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Advances in Neurobiology of the Neuromuscular Junction

Implications for the Anesthesiologist

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THE mammalian neuromuscular junction (NMJ) is one of the most studied and best understood synapses. Recent work has brought forth new information as to development, maturation, and function of this fundamental synapse, both in health and disease. The healthy function of the NMJ underlies one important measurement of the response to general anesthetics, immobility. "Neuromuscular blockers" acting directly at the NMJ are used as a component of many balanced anesthetic techniques, and the health of the NMJ profoundly influences anesthetic technique. For these reasons, it is imperative that anesthesiologists be aware of new developments in the field.

The normal development, maturation, and function of the NMJ are discussed. Diseases of the NMJ are also reviewed with emphasis on new etiologic, pathologic, and treatment-oriented information.

Neuromuscular Junction Development and Structural Maturation

Ease of experimental study makes the vertebrate NMJ the synapse whose formation and function is best understood. The first part of this section summarizes molecular mechanisms involved in NMJ formation, with emphasis on the regulation of acetylcholine receptor (AChR) expression in the subsynaptic membrane. For more extensive accounts, see recent reviews by Sanes and Lichtman[1] and Duclert and Changeux.[2] The issue of how motor axons are guided to innervate the correct muscle fibers is not addressed in this review.

Development of the Neuromuscular Junction

Committed myogenic cells and motor neurites arrive at the region where a muscle is to be formed at approximately the same time during development. Myogenic cells of mesodermal origin, after migration to such sites, divide to fuse into multinucleated myotubes expressing many contractile and synaptic proteins as part of their developmental program. Motor axons extending along peripheral nerves are followed by Schwann cells.[3] Synaptic transmission begins within minutes after the growth cone contacts a myotube and is mediated initially by nicotinic AChRs (nAChRs) expressed constitutively along the entire myotube surface. Such nAChRs, termed "fetal" because of their expression early in development, are assembled from five subunits termed α, β, γ, and δ, each encoded by a different gene.[4] In response to bound acetylcholine, nAChRs flicker rapidly between open and closed states for approximately 5 ms (apparent open times) and allow Na^+, K^+, and Ca^{2+} ions to flow across the muscle membrane down their electrochemical gradients. Dissociation of acetylcholine closes the channel.[5] The long burst duration of the fetal nAChRs' channel combined with the high electrical input resistance of the myotubes allows single acetylcholine quanta to elicit action potentials in the myotubes (fig. 1).[6] At early stages of neuromuscular development, muscle fibers receive input from several motor axons at a single synaptic site. All neural inputs except a single motor nerve withdraw as the NMJ matures.[7]

Synapse maturation involves the formation of a motor nerve terminal with densely packed synaptic vesicles containing the transmitter acetylcholine. Postsynaptic differentiation is characterized by the formation of a postsynaptic apparatus anchoring nAChRs at a density of $10{,}000/\mu m^2$ in the subsynaptic muscle membrane. Un-

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like nAChRs in the nonsynaptic membrane, synaptic nAChRs become metabolically stabilized, their half-lives in the membrane increasing from approximately 1 to 10 days.[8] The basal lamina (BL) enveloping the muscle fiber contains molecular components important to synapse formation, maintenance, and function. The postsynaptic region is further characterized by the presence of cytoskeletal and membrane proteins thought to be involved in its structural maintenance, the anchoring of AChRs and of voltage-activated sodium channels, as well as by the accumulation of several myonuclei. Subsynaptic myonuclei selectively begin to express a new nAChR subunit, ε, at the synapse,[9,10] giving rise to a new, functionally distinct nAChR subtype (termed "adult") with the subunit composition $\alpha_2\beta\varepsilon\delta$ in the synaptic muscle membrane.[4] This mature nAChR has shorter burst duration and a higher conductance to Na^+, K^+, and Ca^{2+} than the fetal nAChR.[11] As discussed below, fetal nAChRs gradually disappear both from synaptic and nonsynaptic muscle membranes. Schwann cells cap the entire synaptic structure.

Synapse Formation

Signals from the Nerve. Signals from the nerve are twofold: (1) the nerve-induced propagated action potentials affect muscle fibers along their entire length, and (2) released or membrane-bound molecules act locally in the region of the NMJ.

Electrical activity down-regulates the synthesis of nAChRs in all but the subsynaptic myonuclei.[12] Electrical activity also induces Ca^{2+} influx through L-type Ca^{2+} channels, which mediates metabolic stabilization of the synaptic nAChRs[13] *via* unknown mechanisms. Impulse activity also affects synapse elimination. Specifically, blockade of the electrical activity in the motor nerve delays the withdrawal and thus the reduction of synaptic inputs converging on a single fiber. One factor involved appears to be the relative synaptic strength of the competing axons. Pharmacologic blockade of nAChRs selectively in the subsynaptic domain occupied by one contending terminal will cause that input to withdraw,[14] and during normal synapse elimination, the axon withdrawal is preceded by loss of its subsynaptic domain.[15] This is consistent with the notion that activation of nAChRs and the associated Ca^{2+} influx may result in a competitive advantage. Both a reduction in acetylcholine release[16] and the activity-dependent rearrangement of subsynaptic cartels that can occur independently of the presence of a nerve[17] may shift the balance between inputs. Accordingly, synchronous stimulation of all inputs converging on a myofiber suppresses elimination of polyinnervation.[18]

Signaling molecules, believed to be of neural origin, regulate the differentiation of a presynaptic nerve terminal and a subsynaptic apparatus. In particular, agrin and neuregulin bind to the synaptic portion of the muscle fiber BL.[19,20]

Nitkin *et al.*[21] originally purified agrin from BL of the synapse-rich electric organ of *Torpedo californica* based on its ability to induce aggregates or clusters of nAChRs expressed constitutively in the membrane of cultured myotubes. Colocalized with nAChR clusters were several components of the postsynaptic apparatus as well as acetylcholinesterase, suggesting a role for agrin in the regulation of postsynaptic differentiation.[22] However, soluble agrin did not affect nAChR gene transcription.

