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Increased expression of the nicotinic acetylcholine receptor in stimulated muscle

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Abstract

Chronic low-frequency stimulation has been used as a model for investigating responses of skeletal muscle fibres to enhanced neuromuscular activity under conditions of maximum activation. Fast-to-slow isoform shifting of markers of the sarcoplasmic reticulum and the contractile apparatus demonstrated successful fibre transitions prior to studying the effect of chronic electro-stimulation on the expression of the nicotinic acetylcholine receptor. Comparative immunoblotting revealed that the α- and δ-subunits of the receptor were increased in 10–78 day stimulated specimens, while an associated component of the surface utrophin–glycoprotein complex, β-dystroglycan, was not drastically changed in stimulated fast skeletal muscle. Previous studies have shown that electro-stimulation induces degeneration of fast glycolytic fibres, trans-differentiation leading to fast-to-slow fibre transitions and activation of muscle precursor cells. In analogy, our results indicate a molecular modification of the central functional unit of the post-synaptic muscle surface within existing neuromuscular junctions and/or during remodelling of nerve–muscle contacts.
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Keywords: Low-frequency stimulation; Skeletal muscle; Fast-to-slow transition; Nicotinic acetylcholine receptor; Neuromuscular junction; Calcium-ATPase; Calsequestrin; Agrin; Dystroglycan; Dystrophin

The various types of skeletal muscle fibres represent a class of tissue that is highly versatile and extremely plastic making muscles highly adaptable to changed functional demands, e.g., enhanced contractile activity triggers many structural, functional, and metabolic changes in order to adapt to altered physiological requirements [1]. The cellular heterogeneity of skeletal muscle fibres is reflected on the molecular level by the existence of numerous protein isoforms in the contractile apparatus [2] and the Ca^{2+}-handling system [3]. Although all skeletal muscle cells generate contractile forces by means of organised filament systems, the specific combinatorial isoform expression patterns of regulatory and contractile elements are not identical in developing, fast twitching adult, slower contracting mature, and ageing fibres [2]. An interesting experimental model system for investigating responses of skeletal muscles to enhanced neuromuscular activity under conditions of maximum activation is chronic low-frequency stimulation [4]. Studying this system, it was shown that fast-to-slow transitions are due to trans-differentiation from fast to predominantly slow muscle cells, the recruitment of satellite cells, and a certain degree of cellular destruction and regeneration [5]. Muscle cells produced by long-term low-frequency stimulation exhibit a decreased caliber, show an elevation of their aerobic-oxidative capacity, are more resistant to fatigue, and show an increase in the time to peak twitch tension and half-relaxation time [6]. On the biochemical level, this adaptive process is reflected by a gradual replacement of myosin heavy chains (MHC) from the MHCIIb isoform to MHCIId(x) to MHCIIa to MHCI [2]. Drastic changes in the isoform expression pattern of many Ca^{2+}-regulatory membrane proteins occur, including shifts to slower isoforms of the voltage-sensing

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dihydropyridine receptor, the ryanodine receptor Ca^{2+}-release channel of the junctional sarcoplasmic reticulum, the luminal Ca^{2+}-reservoir element calsequestrin, and the relaxation-inducing Ca^{2+}-ATPases of the longitudinal tubules and terminal cisternae [7–9].

Besides studying the effect of chronic electro-stimulation on the expression of the acetylcholinesterase [10], the fate of markers of the neuromuscular junction during transitions from a fast to a slower muscle phenotype has not been determined. In order to discover biochemical parameters that may reflect a molecular modification of the neuromuscular transmission machinery within existing motor units and/or during restructuring of nerve–muscle connections following muscle conditioning, we have performed an immunoblot analysis of the nicotinic acetylcholine receptor in chronic low-frequency stimulated fast muscle. The nicotinic acetylcholine receptor in skeletal muscle is a typical ionotropic receptor involved in fast synaptic transmission [11,12]. Its ligand-gated ion channel consists of five subunits (α–α–β–γ–δ/ε) that form a cluster surrounding a central cation-selective transmembrane pore [13]. The neurotransmitter binding sites in the extracellular portion of the receptor complex are believed to be located at the interface between the α-subunit and adjoining δ/ε- and γ-subunits [14]. Within the neuromuscular junction, synaptic transmission is mediated by the release of acetylcholine from synaptic vesicles, diffusion across the subsynaptic cleft, and interaction of one neurotransmitter molecule each with the two α-subunit associated binding sites [11,12]. Activation of the post-synaptic acetylcholine receptor involves a switch in the conformation of the principal α-subunit [15]. This process allows for the efficient transmission of an action potential from the innervating motor neuron into depolarisation of the post-synaptic muscle surface membrane. Many proteins are involved in the formation and maintenance of the neuromuscular junction, including the muscle-specific kinase termed MuSK, rapsyn, agrin, and the utrophin–glycoprotein complex [16–18]. Hence, in order to determine potential changes in the neuromuscular junction following chronic electro-stimulation, we have performed a comparative immunoblot analysis of the α- and δ-subunits of the nicotinic acetylcholine receptor and markers of the utrophin–glycoprotein complex in 5–78 day stimulated muscle specimens. Successful fibre transitions were demonstrated by the well-established isoform shifting of contractile and sarcoplasmic reticulum proteins.

