THE NEURONAL CONTROL OF MUSCLE PROPERTIES

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Skeletal muscle of the pig has a unique grouped arrangement of fibers with 3-70 type I fibers occurring in a tight cluster and being surrounded by type II fibers. A modified acetylcholinesterase-silver nitrate staining method was employed to examine intramuscular nerves, subterminal axons and motor end plates. The terminal innervation ratio of muscle in normal pigs was near 1.00 indicating a one-to-one relationship between subterminal axons and the muscle fibers they innervate. Reinnervation studies were conducted by crushing the upper sciatic nerves in young pigs and subsequently examining the affected muscles. The nerve crush caused distal degeneration of the nerve and loss of the normal spatial distribution of fiber types. Subsequent reinnervation caused extensive collateral ramification of subterminal axons. We concluded that such collateral reinnervation imposed a neuronal influence on muscle fibers which dictated the transformation of all muscle fibers innervated by a single subterminal axon to a uniform histochemical profile. In the broadest sense our results strongly support the concept that the neurone dictates the characteristics of the muscle fiber. The application of this finding to the improvement of meat quality presents an interesting possibility.

LE CONTROLE DES PROPRIETES DU MUSCLE PAR LES NEURONES

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Le muscle squelettique du porc montre un unique modèle de répartition des fibres musculaires, avec par unité, 3-70 fibres de type I rassemblées en un faisceau serré et entourées de fibres de type II. Les nerfs intramusculaires, les axones subterminaux et les plaques motrices ont été etudiées en utilisant une modification de la méthode de coloration à l'acétylcholinesterase-nitrate d'argent. Le rapport du nombre des innervationes terminales au nombre de fibres musculaires est voisin de 1,00, ce qui indique l'existence de liaisons univoques entre les axones subterminaux et les fibres musculaires qu ils innervent. Les études de réinnervation ont été réalisées par écrasement de la racine dorsale du nerf sciatique chez de jeunes porcs et en en examinant les conséquences sur les muscles affectés. L'écrasement entraine une dégénérescence distale du nerf et la disparition de la répartition spatiale normale des fibres des différents types. La réinnervation ultérieure, par régénération naturelle, se fait avec une ramification collatérale importante des axones subterminaux. Nous en concluons que c'est la dépendance neuronale imposée aux fibres musculaires par une telle réinnervation collatérale qui entraine l'évolution vers un profil histologique unique de toutes les fibres innervées par un axone subterminal unique. Dans leur signification la plus large, nos résultats renforcent le concept du neurone fixant les caractéristiques de la fibre musculaire. L'application de ce résultat a l'amélioration de la qualité de la viande montre d'intéressantes possibilités.

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NEURONALE KONTROLLE DER MUSKELEIGENSCHAFTEN

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Der Skelettmuskel des Schweines het eine einzigartig zusammengesetzte Anordnung von Fasern mit 3 - 70 typ I Fasern, die in einem dicken Bündel vorkommen und umgeben sind von Fasern des Typ II. Ein modifiziertes Färbungsverfahren mit acetylcholinesterase-silber Nitrat wurde angewandt, um die intramuskulären Nerven, die am Ende befindlichen Neuriten und motorischen Endplatten (motor end plates) zu untersuchen. Das begrenzte Innervationsverhältnis des Muskels in normalen Schweinen war nahezu 1.00. somit anzeigend, daß eine eins-zu-eins Beziehung zwischen den am Ende befindlichen Neuriten und den Muskelfasern, die sie mit Nerven versorgen, besteht. Weitere Innervationsstudien wurden gemacht, indem die oberen Hüftnerven von jungen Schweinen zerdrückt und später die davon betroffenen Muskeln untersucht wurden.

Das Zerdrücken der Nerven verursachte fremdartige Entartung des Nervs und den Verlust einer normalen räumlichen Verteilung der Fasertypen. Eine weitere Reinnervation verursachte erhebliche Nebenverzweigung (collateral ramification) der End-neuriten.

Wir folgerten daraus, daß solche Neben-reinnervation einen neuronalen Einfluß auf Muskelfasern ausübt und eine Veränderung (transformation) an allen Muskelfasern bestimmt, die, von einem einzigen am Ende befindlichen Neuriten mit Nerven versorgt, zu einheitlichem gewebechemischen Profil werden.