Molecular cloning showed that agrin is a 200-kd protein that, in its native form, is expressed as a 600-kd heparansulfate proteoglycan.[23–25] Splice variants of agrin have different abilities to cluster nAChRs in myotubes.[26,27] Specifically, neurally derived agrin cluster nAChRs, whereas isoforms expressed in skeletal muscle, kidney, and blood vessels do not induce myotubes to form nAChR clusters. Functional mapping shows that an 8, 11, or 19 amino acid splice insert within the C-terminal-most 20 kd of agrin is essential to nAChR clustering activity.[28] Alternative splicing at the N-terminus results either in a secreted isoform exhibiting strong binding to laminin, *i.e.*, to the BL,[29] or in an isoform that is inserted into the cell membrane and whose function is not known.[30]

A receptor-coupled tyrosine kinase, termed MuSK (for muscle-specific kinase), appears to mediate agrin-induced clustering of nAChRs.[31] Mice lacking MuSK and mice lacking agrin have very similar phenotypes.[32,33] They lack NMJs, and their motor axons, rather than forming short branches from a central nerve trunk, wander along the entire length of muscle fibers without making synapses. The pathway downstream of MuSK mediating agrin-induced nAChR clustering is not known. Activation of MuSK by agrin phosphorylates nAChR β subunits, but this is not sufficient for clustering.[34]

An important role is played by rapsyn, a 43-kd peripheral cytoplasmic membrane protein that is associated in a 1:1 ratio with the β subunit of synaptic nAChRs.[35] When coexpressed with nAChRs, rapsyn causes their clustering. Furthermore, mice lacking rapsyn die at birth because their NMJs do not cluster nAChRs and lack several other components of the subsynaptic membrane and cytoskeleton.[36] Synapse-specific aggregation of MuSK, and of synaptic BL components as well as synaptic nAChR gene expression, appear normal, consistent with the idea that MuSK forms a primary scaffold to which other components are attached by rapsyn. Rapsyn may also serve to link components of the signaling pathway activated by agrin.[37]

Studies of mouse mutants suggest roles for several other synapse-specific membrane and cytoskeletal proteins in NMJ development and maintenance. For example, the membrane-spanning dystrophin–glycoprotein complex (DGC), comprising α and β dystroglycans as

well as several other components, bind extracellularly to laminin and intracellularly to dystrophin to link the extracellular matrix to the cytoskeleton. This provides mechanical stability to the muscle along its entire length, with mutations causing different forms of muscular dystrophy.[38] Agrin and several synapse-specific isoforms of laminin[39] as well as rapsyn bind to the DGC. Deletion of distinct components of the synaptic DGC demonstrate the implication of the DGC in the maintenance of the synapse.[40]

Although the molecular signals mediating the selective stabilization of one and the elimination of other nerve inputs to the developing NMJ are not well understood, thrombin derived from muscle prothrombin, the endogenous thrombin inhibitor nexin-1, as well as thrombin receptors may shape these neural inputs.[41,42] Evidence supporting this hypothesis is derived from *in vivo*[43] and *in vitro* studies.[44] The latter suggest that muscle-derived thrombin activates a protease activated receptor (PAR-1).[45] This G-protein–coupled receptor may then activate protein kinase C, which leads to reduced insertion and stability of nAChRs at the endplate surface.[46] According to the hypothesis of Balice-Gordon and Lichtman,[14] endplate areas undergoing loss of nAChRs would also lose neuronal inputs. In addition to thrombin, Ca^{2+}-sensitive proteases may shape the NMJ.[47] This suggests a role for Ca^{2+} influx through the ε nAChR in stabilization of the NMJ.

As indicated above, muscle electrical activity downregulates expression of AChR subunit genes. Therefore, the maintenance of a high concentration of nAChRs in the subsynaptic muscle membrane requires that the nAChR subunit genes α, β, δ, and ε are transcribed selectively in subsynaptic myonuclei of innervated, electrically active muscle fibers. A signal from the nerve and bound to the synaptic portion of the BL[48,49] appears to regulate this transcription. Neuregulin 1 (NRG-1),[50] a product of the *nrg-1* gene, is believed to be the nerve signal. By alternative mRNA splicing, this gene codes for a number of growth and differentiation factors with many different functions in development. They all share one epidermal growth factor–like domain that mediates their biologic activity by activating receptor kinases termed ErbBs. ErbB receptors are concentrated in the subsynaptic muscle membrane.[51] NRG-1 isoforms are expressed by motor neurons[52] as well as muscle fibers.[53] NRG-1 isoforms accumulate in the synaptic BL, probably by binding to agrin and other heparansulphate proteoglycans[54] that are induced by agrin.[53] NRG-1–activated nAChR subunit gene transcription is mediated *via* the activation of phosphatidylinositol 3-kinase and mitogen-activated protein kinases[55,56] and regulatory elements, termed N-box, in their promoters that are similar to those conferring nerve-induced, synapse-specific expression to reporter genes *in vivo*.[57–59] The DNA binding factors involved are growth-associated binding proteins (GABPα/β), members of the Ets family of transcription factors.[60,61] NRG-1 also induces the expression of voltage-gated sodium channels.[62]

Surprisingly, neural agrin alone, when attached to culture substrate or to BL, but not when applied in soluble form, can induce nAChR gene transcription in cultured myotubes or in nonsynaptic muscle region *in vivo*, respectively. Importantly, this occurs in the absence of a nerve terminal and thus of NRG-1 from neurons.[63,64] Neural agrin further induces the formation of a postsynaptic-like membrane exhibiting all the hallmarks of a normal postsynaptic apparatus, including the formation of folds, the accumulation of myonuclei and membrane and cytoskeletal proteins, as well as MuSK, NRG-1, and ErbB receptors[65–67] (fig. 2). The inhibition of agrin-induced transcription of nAChR ε subunit gene by forced overexpression of inactive mutants of ErbB2 in cultured myotubes is consistent with the idea that agrin organizes a NRG–ErbB receptor pathway that, in turn, activates nAChR gene transcription, with NRG-1 originating from muscle.[53] The multiple effects of agrin are all mediated by activation of MuSK.[68] Recent experiments suggest that activation of MuSK induces not only the clustering of MuSK and ErbBs, but also of the transcription of their genes (Moore C [Diploma Biology, Basel, Switzerland], Brenner HR [Department of Physiology, University of Basel, Switzerland], unpublished observations, October 2000).

In summary, neural agrin alone acting *via* MuSK can organize the induction of a postsynaptic apparatus, including the synthesis of proteins that control the synthesis of other synaptic components. Agrin is therefore the only master organizer of synaptic development to be identified. However, it is not clear whether NMJ development depends on the supply of NRG-1 from motor neurons or whether NRG-1 is supplied by the muscle fiber. Furthermore, other neural factors are likely involved in subsynaptic differentiation, consistent with the observation that nAChR density in ectopic, nerve-free postsynaptic membranes induced by agrin appears lower than at normal synapses (Brenner HR [Department of Physiology, University of Basel, Switzerland], unpublished observations, July 1996).