Materials and methods

Materials. Primary antibodies were obtained from Affinity Bioreagents, Golden, CO (mAb 88B to the δ-subunit of the nicotinic acetylcholine receptor from skeletal muscle; mAb IIH11 to the fast Ca^{2+}-ATPase isoform SERCA1; mAb IID8 to the slow Ca^{2+}-ATPase isoform SERCA2; mAb VIIID1_2 against fast calsequestrin; and mAb VF1c against the 90 kDa JFP), Upstate Biotechnology, Lake Placid, NY (polyclonal antibody to slow/cardiac calsequestrin), Chemicon International, Temecula, CA (mAb 374 to glyceraldehyde 3-phosphate dehydrogenase), Sigma Chemical Company, Dorset, UK (mAb MY32 to the fast myosin heavy chain; polyclonal antibody to laminin), Calbiochem, Nottingham, UK (mAb AGR-33 against agrin), and Novocastra Laboratories, Newcastle upon Tyne, UK (mAb NCL-a-SARCG against α-sarcoglycan; mAb NCL-b-DG to β-dystroglycan; and mAb DYS-1 to dystrophin). Polyclonal antibodies to the utrophin isoform Up395 and the α-subunit of the nicotinic acetylcholine receptor were gifts from Drs. Steve Winder (University of Glasgow, Scotland) and Jakob Schmidt (State University of New York at Stony Brook), respectively. Peroxidase-conjugated secondary antibodies were from Chemicon International (Temecula, CA). Ultrapure Protogel acrylamide stock solutions were purchased from National Diagnostics (Atlanta, GA). Chemiluminescence substrates and the Immunoprecipitation Protein G Kit were from Pierce and Warriner Limited (Chester, UK). Immobilon NC nitrocellulose membranes were obtained from Millipore Corporation (Bedford, MA). All other chemicals were of analytical grade and purchased from Sigma Chemical (Dorset, UK).

Chronic low-frequency stimulation. To study the potential effect of skeletal muscle transformation to a slower twitching phenotype on the relative expression levels of the nicotinic acetylcholine receptor, the left hind limb of adult male New Zealand white rabbits was chronically telestimulated through the peroneal nerve in the Animal Facility of the University of Konstanz [19]. Successful fast-to-slow transition was demonstrated by isoform switching of established markers of the sarcoplasmic reticulum (calsequestrin, Ca^{2+}-ATPase, and junctional face membrane protein of 90 kDa) and the contractile apparatus (myosin heavy chain). Muscles were continuously (24 h daily) stimulated at 10 Hz and the stimulation voltage was individually adjusted to yield maximal contractions of the ankle dorsiflexors [6]. Following chronic low-frequency stimulation for 5, 10, 20, 46, and 78 days, the tibialis anterior muscles were excised, cut into several longitudinal sections, and then quick-frozen in liquid nitrogen. Muscle specimens were transported on dry ice and stored at −70 °C prior to further usage.

Preparation of crude microsomes. In order to prevent proteolytic degradation, all preparative steps were performed at 0–4 °C in the presence of a protease inhibitor cocktail [20]. Crude microsomal vesicles for electrophoretic separation and subsequent immunoblot analysis were isolated from tibialis anterior homogenates by a method previously optimised in our laboratory [21]. The protein concentration of isolated microsomes was determined according to Bradford [22] using myofibrillar proteins as a standard.

