截斷神經對大白鼠骨骼肌之巨大肌原纖維蛋白質的影響

Effect of Denervation on the Giant Myofibrillar Proteins in Rat Skeletal Muscle

中文摘要

周邊組織以骨骼肌爲主，約佔總體重的 40%，包含著複雜且有效率的精密細胞骨架。橫紋肌的肌原纖維是由許多稱爲肌節的基本結構單位所構成，肌節也是負責肌肉收縮的基本功能單位。肌節除了以肌凝蛋白質爲主的粗肌絲與以肌動蛋白質爲主的細肌絲之外，肌節尙包含第三種肌絲，即彈性蛋白質 titin (也稱爲 connectin)。在自然界，迄今被鑑定出來的所有蛋白質，titin 是最大的蛋白質(分子量爲 3000-4000 kDa)。單一 titin 分子延伸半個肌節，其 N 端埋入 Z 線之中，其 C 端則位於 M 線上。Titin 的主要功能爲產生被動張力和維持橫紋肌的肌節結構。Nebulin (又稱爲第三帶蛋白質)是另一巨大的肌原纖微蛋白質(分子量爲 600-900 kDa)，其纏繞著整個細肌絲而形成骨骼肌的第四種肌絲。目前認爲 nebulin 爲蛋白質尺規，以維持細肌絲長度的穩定，並且在訊息傳遞和收縮調節作用方面扮演著重要的角色。

近來，科學家正在研究起因於肌原纖維蛋白質的改變而造成的骨骼肌病變之生理病理學，以及探討肌原纖維蛋白質在肌肉結構和活性的重要性。由於 titin 與 nebulin 在調節肌節的結構和功能上是不可或缺的角色，因此可能和骨骼肌疾病有所牽連。然而，截斷神經引發肌肉萎縮的效應對肌肉內 titin 與 nebulin 的影響仍不清楚。爲此目的，我們以截斷坐骨神經而引起脛前肌萎縮作爲動物實驗模式，並檢測骨骼肌之肌原纖維蛋白質的改變情形，特別是深入探討 titin、nebulin、肌凝蛋白質重鏈和肌動蛋白質等四種肌原纖維蛋白質的變化情形。

首先，由於肌動蛋白質、肌凝蛋白質重鏈、nebulin 和 titin 四者的分子量範圍差距很大(從 42 到 4000 kDa)，很難同時在同一片微小膠體上作分析，因此我們研製一套改良式的微小膠體，以達到快速偵測骨骼肌之肌原纖維蛋白質的目的。方法是藉由一個塑膠針筒取代市面上販售的漸層鑄膠器，建立一套具有模糊界面的梯度漸層微小膠體，經由膠體染色和免疫轉漬的方法，顯示四種肌原纖維蛋白質可以成功地被分離和進行免疫點墨分析。此套微小膠體系統具有容易製備且易於與操作之優點，乃爲一個值得信賴的方法，可以提供分析肌原纖維蛋白質或是其他具有大範圍分子量的蛋白質混合物。

許多研究指出萎縮肌肉出現被動張力減弱且肌節排列不規則的現象。然而，在截斷神經之後 titin 的變化仍未被探討。因此我們應用研發的微小膠體以進行電泳和免疫螢光染色，以檢測大白鼠的對照組和截斷神經組之脛前肌內 titin 含量與型態變化。依據我們的數據顯示，截斷神經組肌肉內的 titin 與肌凝蛋白質重鏈比值(titin/MHC)以及 titin 與肌動蛋白質比值(titin/actin)明顯地減少。截斷神經後的 titin 含量和肌凝蛋白質重鏈與肌動蛋白質比較起來，呈現較大程度的降

解。另外，分別以電子顯微鏡與免疫螢光等方法，以檢定肌節的超微構造與靠近 Z 線的 titin 表型變化。型態學的觀察顯示截斷神經組肌肉的肌原纖維呈現不規則排列以及 titin 表型發生轉位的現象。歸納我們的結果推測截斷神經之後 titin 的蛋白質裂解作用較肌凝蛋白質重鏈與肌動蛋白質更爲敏感，並且，截斷神經組肌肉之 titin 的衰微，可能因而造成 titin 爲主的肌節結構不完整。

此外，我們在也探究截斷神經效應對 nebulin 改變情形，利用自製的微小膠體以進行電泳，以定量大白鼠的對照組和截斷神經組之脛前肌內 nebulin、肌凝蛋白質重鏈、肌動蛋白質和 titin 的變化。我們的數據顯示，在截斷神經之肌肉組的 nebulin 以時間依賴性的型式減少，特別是在截斷神經的二十八天與五十六天之後，和對照組之肌肉比較，截斷神經組之肌肉的 nebulin 分別地明顯減少 24.6%與 40.2%。Nebulin 與肌凝蛋白質重鏈比值(nebulin/MHC)、nebulin 與肌動蛋白質(nebulin/actin)以及 nebulin 與 titin 比值(nebulin/titin)均顯著地下降，顯示在萎縮之肌肉組內，nebulin 降解的速度比肌凝蛋白質重鏈、肌動蛋白質與 titin 的降解速度更爲快速。我們推測 nebulin 的降低可能影響截斷神經引起的棄用型萎縮之肌節重塑作用。然而仍有待後續的研究以明瞭確實的機制。