Signals from Muscle. Little is known regarding the identity of factors affecting presynaptic differentiation, but three candidates with activities on cultured neurons consistent with such roles are present in synaptic BL. Fibroblast growth factor 2, when coated to beads and muscle agrin, stimulates the accumulation of vesicles in cultured neurites.[69–71] However, mice lacking muscle agrin have normal NMJs.[72] A laminin β chain, β2, in the context of synapse-specific laminin-11,[73] stops motor neurite outgrowth[39] and, *in vivo*, prevents glial entry into the synaptic cleft.[73] Synapse-specific accumulation of laminin β2 is regulated by neural agrin,[65] again mediated *via* MuSK activation.[68] Neurotrophins secreted by muscle fibers, activating *trk*B receptors localized in the

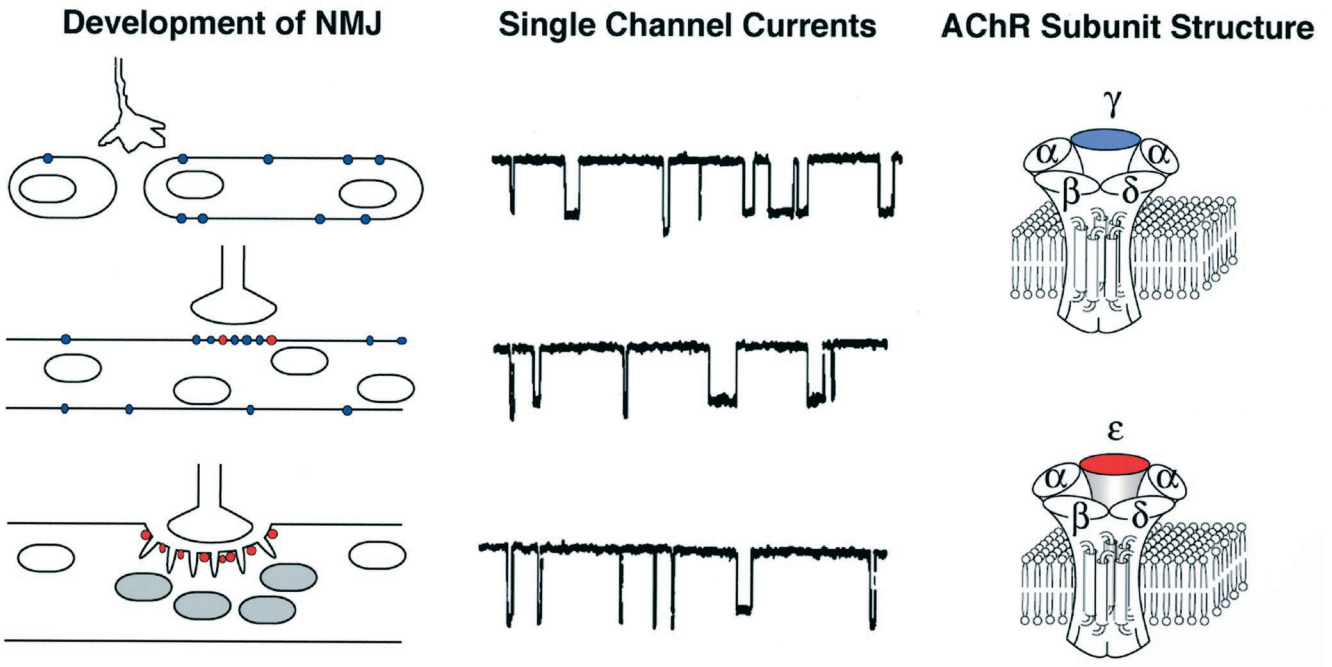

Fig. 1. Development of the neuromuscular junction. (*Left*) Motor neuron growth cones contact myotubes as they fuse from myoblasts and express mostly fetal nicotinic acetylcholine receptors (nAChRs; marked in blue) in their surface membranes. In adult muscle, adult nAChRs (marked in red) predominate and are largely concentrated at the neuromuscular junction. (*Center*) Records of AChR channel openings from muscle membranes at different stages of neuromuscular development. Fetal (*top*) and adult nAChRs (*bottom*) are activated by acetylcholine to form ion channels of different conductance and gating properties. (*Right*) Subunit composition of fetal and adult AChR subtypes. Fetal and adult AChR subtypes are characterized by the presence of a γ and ε subunit, respectively.

synaptic muscle membrane, are required for the maintenance of the postsynaptic nAChR-rich region.[74]

Role of Schwann Cells

Unlike motor neurons, Schwann cells do express ErbB receptors, and they depend on neuronal NRG-1 for their survival required for the maintenance of motor neurons.[3] Consequently, NRG-1 expressed by subsynaptic muscle regions may influence synapse formation indirectly. Consistent with this notion, injection of NRG-1 into neonatal muscle causes a redistribution of Schwann cells, a loss of synaptic sites, and growth of motor neu-

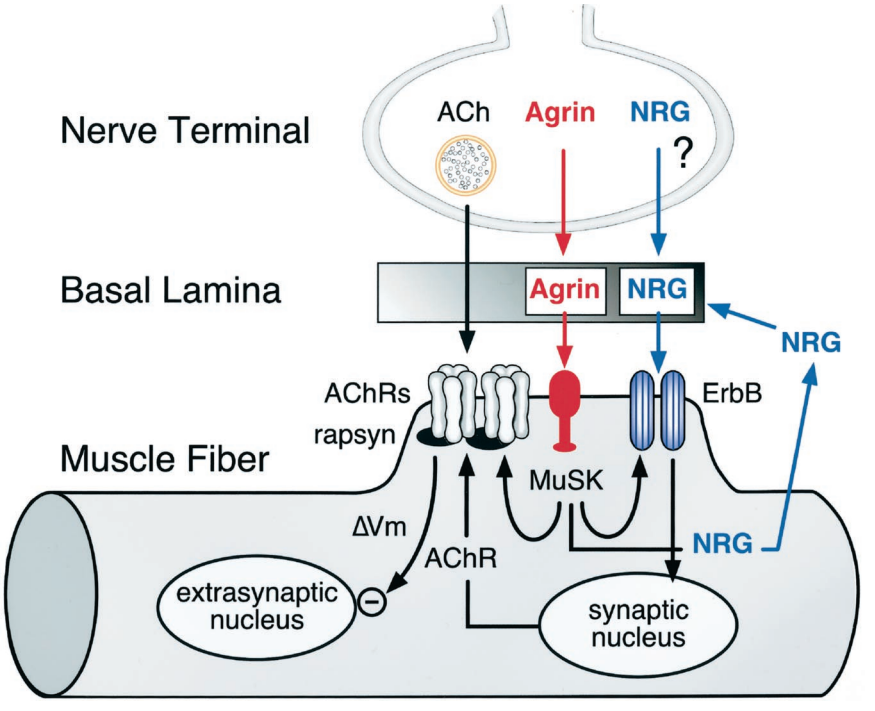

Fig. 2. Neural control of acetylcholine receptor (AChR) expression at the neuromuscular junction. AChR subunit genes are expressed selectively by subsynaptic nuclei. Control is mediated by (1) neural agrin organizing *via* the activation of muscle-specific kinase (MuSK), a neuregulin–ErbB receptor pathway across the subsynaptic membrane, with neuregulin 1 (NRG-1) originating from muscle, or (2) by NRG-1 derived from the nerve. Nicotinic AChRs are clustered in the subsynaptic membrane by activation of MuSK. AChR gene expression in nonsynaptic muscle nuclei is down-regulated by electrical muscle activity induced by acetylcholine released from the nerve terminal and activating subsynaptic nAChRs (modified from Sanes[379] with permission from Elsevier Science).