Immunoblot analysis. Since only restricted amounts of microsomal membranes can be extracted from chronic electro-stimulated tibialis anterior muscles, a Mini-MP3 electrophoresis system and mini blotting cell from Bio-Rad Laboratories (Hempel Hempstead, Herts., UK) were employed. For the gel electrophoretic separation of microsomal muscle proteins, SDS–PAGE under reducing or non-reducing conditions was carried out by standard methodology [23] using 7% resolving gels and 20 μg protein per lane [24]. Following electrophoretic separation, gels were transferred onto Immobilon NC membranes according to Towbin et al. [25]. Blocking and incubation of nitrocellulose sheets with primary and secondary antibodies were carried out by standard techniques [26]. The enhanced chemiluminescence detection method was employed to visualise immunodecorated protein bands. Densitometric scanning of enhanced chemiluminescence blots was performed on a Molecular Dynamics 300S computing densitometer (Sunnyvale, CA) with ImageQuant V3.0 software. Immunoprecipitation experiments with monoclonal antibody NCL-b-DG to β-dystroglycan were carried out as previously described [24] using the Pierce Protein G IP Kit.

Results and discussion

Chronic low-frequency stimulation represents a model system for investigating responses of skeletal muscle fibres to enhanced neuromuscular activity under experimentally controlled conditions of maximum activation. In analogy to well-established changes in the abundance of muscle marker proteins and shifts in the isoform expression pattern of elements involved in contraction, energy metabolism, and ion homeostasis [2], we can show here that chronic electrostimulation induces a drastically increased expression of specific subunits of the nicotinic acetylcholine receptor. Taking the receptor as a marker of the post-synaptic muscle surface membrane, its enhanced expression during the fast-to-slow transformation process suggests a molecular modification of the central functional unit of the post-synaptic muscle surface within existing neuromuscular junctions and/or during remodelling of nerve–muscle contacts. Our analysis encompassed the demonstration of successful fibre transition following chronic low-frequency stimulation (Fig. 1), the immunoprecipitation analysis of the supramolecular neuromuscular junction complex (Fig. 2) and the immunoblotting of subunits of the nicotinic acetylcholine receptor and associated components in 5–78 day electro-stimulated muscle specimens (Fig. 3).