Im weitesten Sinne bekräftigen unsere Resultate nachdrüklich den Gedanken, daß das Neuron die Eigenschaften der Muskelfaser bestimmt. Die Anwendung dieses Befundes bei der Verbesserung der Fleischqualität bietet interessante Möglichkeiten.

Нейронный контроль мышечных свойств

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Лаборатория по биологии мышец

Скелетная мышца свиньи имеет особенное сгрупированное расположение волокон с 3 - 70 волокон типа I, встречающихся в туго завязанных пучках, и окруженных волокнами типа II. Модифицированный метод окрашивания способом ацетилхолинэстеразы-азотнокислого серебра был использован с целью исследования внутримышечных нервов, терминальных аксонов, и двигательных бляжек. Коэффициент иннервации окончания мышцы здоровых свиньей был близок к 1.00, показывающий отношение одного к одному между терминальными аксонами и мышечными волокнами ими иннервированными. Изучения реиннервации производились путем сокрушения верхних седалищных нервов у молодых свиньей, сопровожденные последующим рассматриванием затронутых мышец. Сокрушение нервов привело к дистальному перерождению нервов и потери нормального пространственного расположения типов волокон. Последующая реиннервация привела к экстенсивному побочному разветвлению терминальных аксонов. Мы пришли к заключению, что такая побочная реиннервация произвела сильное нейронное влияние на мышечные волокна. Это реиннервация привела к превращению всех мышечных волокон иннервированных одним терминальным аксоном. Это явление привело к единообразному гистохимическому профилю. В общем, итоги нашего эксперимента строго подтверждают то понятие, что нейрон определяет характерные черты мышечного волокна. Применение полученных данных к улучшению качества мяса представляет собой интересную возможность.

THE NEURONAL CONTROL OF MUSCLE PROPERTIES[1]

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INTRODUCTION

The nervous system exerts a strong regulatory effect on muscle; neuronal control of meat production is therefore a proposition to consider. Even though this topic has been largely overlooked by meat research workers, it represents an exciting new avenue of approach and is especially timely in view of the current and developing world food situation.

That the function and biochemical properties of individual myofibers are controlled by the motor neuron is now widely accepted. The trophic factor, while still an unidentified entity, has been discussed in detail (Guth, 1968; Close, 1972).

Swatland and Cassens (1974) have considered the role of innervation in muscle development and function. The New Zealand workers (Chrystall and Hagyard, 1975) have utilized electrical shocking of muscle, in slaughtered animals, to control post-mortem change, and Swatland (1975) has found that intact neural pathways from spinal motor neurons to muscle fibers survive for at least 13 to 18 minutes following exsanguination. Some consideration has therefore been given to the regulatory influence of the nervous system on meat production at both the time of fetal development and at the time of slaughter.

Skeletal muscle of the pig affords an unique opportunity to study the basis for neuronal control of the properties of individual myofibers because Type I myofibers are grouped in the center of fasciculi and are surrounded by Type II myofibers. In other normal mammalian skeletal muscle the spatial distribution pattern of myofiber types appears random. Specific exceptions are a "uniform" pattern of myofiber types or type predominance. Type grouping in skeletal muscle, however, is usually connected with myopathies, diseases of the lower motor neuron and other neuromuscular disorders.

Type grouping can be caused by experimental denervation of muscle with subsequent self-reinnervation (Karpati and Engel, 1968), and is thought to be the result of reinnervation of denervated myofibers by collaterals from persisting healthy axons or regenerating axons (Morris, 1969).

The objective of our work was to study the effect of reinnervation on the spatial distribution and arrangement of myofiber types in muscle of the pig. The rationale was to establish if the usual type grouping pattern persisted or was altered so that conclusions could be made about the relative importance of the exogenous nervous system and the endogenous genetic information within the myofiber in controlling the properties of the myofiber.

MATERIALS AND METHODS

Eighteen castrated male Hampshire and Poland China pigs (12.3 kg average body weight) were anesthetized and the upper sciatic nerve was isolated between the superficial gluteus and biceps femoris muscles. The nerve was crushed for 15 seconds by full closure of an eight-inch hemostatic forceps, the ends of which were covered with tygon tubing. The sciatic nerve was isolated but not crushed in four sham-operated animals. Five non-operated animals and the contralateral limbs of animals which received a nerve crush also served as controls. Four animals which received a nerve crush were sacrificed at one week post nerve crush and the remaining animals were sampled at 10, 15, 20, 25, 27 and 31 weeks after nerve crush.