本論文爲有意義的實證表示巨大的肌原纖維蛋白質 titin 與 nebulin 較肌凝蛋白質重鏈與肌動蛋白質對截斷神經效應更爲敏感，以及更瞭解 titin 與 nebulin 在萎縮肌肉的重要角色。而且，我們推測若是延緩 titin 與 nebulin 的降減作用將可作爲骨骼肌萎縮症的治療介入之考量。

英文摘要

Skeletal muscle is a major peripheral tissue that accounts for approximately 40% of the total body weight, and is also an intricate and efficient machine that contains precise cytoskeletal networks. Striated myofibrils are composed of numerous basic units, sarcomeres, and are responsible for muscle contraction. In addition to myosin-based thick filaments and actin-based thin filaments, sarcomeres contain a third filament system, which is formed by the elastic protein, titin (connectin). In nature, titin is the largest protein (3000-4000 kDa) identified to date. A titin molecule extends for one-half of the sarcomeric length and its N-terminus is embedded within the Z-line and its C-terminus within the M-line. The principal function of titin is to generate passive tension and maintain the sarcomere structure in striated muscles. Nebulin, another giant myofibrillar protein (600-900 kDa), spans the length of actin filaments and forms the fourth filament system in skeletal muscle. Nebulin acts as a protein ruler to maintain the lattice arrays of thin filaments, and plays a role in signal transduction and contractile regulation.

Currently, tremendous efforts are being devoted to understanding the physiopathology of several skeletal muscle myopathies that result from changes in myofibrillar

proteins, highlighting their importance in muscle architecture and activity. Due to the essential roles of titin and nebulin in regulating the structure and function of sarcomeres, it has been suspected for some time that titin and nebulin may be implicated in skeletal muscle disorders. However, the effect of denervation on the changes of titin and nebulin is unclear. For this purpose, we cut the sciatic nerve to elicit tibialis anterior (TA) muscle atrophy in an animal experimental model, and then examined the changes in myofibrillar proteins of skeletal muscle, by especially focusing on titin, nebulin, myosin heavy chain (MHC), and actin.

First, it is difficult to simultaneously analyze actin, MHC, titin, and nebulin on the same minigel due to their broad range of molecular weights (from 42 to 4000 kDa). We developed an improved minigel with an ambiguous interface to detect myofibrillar proteins of skeletal muscle. By employing a plastic syringe instead of the commercial gradient formers, we established a step gradient minigel with an ambiguous interface. Coomassie brillant blue R-250 staining and immunoblotting revealed that the four proteins were successfully separated and transferred for analysis. With this minigel system, one can simply, easily, and reliably analyze myofibrillar proteins or other protein mixtures with broad molecular masses.

Second, several studies have reported attenuation of passive tension and disorganization of sarcomeres in atrophic muscles, but changes in titin after denervation have not been investigated. We utilized the minigel we developed to perform gel electrophoresis and immunofluorescent staining to examine titin’s properties in innervated and denervated TA muscles of the rat. Our data indicated that a greater loss of titin content occurs compared to MHC and actin following denervation. Moreover, the ratios of titin/MHC and titin/actin significantly decreased in denervated muscles. In addition, the ultrastructure of sarcomeres and changes in the titin epitope near the Z-line were respectively examined by electron microscopy and immunofluorescence. Morphological observations showed the disorganized arrangement of myofilaments and translocation of the titin epitope in denervated muscle. Taken together, our results provide significant evidence that titin is more sensitive to degradation than MHC and actin after denervation. The titin decline results in the loss of titin-based sarcomeric integrity in denervated muscle.

Finally, we investigated the effect of denervation on changes in nebulin content in atrophic muscle of the hindlimb. Herein, we also used our homemade minigel to quantify levels of nebulin, MHC, actin, and titin in innervated and denervated TA muscles of the rat. Our preliminary results showed the nebulin of denervated muscle gradually decreased in a time-dependent manner. Particularly after denervation for 28 and 56 days, the nebulin content in denervated muscles markedly decreased by 24.6% and 40.2% in comparison with innervated muscle, respectively. The obvious loss in

the ratios of nebulin/MHC, nebulin/actin, and nebulin/titin suggested a greater drop in nebulin compared to MHC, actin, and titin in atrophic muscle. We speculated that the reduction in nebulin might affect sarcomeric plastisity to denervation-induced disuse atrophy. However, elucidation of the detailed mechanism required further investigation.

This dissertation provides significant evidence that titin and nebulin are more sensitive to the effect of denervation than MHC and actin, and foster a better understanding of the major roles of titin and nebulin in atrophic muscle. Moreover, our data raise the intriguing possibility that retarding the declines in titin and nebulin can be considered as options for therapeutic interventions in skeletal muscle atrophy.