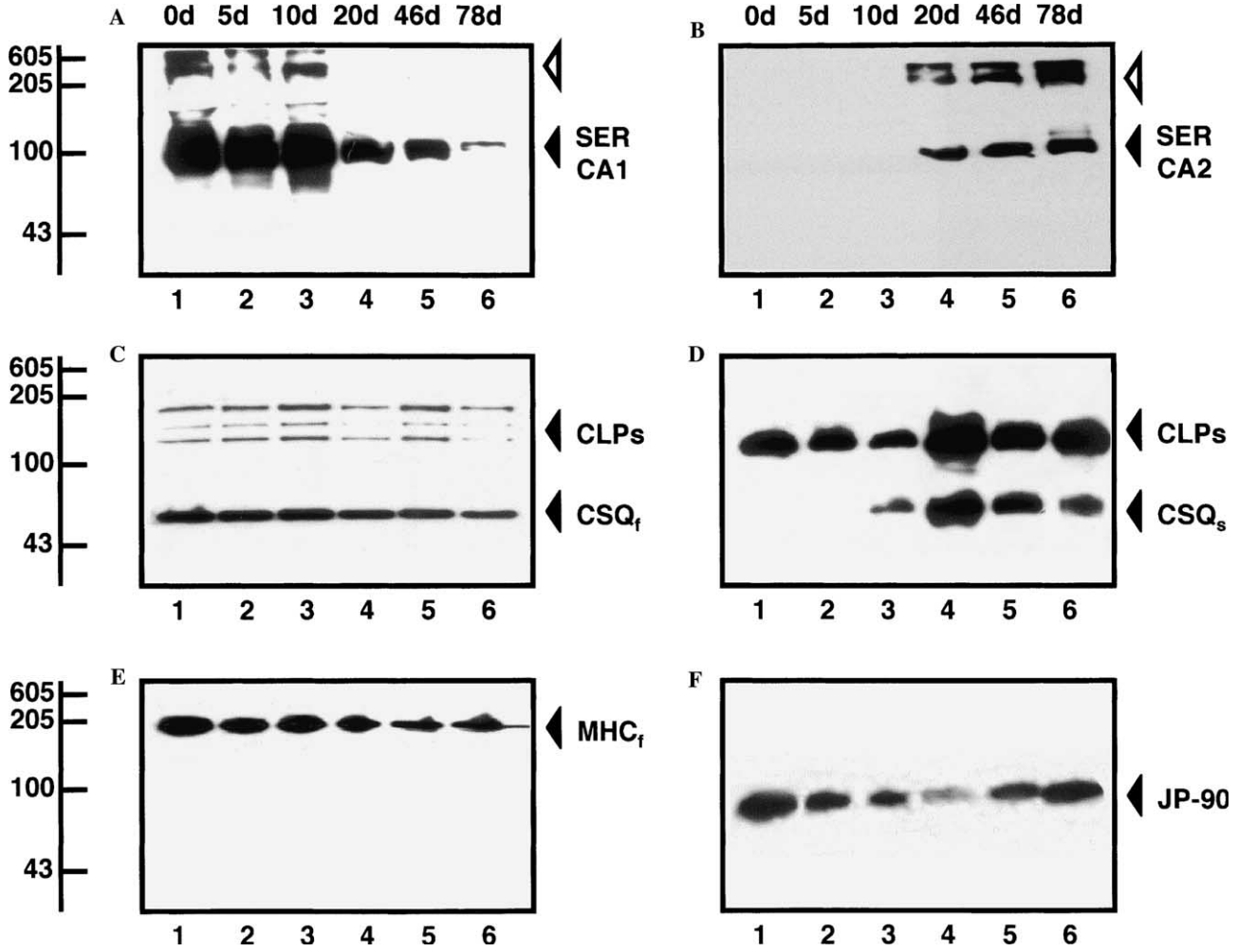

Fig. 1. Immunoblot analysis of muscle marker isoform shifting following chronic low-frequency stimulation. Shown are representative immunoblots of microsomal membranes labelled with antibodies to the fast Ca^{2+}-ATPase isoform SERCA1 (A), the slow Ca^{2+}-ATPase isoform SERCA2 (B), the fast calsequestrin isoform CSQ_f and fast calsequestrin-like proteins (CLPs) (C), the slow/cardiac calsequestrin isoform CSQ_s and slow CLPs (D), the fast myosin heavy chain (MHC_f) (E), and the junctional triad protein of apparent 90 kDa (JP-90) (F). Lane 1 represents membranes from unstimulated contralateral muscles (0 day) and lanes 2–6 show microsomes prepared from rabbit tibialis anterior muscles exposed to continuous (24 h per day) low-frequency (10 Hz) stimulation for 5, 10, 20, 46, and 78 days (5–78 days), respectively. Immunodecorated bands are indicated by closed arrowheads. In panels (A) and (B), open arrowheads mark the position of oligomeric Ca^{2+}-ATPase species, estimated to be dimers and tetramers. Sizes of molecular mass standards (in kDa), as deduced from rat myofibrillar proteins, are indicated in the left.

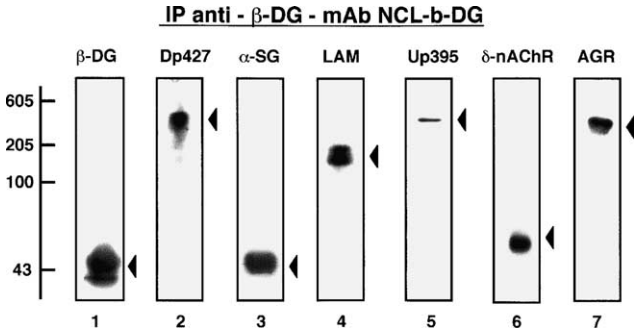

Fig. 2. Immunoprecipitation analysis of the supramolecular neuromuscular junction complex. Shown are immunoblots of solubilised microsomal membranes from normal unstimulated rabbit skeletal muscle immunoprecipitated (IP) with monoclonal antibody NCL-b-DG to β-dystroglycan of apparent 43 kDa (β-DG). Lanes 1–7 represent the immunoprecipitated fraction immunolabelled with antibodies to β-dystroglycan (β-DG), the dystrophin isoform Dp427, α-sarcoglycan (α-SG), laminin (LAM), the utrophin isoform Up395, the δ-subunit of the nicotinic acetylcholine receptor (δ-nAChR), and agrin (AGR), respectively. For proper immunodecoration, the gel in lane 7 had to be run under non-reducing conditions. The high-molecular-mass band represents an oligomeric species containing agrin. The position of immunodecorated protein bands is marked by closed arrowheads. Sizes of molecular mass standards (in kDa), as deduced from rat myofibrillar proteins, are indicated in the left.