Samples were excised from deep and superficial portions of the semitendinosus muscle and processed for histochemistry. Type I and Type II myofibers were classified according to Engel (1974) and Brooke and Kaiser (1970). Type I myofibers exhibited low alkaline ATPase and phosphorylase activity and high acid ATPase and NADH-tetrazolium reductase activity while Type II myofibers exhibited the opposite characteristics. Type II myofibers stained by the alkaline ATPase procedure had subclasses of dark and intermediate staining myofibers, but both were classified as Type II myofibers.

A modified acetylcholinesterase-silver nitrate method (Beermann and Cassens, 1976) was used to stain for intramuscular nerves, subterminal axons and motor end plates. Functional and absolute terminal innervation ratios were determined with a minimum of 200 axons for each sample.

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RESULTS

Nerve crush blocked muscle contraction as elicited by stimulation of the nerve proximal to the site of crush and also caused marked demyelination of the nerve by one week post crush. Hyperextension of the posterior limb and gross atrophy of the posterior limb muscles were evident in 2 weeks following crush and appeared to be most severe at 2 to 3 months after crush. Five to six months after the sciatic nerve was crushed, recovery of the denervated muscles was clinically good.

No changes were found in the histochemistry or morphology of myofibers at 1 week post nerve crush but at 10 -11 weeks the differential staining characteristics of the NADH-TR and phosphorylase reactions were replaced by a broad spectrum of staining intensities. Differentiation of myofiber types was still afforded with both ATPase reactions and atrophy of myofibers was apparent. At 15 weeks post crush reinnervated muscle samples stained with more gradations of intensity and with less overall intensity than controls. The two types of myofibers could be perceived and an apparent transformation had occurred. Large groups (40-50 myofibers) of Type I and Type II myofibers were visible in deep semitendinosus and small groups of Type I myofibers were observed in superficial semitendinosus. A moderate degree of fat infiltration was also evident at this time. At later stages, the differentiation of myofiber types became clearer.

There was no significant difference in percentage of Type I and Type II myofibers between control and reinnervated samples.

Denervation and subsequent reinnervation caused myofibers to atrophy, become more homogeneous histochemically and then regain their distinguishing histochemical properties. During transformation the normal type grouping pattern of pig muscle was destroyed and replaced by a grouping pattern previously described to result from reinnervation in other mammalian muscle. The proportion of myofiber types did not change even though the original spatial distribution was completely obliterated and even though the myofibers lost their distinguishing characteristics during the transformation.

The Functional Terminal Innervation Ratio for reinnervated samples ranged from 1.45 to 2.15 compared to about 1.00 for controls. All reinnervated muscle samples had collateral, ultraterminal and terminal branching of the subterminal axons which resulted in the formation of more than one motor end plate on one myofiber or in the innervation of more than one myofiber.

DISCUSSION

A simple crush of the upper sciatic nerve was sufficient to evoke a complete rearrangement of the original spatial distribution of myofiber types. One subterminal axon innervated one myofiber on the average in normal pig muscle, but reinnervated muscle exhibited collateral branching which gave rise to simultaneous innervation of up to 15 myofibers by a single subterminal axon. Myofibers became more histochemically homogeneous following denervation. This was probably due to the loss of trophic influence brought about by nerve crush. Only after return of this trophic influence by extensive collateral reinnervation did myofibers regain their distinguishing histochemical properties.

Our results argue against the idea that certain stem lines of myofibers attract preferentially a given type of axon. If this were the case then the normally occurring clusters of Type I myofibers should have been maintained. In the broad sense, our results add strong support to the idea that a neuron dictates the characteristics of a myofiber.

CONCLUSIONS

(1) Nerve crush causes distal degeneration of the nerve and loss of the normal myofiber type pattern.
(2) Denervation and subsequent reinnervation causes extensive collateral ramification of subterminal axons.
(3) Collateral reinnervation imposes neuronal influence on myofibers which dictates transformation of all myofibers innervated by a single subterminal axon to a uniform histochemical profile.
(4) The type grouping observed in normal porcine muscle is not a result of neuronal influence mediated by collateral branching of subterminal axons, but, rather, may be the manifestation of a unique motor unit topography.

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