours before enzymatic reaction.

DEMONSTRATION, COUNTING & MEASUREMENT OF MOTONEURONS

Histo chemical demonstration of HRP was done according to the method of mesulam[8]. The total number of Labeled motoneurons forming the Tibial Nerve (TN) were counted at magnification of X100 and X400. Measurement of somal size of labeled motoneurons was done according to the method of Burke, et al[9].

LOCALIZATION OF MOTOR END PLATE

The hindlimb muscles supplied by the Tibial Nerve were removed surgically with almost care without any damage to the muscle fiber; transferred to the labeled bottles containing buffered formalin and stored at 4°C. The sections of the muscles were cut longitudinally on freezing microtome at thickness of 100 um collected in staining tray containing de-ionized water and were incubated for the demonstration of cholinesterase activity at motor end plates of skeletal myofibers with bromoindoxyl acetate[10].

After incubation, the sections were transferred to the albuminized slides and air dried. Few sections of each muscle mounted on slides were processed in Urea-Silver nitrate for the demonstration of axons. On skeletal myofibers, the end-plate stained as rounded or elongated elevations were counted under light microscope with x10 objective in all the muscles.

MOTOR UNIT

The motor units supplied by the Tibial Nerve were estimated by the integration of data of motoneurons and the motor end-plates of muscles supplied by the Tibial Nerve.

OBSERVATIONS AND RESULTS

MOTONEURONS

The motoneurons forming the Tibial Nerve extended between L3 - S1 segments of the spinal cord in all animals. The motoneuronal density varied at different segment levels, showing sparse distribution in more rostral and caudal segments but dense population in L4 L5 segments in all the animals situated.

The motoneurons forming the Tibial Nerve were distributed mainly in the posterolateral and post-posterolateral groups with few of them also distributed in the central groups.

The total number of labelled motoneurons in L3, L4, L5 and S1 segments of spinal cord in all animals are shown in table-1.

The size spectrum of various motoneurons measuring more than 38 um. Presumably alpha motoneurons were 68.76%.

MOTOR END-PLATES

With the use of bromo-indigo and urea silver technique, motor-end plates were displayed in all the sections of muscles supplied by Tibial Nerve. The subneuronal apparatus stained blue, axons innervating the end plates were brown to black. Motor end-plates were found usually in the middle of the skeletal fibers. The motor end-plates on skeletal myofibers supplied by Tibial Nerve in adult albino rats stained with bromoindigo method were 85763 as recorded in table-2.

The number of labelled motoneurons measuring more than 38μm forming the Tibial Nerve were correlated with the number of motor end-plates counted in muscles supplied by that nerve and it was concluded that the average number of end-plates per

motoneuron was about 826.

Table No. 1 **Total cell count and segmental distribution of motoneurons whose axons form the Tibial Nerve (TN) in adult albino rat.**

SEGMENTAL DISTRIBUTION IN SPINAL CORD					
Total Cells	**L3**	**L4**	**L5**	**L6**	**S1**
2266	**239** (10.548%)	**681** (30.052%)	**753** (33.230%)	**415** (18.314%)	**178** (07.855%)
2018	**197** (09.762%)	**574** (28.44%)	**679** (33.647%)	**365** 18.087%)	**203** 10.059%)
1905	**105** (05.511%)	**711** (37.322%)	**658** (34.540%)	**296** (15.538%)	**135** 07.086%)
2246	**371** (16.518%)	**891** (39.670%)	**701** (31.211%)	**178** 07.925%)	**105** 04.674%)
1781	**291** (16.339%)	**403** (22.627%)	**571** (32.060%)	**331** (18.585%)	**185** (10.387%)
2519	**121** (04.803%)	**735** (29.178%)	**879** (34.894%)	**503** (19.968%)	**281** (11.155%)
1837	**099** (05.389%)	**685** (37.289%)	**591** (32.172%)	**306** (16.657%)	**156** (08.492%)
1897	**155** (08.170%)	**701** (36.953%)	**651** (34.317%)	**297** (15.656%)	**93** (04.902%)
2123	**215** (10.127%)	**357** (16.815%)	**792** (37.305%)	**506** (23.834%)	**253** (11.917%)
2355	**198** (08.407%)	**651** (27.643%)	**893** (37.919%)	**417** (17.707%)	**196** (08.322%)

DISCUSSION

The motoneurons forming Tibial Nerve, labelled with retrograde uptake of horse-raddish-peroxidase (HRP) applied to the proximal cut end of the nerve were located in the lateral part of the ventral horn of the spinal cord, our findings are in agreement with the results obtained by Janjua and Leong who reported that the Tibial Nerve motoneurons are located in the lateral part of the ventral horns of spinal cord. This study is also in agreement with the study of Leong et al[12] who observed that the radial motoneurons supplying the extensor muscles of the forelimb in monkey were located in the lateral part of the ventral horn of spinal cord. Our findings are in contrast to the findings of Kaizawa and Takahashi[13] who studied the distribution pattern of motoneurons in the lumbar spinal cord of rat by crushing the axons of the common peroneal nerve (CPN) and found that the CPN motoneurons were situated medially. As the HRP method of localizing neurons is more sensitive than the chromatolytic

method the difference may be attributed to the different techniques used, although species and individual differences may also be contributory factors. In the present study, the average somal diameters of motoneurons forming Tibial Nerve range between 12 and 63µm which is not in agreement with observations of Janjua and Leong who found the size range of CPN motoneurons between 14 and 76µm in monkeys. The difference may be due to different species used. In the present study, the percentage of alpha motoneurons forming Tibial Nerve was 68.76% which corresponds to the findings of Burke et al[9] who observed 70-75% of alpha motoneurons after intra muscular injection of HRP into gastrocnemius muscle in cat.

MOTOR END - PLATES

From the results of present study, it is clear that the motor end-plates are located usually in the middle half of the skeletal fibers. Motor end-plates in flexor carpi-radials muscle of cat were found predominantly near the middle of the fibers while a few occurring near the proximal or distal third[14]. In human biceps brachii muscle, the motor end-plates have been found in the middle. Our findings are in agreement with the results obtained in these studies.

MOTOR UNIT

The present study revealed that there are 826 motor units with the motor unit size of 137 with relation to the muscles innervated by Tibial Nerve in adult Albino rats.

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