Immunoblot analysis of muscle marker isoform shifting following chronic low-frequency stimulation

Prior to investigating the potential effect of chronic electro-stimulation on the relative expression of the nicotinic acetylcholine receptor, the successful transformation of fast tibialis anterior fibres to a slower twitching phenotype required demonstration. Because major differences exist in the physiological organisation of Ca^{2+}-uptake and luminal Ca^{2+}-binding mechanisms between fast and slow muscles [8], the relative abundance and isoform expression pattern of the two major muscle Ca^{2+}-ATPases of the sarcoplasmic reticulum, fast SERCA1, and slow SERCA2 [27], and the Ca^{2+}-reservoir markers, fast calsequestrin CSQ_f and slow/cardiac calsequestrin CSQ_s [28], were investigated to establish proper muscle transformation. As illustrated in Figs. 1A–D, during 11 weeks of chronic electro-stimulation a switch between fast and slow isoforms occurred for both Ca^{2+}-regulatory elements. Immunolabelling of the SERCA1 Ca^{2+}-ATPase by monoclonal antibody IIH11 shows a drastically reduced expression of the 110 kDa monomer (Fig. 1A). The high-molecular-mass bands represent dimers and tetramers of this ion pump [9]. In contrast to fast SERCA1, the immunodecoration pattern with monoclonal antibody IID8 to the slow SERCA2 isoform of the sarcoplasmic reticulum Ca^{2+}-ATPase showed a drastic increase of this pump in 20–78 day stimulated specimens (Fig. 1B). Following chronic low-frequency stimulation, the relative abundance of the two isoforms of the major luminal Ca^{2+}-binding protein of the terminal cisternae was investigated. Antibodies to calsequestrin recognise the monomeric form of approximately 60 kDa, as well as several calsequestrin-like proteins (CLPs) of 120–200 kDa [24]. During the fast-to-slow muscle transformation process, the monomer of the fast calsequestrin isoform decreased in its relative abundance (Fig. 1C), while the monomeric slow/cardiac isoform was clearly up-regulated after 10 days of electro-stimulation (Fig. 1D). In contrast to the monomeric CSQ isoforms, the CLPs did not exhibit drastic changes following stimulation.

To demonstrate successful transformation, immunoblotting of the fast myosin heavy chain MHC_f isoform was performed. Although monoclonal antibody MY32 does not differentiate between the various fast-twitch species of the myosin heavy chain, Fig. 1E clearly shows an overall decreased expression of the contractile apparatus marker MHC_f following chronic stimulation. Since it has previously been established that chronic low-frequency stimulation induces a gradual isoform exchange of myofibrillar proteins, such as the replacement of myosin heavy chains in rabbit tibialis anterior in the following sequence: MHCIIb to MHCIId(x) to MHCIIa to MHCI [1], this result can be used as an internal standard for our immunoblot analysis. The decrease in MHC_f clearly established the successful transformation process in the muscle specimens investigated. We could show previously that a microsomal protein of apparent 90 kDa, termed JP-90, is closely associated with the ryanodine receptor Ca^{2+}-release channel [21]. This triadic marker exhibited a decreased expression after 3 days of chronic stimulation, followed by a recovery of its expression at day 60 [21]. Fig. 1F confirms this finding and shows the transient down-regulation of this triad protein between 5 and 46 days of electro-stimulation. Following 78 days of chronic low-frequency simulation, the expression levels of JP-90 are comparable to unstimulated control specimens. This evaluation of the muscle specimens prepared by experimental low-frequency stimulation represents an important prerequisite for the below-described analysis of the nicotinic acetylcholine receptor, since it shows that the proper fast-to-slow transition process had occurred.