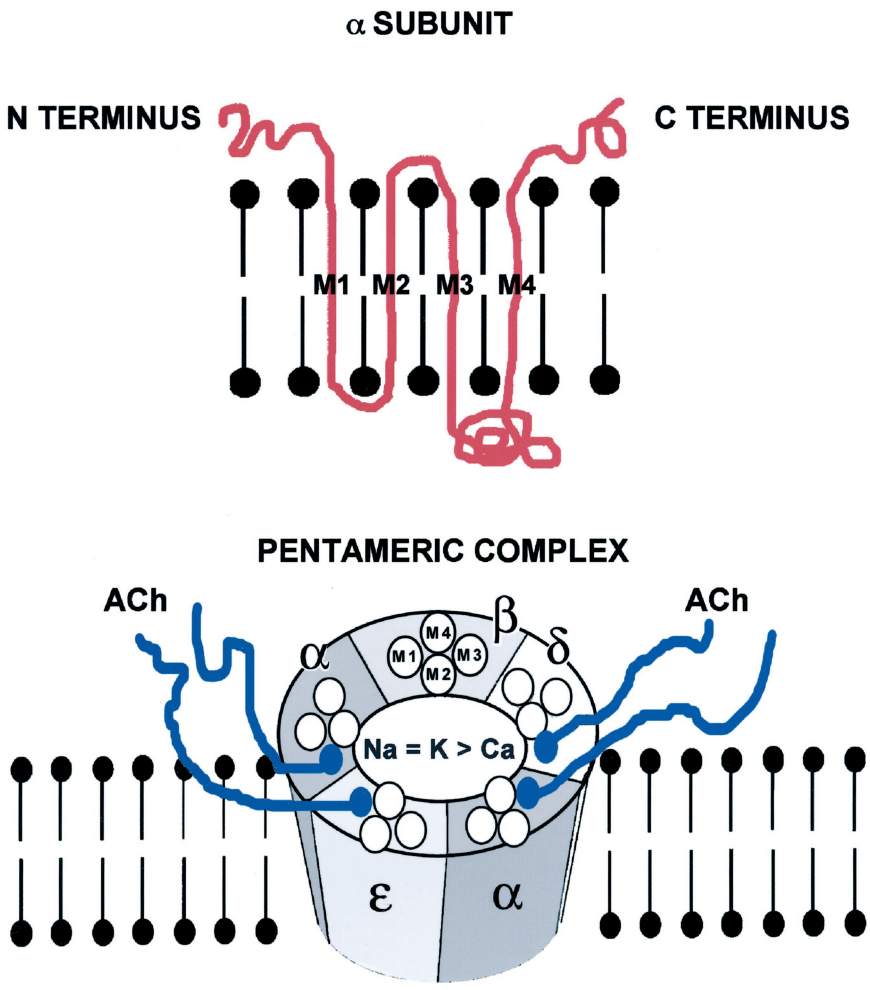

Fig. 3. Subunit composition of the nicotinic acetylcholine receptor (nAChR) in the endplate surface of adult mammalian muscle. The adult AChR is an intrinsic membrane protein with five distinct subunits ($\alpha_2\beta\delta\varepsilon$). Each subunit contains four helical domains labeled M1 to M4. The M2 domain forms the channel pore. (*Top*) A single α subunit (red) with its N and C termini on the extracellular surface of the membrane lipid bilayer (black). Between the N and C termini, the α subunit forms four helices (M1, M2, M3, and M4) that span the membrane bilayer. (*Bottom*) The pentameric structure of the nAChR of adult mammalian muscle. The N termini of two subunits cooperate to form two distinct binding pockets for acetylcholine. These pockets occur at the ε-α and the δ-α subunit interface. The M2 membrane spanning domain of each subunit lines the ion channel. The doubly liganded ion channel has equal permeability to Na^+ and K^+; Ca^{2+} contributes approximately 2.5% to the total permeability.

rons throughout the muscle.[75] The role of Schwann cells is more obvious during reinnervation after nerve cuts. Terminal Schwann cells sprout processes on denervation. These are used by regenerating motor axons as guides to leave the endplate domains and, driven by factors from denervated fibers,[76] reach other synapses. In this way they cause transient polyneuronal innervation of individual endplates.[77]

Structure and Function of the Nicotinic Acetylcholine Receptor

The function of the endplate nAChR depends on five subunit proteins that combine to form the pentameric unit (fig. 3). The α subunit was the first to be purified. Subsequent analyses of amino acid sequence, as well as accessibility of synaptic nAChRs to ligands, revealed that both the N and C termini of the α-subunit protein protrude beyond postsynaptic membranes into the extracellular space. Repeated clusters of hydrophobic amino acid residues suggested that between its N and C termini, the α subunit formed four membrane-spanning helices, M1 through M4.[78] Extensive sequence homology with α facilitated characterization of four additional subunit proteins contributing to nAChR structure.[79,80] We now know that the nAChR of adult mammalian skeletal muscle is a pentameric complex of two α subunits in association with a single β, δ, and ε subunit. These subunits interact to form a transmembrane pore as well as the extracellular binding pockets for acetylcholine and other agonists or antagonists. The M2 transmembrane-spanning segment of each subunit lines the cation selective pore.[81] The extracellular binding sites for acetylcholine and antagonists such as curare form at the interface of the N-terminal domain of the $\alpha\delta$ and the $\alpha\varepsilon$ subunits.[82,83] In the absence of acetylcholine or other agonists, the stable closed state of this pore normally precludes flow of cations down their electrochemical gradient. A major function of the ε and γ subunits is to stabilize this closed state.[84] Simultaneous binding of two acetylcholine molecules to a nAChR[85] initiates conformational changes that open the pore.[86,87] The duration of this open state depends on the duration of dual occupation by acetylcholine.