Immunoprecipitation analysis of the supramolecular neuromuscular junction complex

The formation and maintenance of the neuromuscular junction, including the clustering of the nicotinic acetylcholine receptor on the post-synaptic muscle membrane, involve many different proteins [16–18]. To demonstrate the relatively close linkage between the acetylcholine receptor and the utrophin–glycoprotein complex, we have performed an immunoprecipitation experiment. Using monoclonal antibody NCL-b-DG to the dystrophin/utrophin-associated glycoprotein β-dystroglycan of apparent 43 kDa, we can show here that

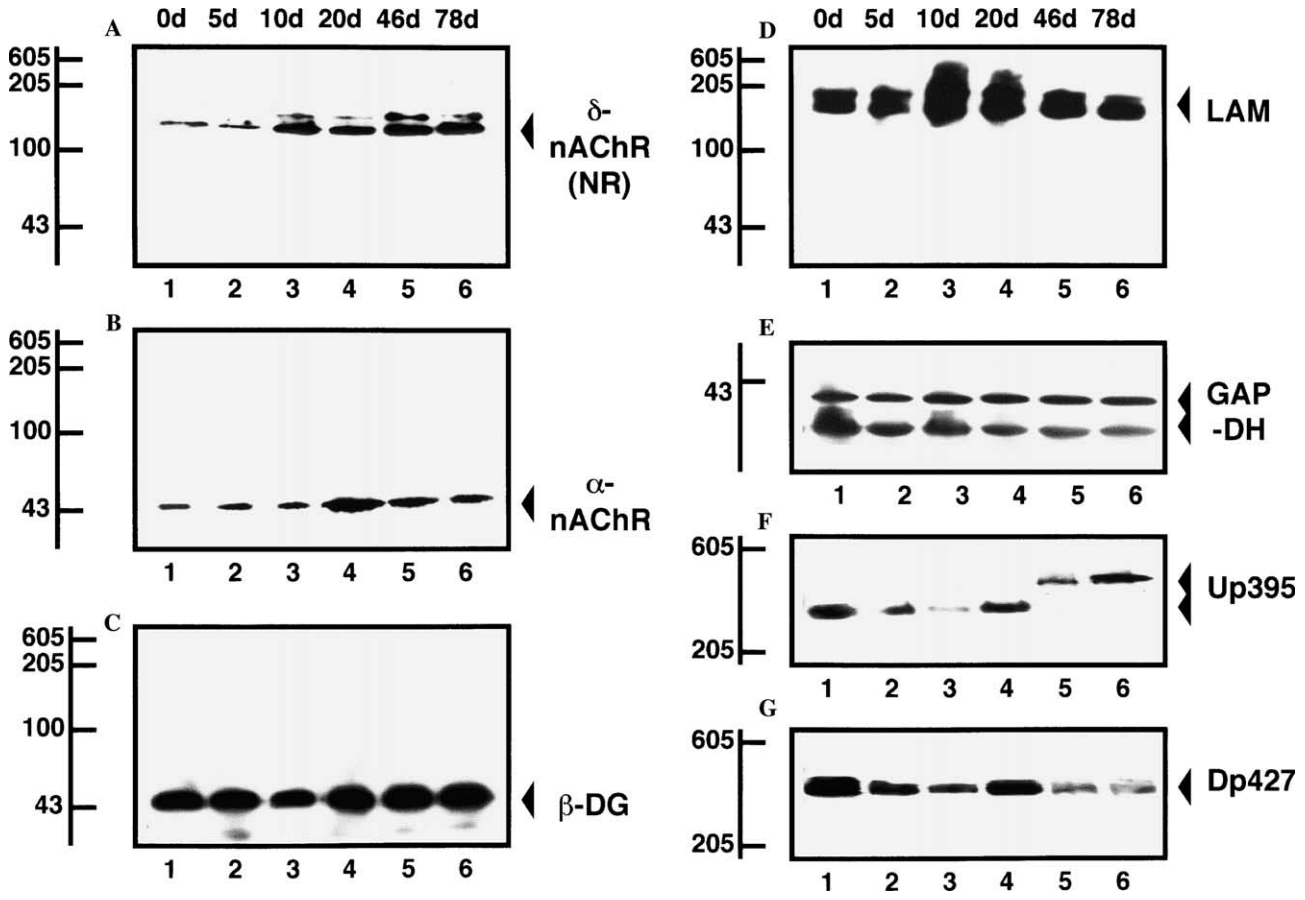

Fig. 3. Immunoblot analysis of the nicotinic acetylcholine receptor following chronic low-frequency stimulation. Shown are representative immunoblots of microsomal membranes labelled with antibodies to the δ-subunit of the nicotinic acetylcholine receptor (δ-nAChR) (A), α-subunit of the nicotinic acetylcholine receptor (α-nAChR) (B), β-dystroglycan (β-DG) (C), laminin (LAM) (D), glyceraldehyde 3-phosphate dehydrogenase (GAP-DH) (E), the utrophin isoform Up395 (F), and the full-length dystrophin isoform Dp427 (G). In panel (A), for a proper immunodecoration signal samples had to be run under non-reducing (NR) conditions. The 130 kDa band of the δ-nAChR band consists probably of dimers. Lane 1 represents membranes from unstimulated contralateral muscles (0 day) and lanes 2–6 show microsomes prepared from rabbit tibialis anterior muscles exposed to continuous (24 h per day) low-frequency (10 Hz) stimulation for 5, 10, 20, 46, and 78 days (5–78 days), respectively. Immunodecorated bands are indicated by closed arrowheads. Sizes of molecular mass standards (in kDa), as deduced from rat myofibrillar proteins, are indicated in the left.

this antibody precipitates, besides its own antigen (Fig. 2), also other surface components. This includes other elements of the dystrophin– and utrophin–glycoprotein complexes, such as the major Dp427 isoform of dystrophin, α-sarcoglycan of apparent 50 kDa, laminin of approximately 200 kDa, and the full-length utrophin isoform Up395 (Fig. 2). Incubation of the immunoprecipitated fraction with antibodies to the abundant sarcoplasmic reticulum Ca^{2+}-ATPase did not reveal any immunolabelling (not shown), confirming the specificity of the immunoprecipitation method. Interestingly, the antibody to β-dystroglycan also precipitated the δ-subunit of the nicotinic acetylcholine receptor and the neuromuscular junction marker protein agrin [29] (Fig. 2). This shows that elements of the utrophin/dystrophin–glycoprotein complex are closely associated with the nicotinic acetylcholine receptor complex and probably form a supramolecular membrane assembly [18]. The co-localisation of the α-subunit of the nicotinic acetylcholine receptor and the full-length utrophin isoform Up395 has previously been documented by immunofluorescence microscopy [30].

Acetylcholine receptor clustering during synapse formation at the vertebrate neuromuscular junction requires the expression of the muscle-specific kinase MuSK for proper pre-patterning of clusters of the post-synaptic neurotransmitter receptor in the endplate region [31]. This early event of neuromuscular synaptogenesis can occur independent of motor neurons [17]. Agrin triggers further clustering of the nicotinic acetylcholine receptor, acting through rapsyn and MuSK [32]. The molecular anchoring of established units of neurotransmitter receptors at the nerve–muscle junction is then stabilised by interactions with the sarcolemma-spanning utrophin/dystrophin–glycoprotein complex [18,33]. These peripheral membrane assemblies provide an indirect linkage between the subsarcolemmal actin cytoskeleton and the extracellular matrix region. The

fact that antibodies to the central linker element of the utrophin/dystrophin–glycoprotein complex, β-dystroglycan, precipitate agrin, and the nicotinic acetylcholine receptor confirms this molecular scenario. While nicotinic acetylcholine receptor clusters represent the functional units of the post-synaptic muscle membrane and are absolutely critical for fast and efficient synaptic transmission, the utrophin-associated surface complex provides the essential structural support for the maintenance of neuromuscular junction architecture [16–18].

Immunoblot analysis of the nicotinic acetylcholine receptor following chronic low-frequency stimulation