The γ to ε Subunit Shift. An interesting, although poorly understood observation is that developing mammalian muscle contains a γ rather than an ε subunit (fig. 1).[4] Although the role, if any, of the developmentally programmed ε for γ subunit shift is not understood,

these subunits determine pharmacologic[88,89] and physiologic[11] properties of the muscle nAChR. Mutations of the human ε subunit gene give rise to congenital forms of myasthenia gravis (MG).[90] In addition, endplates of mice lacking the ε subunit gene begin to degenerate within 2 weeks after birth. This myasthenic condition leads to death within 3 months.[91–93] These findings suggest that understanding the role of the ε subunit will improve therapeutic management of the healthy and diseased NMJ. Furthermore, such understanding may also clarify the significance of subunit changes to the health of central nervous system synapses, the function of which is mediated by ligand-operated ion channels structurally related to the muscle nAChR.[94,95]

Genes coding for the γ and ε subunit map to human chromosomes 2 and 17, respectively.[96] The regulation and timing of human γ and ε gene expression, as well as the subunit shift of the nAChR, is unexplored. Although the γ to ε subunit shift occurs for all mammalian species studied, most information has been acquired from rodents.

Muscle precursor cells of 12-day rat embryos[97] express mRNAs encoding α, β, δ, and γ subunits. Formation of the NMJ at embryonic days 15 to 17 initiates accumulation and decline of these mRNAs, respectively, below the junctional and extrajunctional sarcolemma. Two to three days later, mRNA encoding the ε subunit is first detectable in subendplate nuclei. During the first 2 weeks after birth, the levels of mRNA encoding the γ and ε subunits change in a reciprocal fashion.[98] As previously discussed, neural factors control subunit gene expression. In particular, NRG-1 binds to ErbB receptors located on the endplate membrane.[51,99,100] This activates tyrosine kinase to phosphorylate GABP α/β, which binds to the promoter sequence of the ε subunit gene. A single nucleotide mutation in this promoter sequence reduces its affinity for the GABP. The reduced synaptic specific expression of the ε subunit leads to a myasthenic condition in humans[90] and the mouse.[58]

Although functioning, mature, ε-subunit–containing nAChRs are observed at endplates of 1-day-old rodents[101] (McArdle JJ [Professor of Pharmacology and Physiology, Newark, NJ], unpublished observations, November 2000; data provided in the form of an abstract presented to the Society for Neuroscience), substitution for the γ nAChR is gradually completed within 3 weeks after birth.[11,102,103] Thus, the ε for γ subunit shift occurs during the dynamic phase of synaptogenesis.[1] Copopulation of developing or reinnervating endplates with ε and γ nAChRs causes endplate currents that have a fast and slow component of decay. Because of its briefer apparent open time, the ε nAChR is responsible for the fast component of endplate current decay.[104] At the same time, activation of the ε nAChR will increase Ca^{2+} concentration within the subsynaptic cytoplasm.[105,106] Because Ca^{2+} is an essential second messenger, the ε form of nAChRs may have evolved to allow highly localized Ca^{2+} influx to regulate nearby mechanisms that determine the architecture and function of the NMJ. On the other hand, excessive activation of the ε nAChR, as during cholinesterase inhibition,[107] may overload the endplate with Ca^{2+}, which initiates degenerative processes. Similar to Ca^{2+}-mediated glutamate neurotoxicity,[108] prolonged activation of the ε AChR may increase the concentration of Ca^{2+} in the cytoplasm below the endplate membrane to activate degenerative processes.[109] For example, Ca^{2+}-activated calpain, DNase, or phospholipase may degrade molecules essential to synaptic stability.[110] As in the case of *N*-methyl-D-aspartate–induced neurotoxicity, subendplate mitochondria may be stimulated to produce reactive oxygen species that initiate degenerative processes.[111] Strong support for a necrotic effect of increased influx through the mature nAChR comes from studies of mutations discovered in patients with slow-channel congenital myasthenic syndromes (SCCMS).

Subunit Mutations and the Myasthenic Syndromes. The skeletal muscle weakness and fatigue of SCCMS is associated with degeneration of the motor endplate.[112] Diverse mutations of different nAChR subunits contribute to the SCCMS. Initial studies attributed the SCCMS to mutation within the ε and β subunits, which slow channel closure in the presence and allow spontaneous openings in the absence of acetylcholine.[113] Mutations of the α subunit, which increase the affinity of the nAChR for agonist, decrease the agonist dissociation rate, allowing repeated channel openings.[114] The net effect of these gain-of-function mutations is to prolong the open state of the nAChR. This allows what normally is physiologic activation of the NMJ to overload the postsynaptic region with Ca^{2+} and initiate necrosis. In addition to the resultant loss of junctional clusters of nAChR, depolarization–desensitization block of the endplate occurs because the prolonged synaptic potentials summate temporally. An open channel blocker of the nAChR, quinidine sulfate, is therapeutically efficacious in SCCMS because it normalizes the open duration of slow channel mutants.[115]

In addition to gain-of-function mutations that contribute to SCCMS, the α and ε subunit demonstrate loss-of-function mutations that contribute to another congenital myasthenic syndrome.[113,116] These mutations decrease the rate of channel opening and increase the closure rate. This loss of nAChR function reduces the safety factor for synaptic transmission. Just as for ε subunit knockout mice, the endplate region is simplified in patients with the loss-of-function mutations. However, in contrast to ε knockout mice, expression of the γ subunit is up-regulated in the human condition. This up-regulation preserves the human phenotype. It is interesting to note that up-regulation of the γ subunit does not occur in autoimmune MG.[117]

In addition to the physiologic consequences summarized above, subunit mutations also modify the pharmacologic sensitivity of the nAChR. A striking example is the choline sensitivity of nAChRs in SCCMS.[118] Normal nAChRs do not respond to plasma concentrations of this ordinary metabolite, but mutated nAChRs in SCCMS are activated. Such activation worsens the cationic overload of the motor endplate, which is responsible for endplate degeneration in the SCCMS. Recent evidence suggests that nitric oxide synthase inhibitors may have the potential to provide therapeutic benefit in SCCMS.[119]

Membrane Cholesterol and the Nicotinic Acetylcholine Receptors. In view of the Overton-Meyer lipid theory of general anesthetic action, it is useful to consider biochemical studies suggesting an influence of membrane lipids and cholesterol on the function of the nAChR. Early biochemical studies suggested an influence of cholesterol on the function of the reconstituted nAChR.[120,121] The isolated nAChR has a particularly high affinity for cholesterol.[122] Furthermore, functional insertion of isolated nAChRs into artificial membranes requires cholesterol.[123] The postsynaptic membrane is rich in cholesterol.[124] These observations suggest novel posttranslational processing of newly synthesized nAChRs. Only after nAChRs are inserted into the postsynaptic membrane and charged with cholesterol do they become fully active.[125] Cells deficient in sphingolipid biosynthesis are unable to insert normal concentrations of nAChR into their membrane.[126] Reduction of membrane cholesterol dramatically increases the input resistance of muscle fibers, allowing for greater endplate depolarization in response to acetylcholine.[127,128]

The effect of cholesterol on nAChR function is not attributable to an action on the bulk lipid of the membrane.[129] Rather, cholesterol may interact with either nonannular sites within subunits of the nAChR that are not part of the lipid–protein interface,[122] or with the immobilized lipid-belt region surrounding the nAChR.[130] The α M1 and M4 transmembrane domains and the γ M4 domain appear to form the cholesterol "binding" domain.[131] The lipid-soluble steroid promegestone[132] and organochlorine insecticides[133] may noncompetitively block the nAChR by acting at these protein–lipid interfaces. Furthermore, amino acid substitutions in the vicinity of the protein–lipid alters channel gating kinetics.[134] The specificity of such putative sites for cholesterol is apparently not high since other neutral lipids maintain nAChR function.[135]

The Synthesis and Release of Acetylcholine

It is generally accepted that the synthesis and release of acetylcholine involves a cycle of events (fig. 4). Acetylcholine is first formed in the cytoplasm of the nerve terminal from acetyl coenzyme A and choline in a reaction catalyzed by the soluble enzyme choline acetyltransferase. An energy-dependent "transporter" then accumulates acetylcholine within vesicles. Acetylcholine is packed at superosmotic concentrations (approximately 300 mM) within the lumen of the vesicle, together with adenosine triphosphate (ATP), proteoglycans, H^+, Mg^{2+}, and Ca^{2+} ions.[136] The acetylcholine:ATP molar ratio in synaptic vesicles has been estimated to range from 10:1 to 1:1.[137,138] Each vesicle appears to contain 5,000–10,000 molecules of acetylcholine. The acetylcholine contained in a single vesicle is often referred to as a "quantum" of transmitter. Release of acetylcholine is a Ca^{2+}-dependent process and is triggered by an increase in the concentration of free Ca^{2+} within the nerve terminal. This results from the opening of voltage-gated Ca^{2+} channels by the depolarization of the nerve impulse. In addition to Ca^{2+} channels, several forms of potassium channel are present in the nerve terminal, including voltage-gated and Ca^{2+}-activated potassium channels. The potassium channels are likely to limit the duration of nerve terminal depolarization and hence the extent of Ca^{2+} entry and transmitter release. In addition to acetylcholine, ATP is also released and subsequently hydrolyzed within minutes to adenosine in the junctional cleft.[137] Adenosine in the cleft binds to prejunctional P_1 purinoceptors,[139] which depress neuromuscular transmission *via* a G-protein–mediated Ca^{2+} channel inhibition.[140] P_2 purinoceptors, sensitive to ATP but not to adenosine, have been identified in the muscle.

Synaptic vesicle (SV) exocytosis occurs in successive steps: docking vesicles attach to the presynaptic active zone. SVs then undergo a priming reaction to become capable of responding to a Ca^{2+} signal. The action potential causes membrane depolarization and a sharp increase in internal Ca^{2+} concentration through voltage-gated Ca^{2+} channels and direct release from intracellular Ca^{2+} stores. This Ca^{2+} signal triggers the fusion of SVs with the presynaptic membrane and subsequently exocytosis. The sequence of exocytosis is very rapid (< 0.3 ms). Fusion results in the release of a "quantum" of several thousand acetylcholine molecules into the synaptic cleft. The synaptic cleft is very narrow (around 50 nm), and acetylcholine can diffuse this distance in a few microseconds to reach the postsynaptic membrane. Some of acetylcholine molecules bind to the nAChRs on the postsynaptic membrane, while the rest are rapidly hydrolyzed by the acetylcholinesterase present in the synaptic cleft to choline and acetate. Choline is recycled into the terminal by a high-affinity uptake system, making it available for the resynthesis of acetylcholine. The hemicholinium-3 inhibits the later mechanism. After exocytosis, the membrane components of the SVs are recovered by endocytosis and recycled for future use.

The released acetylcholine binds to α subunits of the AChRs. These ligand-gated cation channels allow sodium to enter and depolarize the muscle cell membrane at synaptic sites. This local depolarization leads to the activation of nearby voltage-gated sodium channels, which