Since the nicotinic acetylcholine receptor represents the central functional unit of the post-synaptic muscle surface during neuromuscular transmission, we were interested in whether fibre type shifting, destruction of fast glycolytic fibres, and the activation of satellite cells during the fast-to-slow transition process involve major changes in the expression of this receptor and its auxiliary membrane components. As illustrated in Figs. 3A and B, both the δ- and α-subunits of the receptor complex showed enhanced expression levels in chronic low-frequency stimulated muscle fibres. Immunoblotting with antibodies to other subunits of the nicotinic acetylcholine receptor and certain associated components, such as agrin, did not cross-react with rabbit skeletal muscle samples or showed only weak immunostaining that could not be used for proper evaluation of stimulation-induced changes in the relative abundance of these proteins (not shown). The δ-subunit of the nicotinic acetylcholine receptor exhibited a drastically increased abundance at day 10 of stimulation and the α-subunit after 20 days of muscle transformation. This agrees with the previous observation of stimulation-induced degeneration/regeneration processes [5]. Following destruction of fast glycolytic fibres and regeneration of slower twitching cells, major molecular modifications appear to occur in the post-synaptic region. In contrast, no drastic changes in β-dystroglycan were observed during the fast-to-slow transition process (Fig. 3C). This is surprising, since it is expected that the expression of this utrophin/dystrophin-associated glycoprotein undergoes major changes at the muscle surface adjacent to motor neurons during neuromuscular restructuring. However, β-dystroglycan is an abundant surface glycoprotein. Therefore the immunoblotting technique may not be sensitive enough to detect modifications in its density at the neuromuscular junction against a background of relatively static expression in other regions of the skeletal muscle periphery.

On the other hand, the expression of the extracellular matrix marker laminin showed a very interesting transient up-regulation during the fast-to-slow transformation process (Fig. 3D). Following 10 and 20 days of chronic electro-stimulation, this component of the dystrophin–glycoprotein complex increased, but showed comparable density levels at 5 and 46 days of conditioning as compared to unstimulated controls. This indicates a restructuring event in extracellular matrix architecture during stimulation-induced fibre type remodelling. In analogy to changes in the nicotinic acetylcholine receptor, the abundance of laminin also increased at a critical time period 2–3 weeks following chronic stimulation. The transiently enhanced expression of laminin during the fast-to-slow transformation process indicates increased extracellular matrix formation during molecular modifications within existing neuromuscular junctions and/or within the context of restructuring of nerve–muscle contact zones. Immunoblotting with antibodies to glyceraldehyde 3-phosphate dehydrogenase did show a decrease in the lower band of the immunodecorated doublet (Fig. 3E). This confirms the previously documented stimulation-induced decrease in the activity of this representative enzyme of anaerobic–glycolytic energy metabolism [1]. The immunoblot analysis of glyceraldehyde 3-phosphate dehydrogenase agrees with the idea that chronic electro-stimulation triggers increased activities of enzymes involved in aerobic-oxidative metabolic pathways and a concomitant down-regulation of anaerobic glucose catabolism [1].

The proper immunodecoration of dystrophin and utrophin in stimulated samples was difficult to perform. Figs. 3F and G show a representative example of our attempts to determine the relative density of the full-length isoforms Dp427 and Up395. The shift in the electrophoretic mobility of utrophin in long-term stimulated specimens and the apparent decrease in Dp427 expression in 46 and 78 days stimulated samples probably represent electrophoretic artifacts. The relatively low abundance of these membrane cytoskeletal elements of the surface membrane may have caused distortion of these high-molecular-mass bands due to partial coverage with other more abundant proteins of low electrophoretic mobility. The covering of antigenic determinants in dystrophin and utrophin may have led to a diminished exposure to antibodies and thereby a decreased immunodecoration signal. Due to these technical problems, we could not study potential rearrangements at the level of the subsarcolemmal membrane cytoskeleton following electro-stimulation. However, previous studies indicate that dystrophin expression is not affected during fibre type shifting [6,34].

In conclusion, the nicotinic acetylcholine receptor forms an integral part of a gigantic surface complex mediating signal transmission at the neuromuscular junction, thus representing the first regulatory element of excitation–contraction coupling in skeletal muscle fibres. In analogy to the supramolecular voltage sensor complex of the transverse tubules, the junctional ryanodine receptor Ca^{2+}-release channel complex, and the sarcolemmal dystrophin–glycoprotein complex, the

neurotransmitter receptor of the muscle periphery also forms a large membrane assembly. Fibre type shifting clearly has an effect on synapse formation, which appears to involve rearrangements at the level of the extracellular matrix and the surface membrane. Our analysis of the nicotinic acetylcholine receptor in chronic low-frequency stimulated fast fibres shows that differentiated fast skeletal muscle fibres do not represent invariable physiological entities, but exhibit a relatively high capacity to adapt to altered functional demands.

Acknowledgments

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