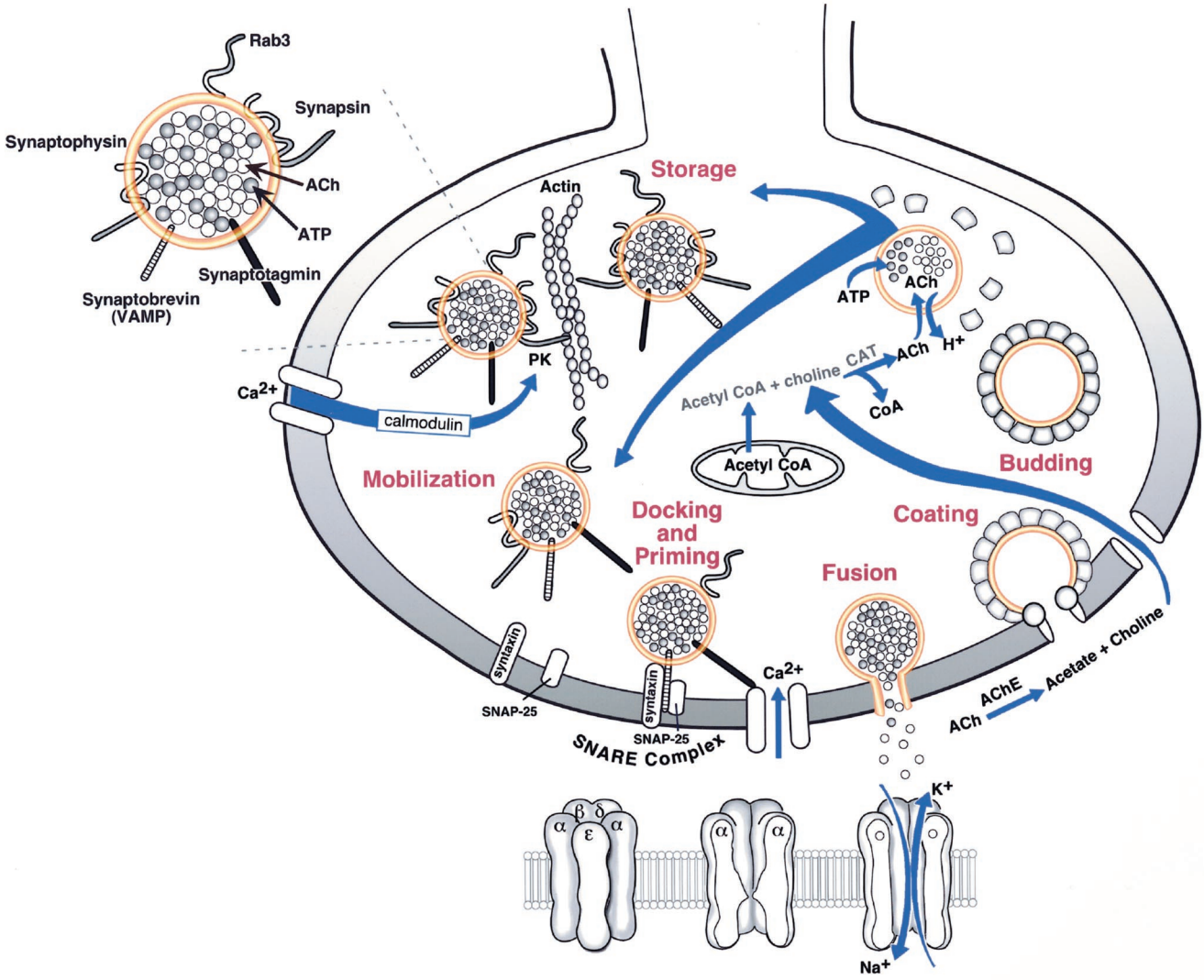

Fig. 4. The synaptic vesicle exocytosis–endocytosis cycle. After an action potential and Ca^{2+} influx, phosphorylation of synapsin is activated by calcium-calmodulin activated protein kinases I and II. This results in the mobilization of synaptic vesicles (SVs) from the cytomatrix toward the plasma membrane. The formation of the SNARE complex is an essential step for the docking process. After fusion of SVs with the presynaptic plasma membrane, acetylcholine (ACh) is released into the synaptic cleft. Some of the released acetylcholine molecules bind to the nicotinic acetylcholine receptors (nAChRs) on the postsynaptic membrane, while the rest is rapidly hydrolyzed by the acetylcholinesterase (AChE) present in the synaptic cleft to choline and acetate. Choline is recycled into the terminal by a high-affinity uptake system, making it available for the resynthesis of acetylcholine. Exocytosis is followed by endocytosis in a process dependent on the formation of a clathrin coat and of action of dynamin. After recovering of SV membrane, the coated vesicle uncoats and another cycle starts again. See text for details. Acetyl CoA = acetylcoenzyme A; CAT = choline acetyltransferase; PK = protein kinase.

amplify and propagate action potentials across the surface of the muscle fiber and into the transverse tubules where Ca^{2+} channels are present at high density.[141] The dihydropyridine receptors (DHPRs) in the transverse system membrane act as voltage sensors, detecting the depolarization and opening adjacent type-1 ryanodine receptor (RyR1)–Ca^{2+}-gated, Ca^{2+}-release channels in the apposing sarcoplasmic reticulum membrane by some protein–protein interaction.[142,143] DHPR-RyR1 coupling is not yet fully elucidated.[144] Several endogenous effectors, such as Ca^{2+}, Mg^{2+}, adenine nucleotides, calmodulin, and nitric oxide, are known to regulate RyR1 function.[143,145] After DHPR-RyR1 coupling, the RyR1 release large amounts of Ca^{2+} from the sarcoplasmic reticulum, resulting in muscle contraction. The translation of electrical signaling at the surface membrane into intracellular Ca^{2+} release from the sarcoplasmic reticulum is known as excitation–contraction coupling.[146] Binding of Ca^{2+} to the troponin complex alters the interactions between tropomyosin and the contractile machinery, allowing the proper interaction between actin molecules and myosin heads. Thus, muscle contraction occurs *via* myofilament sliding. As the sodium channel openings subside, chloride enters the cell through more slowly opening voltage-sensitive chloride channels, to return the muscle membrane potential to its

resting level (approximately −70 to −90 mV).[141] Molecular leakage (nonquantal leakage) and quantal leakage of acetylcholine from the nerve are events that are unrelated to nerve impulse.[147]

The Synaptic Vesicle Recycling Pathway

Storage of Synaptic Vesicles. At the NMJ, SVs are specialized secretory organelles used for fast signaling between nerve and muscle. There are two pools of vesicles, a readily releasable store (active pool) and a reserve store. Electron microscopic studies demonstrate that the majority of SVs are sequestered in the reserve pool in a filamentous network believed to be composed mainly of actin, synapsin (an actin-binding protein), and spectrin (fig. 4).[148,149] Synapsin I binds vesicles to the presynaptic cytoskeleton (actin filaments and microtubules).[148] Mice lacking synapsins are viable and fertile with no gross anatomic abnormalities, but they are prone to seizures and are unable to properly regulate synaptic transmission. For example, repetitive stimulation of synapses at physiologic frequencies result in massive synaptic depression.[148,150] This suggests that the SV cycle is unable to mobilize appropriately during repetitive stimulation.[148,150]

The synaptic vesicles possess a diverse set of specialized proteins that can be divided into two functional classes: proteins involved in the uptake of neurotransmitters (transport proteins) and proteins that mediate SV membrane traffic such as docking, fusion, and budding.[151] It is believed that intrinsic and peripheral membrane proteins of SVs are imported from the cell body *via* axonal transport.[152] Sudhof[151] developed a structural model of the vesicle membrane (fig. 4). Although many proteins have been implicated in the process of exocytosis, the overall mechanism is still not completely understood.

Vesicle Mobilization and Docking. After an action potential and Ca^{2+} influx, phosphorylation of synapsin I is activated by cyclic adenosine monophosphate–dependent protein kinase and by calcium-calmodulin activated protein kinases I and II (fig. 4).[153] This weakens binding between SVs and the cytomatrix, allowing mobilization of SVs from the reserve pool into the active pool lying close to the plasma membrane. SVs then attach to the presynaptic plasma membrane in a process known as docking. Synaptotagmins, synaptophysins, and the SV associated membrane protein (VAMP, or synaptobrevin) are integral vesicular membrane proteins involved in the docking process of SVs within a specialized region termed the active zone. The active zone is characterized by the presence of electron-dense regions on both the presynaptic and postsynaptic plasma membrane that contain clusters of Ca^{2+} channels.[154]

Synaptotagmin I is believed to be the main Ca^{2+}-binding protein, and it has the ability to bind multiple Ca^{2+} ions.[155] Synaptotagmin I is involved in localizing SVs to synaptic zones rich in voltage-gated Ca^{2+} channels[156] or stabilizing vesicles in the docked state at the presynaptic membrane.[157] Synaptotagmin I is therefore essential for the fast component of neurotransmitter release.[155] Mice deficient in synaptotagmin I lack fast, but not slow neurotransmission.[155]

The formation of a core complex of three synaptic proteins (the SNARE complex) is an essential step for the docking process. Two of these proteins are from the plasma membrane: SNAP25 (synaptosome-associated membrane protein of 25 kd) and syntaxin 1 (or HPC1). The third protein is from SVs (synaptobrevin) (fig. 4).[151,158] The core complex forms the anchor for a cascade of protein–protein interactions required for exocytosis to occur. However, controversy exists as to which proteins function in docking, fusion, or both.[159] Recent evidence suggests that the SNARE complex is perhaps only one of several protein complexes involved in vesicle targeting and fusion. Synaptotagmin I also interacts with the plasma membrane proteins syntaxin[160] and neurexins.[161]

The SV proteins are common targets for environmental toxins. The neurexins include one of the receptors for α-latrotoxin (black widow spider venom), a toxin that causes massive neurotransmitter release. Cleavage of SNAP25, syntaxin 1, or synaptobrevin by clostridial neurotoxins (which include tetanus and botulinum toxins) results in inhibition of exocytosis.[159] Botulinum toxins are zinc endoproteases that are used clinically for treatment of muscle dystonia and for spastic disorders. Since approval of type-A botulinum toxin by the US Food and Drug Administration in December 1989 for three disorders (strabismus, blepharospasm, and hemifacial spasm), the number of indications for its use has increased greatly and now includes numerous focal dystonias, spasticity, tremors, cosmetic applications, and migraine and tension headaches.[162] Treatments can be repeated several times without major side effects, such as the development of an immune response. Synaptotagmin is not a known substrate for any neurotoxin, but it may be targeted by antibodies found in Lambert-Eaton myasthenic syndrome (LEMS).[163]

Vesicle Priming. Further "mutation" or "priming" events are required to convert a docked vesicle into a fusion-competent, readily releasable vesicle. At the priming stage, the system becomes competent to undergo fusion on an increase in Ca^{2+} concentration. A family of low-molecular-weight guanosine triphosphate–binding proteins, termed rabs, are involved in vesicle attachment to acceptor membranes.[164] Rab3A is required to maintain a normal reserve of SVs, to facilitate accelerated exocytosis during repetitive stimulation when SV recycling becomes rate limiting. Triggering SV exocytosis leads to dissociation of rab3A from SVs. This dissociation is inhibited by botulinum and tetanus toxins. In mice lacking rab3A, synaptic transmission persists but is more

susceptible to fatigue and is less plastic, a phenotype consistent with altered vesicle availability at active zones.[164]

Vesicle Fusion. A fundamental step in synaptic transmission is the fusion of SVs with the plasma membrane and the release of their content. Fusion occurs within a few hundred microseconds of Ca^{2+} entering the nerve terminal *via* presynaptic voltage-gated Ca^{2+} channels.[165] Ca^{2+} triggers exocytosis by participating in one or more reactions that catalyze vesicle fusion. Recent evidence suggests that vesicle fusion is mediated by two proteins with opposite actions: synaptotagmin, which probably serves as the Ca^{2+} sensor,[151] and rab3, which limits the number of vesicles that can be fused as a function of Ca^{2+} to allow a temporally limited, repeatable signal. However, it is not yet clear how vesicle fusion is triggered by Ca^{2+}-bound synaptotagmin, and it is possible that one of the several proposed interactions with SNARE proteins could be important.[166] At the NMJ, the release of acetylcholine contained inside one vesicle causes a miniature endplate potential. These miniature endplate potentials have small amplitudes (0.5–1 mV) that are normally insufficient to trigger action potentials. A nerve impulse causes the release of approximately 20–200 quanta, depending on the species, within a fraction of a millisecond. The endplate potential is generated by electrical summation of many miniature endplate potentials synchronously discharged from the active zones. The peak amplitude of the endplate potential is 15–20 mV.

Vesicle Endocytosis. After fusion, the SV membrane is recovered *via* endocytosis. However, because exocytotic vesicle membranes contain unique proteins, endocytosis must retrieve them selectively. Three mechanisms have been proposed.[167] The first mechanism suggests that endocytosis in nerve terminals is based on a membrane-budding process that requires the formation of coated pits and coated vesicles. This seems to involve some sort of coating protein that is widely assumed to be clathrin or "accessory" proteins (dynamin, endophilin, and synaptojanin). Many of these proteins have now been characterized in considerable detail.[168] Synaptotagmin also appears to be involved in this proposed mechanism. After pinching off the membrane, the clathrin-coated vesicles uncoat and another cycle starts again. The second mechanism also proposes that clathrin-coated vesicles transit through endosomes and other intermediates, from which functional SVs are then formed. The third proposed mechanism is the "kiss-and-run" hypothesis. It attempts to explain the rapid retrieval of SVs after exocytosis. According to this hypothesis, the SVs empty within fractions of a millisecond as their low-molecular-weight contents escape through the fusion pore. The fusion pore then closes, the vesicle reaccumulates transmitter from the cytoplasm, and is once again ready to participate in synaptic transmission.[169] This model implies that vesicles do not lose their identity during exocytosis and that a new vesicle is formed by the rapid reclosure of a transient fusion pore.[170] SVs then accumulate acetylcholine by active transport. Recent data suggest that kiss-and-run operates in parallel with the classic coated-vesicle recycling.[171] Recycling SVs appear to be incorporated into the releasable pool from which they have roughly the same probability of release as the preexisting vesicles.[172] The entire SV cycle takes approximately 1 min.[173]

Acetylcholinesterase at the Neuromuscular Junction

At the NMJ, acetylcholinesterase (enzyme classification 3.1.1.7) is a type-B carboxylesterase enzyme responsible for rapid hydrolysis of released acetylcholine, thereby controlling the duration of receptor activation.[174] Approximately 50% of the released acetylcholine is hydrolyzed during the time of diffusion across the synaptic cleft before reaching nAChRs. The efficiency of acetylcholinesterase depends on its fast catalytic activity. Acetylcholinesterase ranks as one of the highest catalytic efficiencies known. It can catalyze acetylcholine hydrolysis (4,000 molecules of acetylcholine hydrolyzed per active site per second) at near diffusion-limited rates.[174] The active site lies near the bottom of a deep and narrow cleft that reaches halfway into the protein. Acetylcholine must enter this cleft in the enzyme that is blocked by a mobile ring of molecules more than 97% of the time. Molecular dynamics simulations showed that the entrance to the cleft opens and shuts so frequently that any acetylcholine molecules lingering nearby have ample chances to diffuse in.[175] The molecular dynamics simulations also showed that the motions of the channel extend from the region outside the acetylcholinesterase enzyme to the active site. These fluctuations in the width of the channel are required to allow acetylcholine to move from the outside into the active site. They also contribute to the selectivity of the enzyme, by slowing the entrance of substrates that are larger than acetylcholine.[175]

Acetylcholinesterase is highly concentrated at the NMJ but present in a lower concentration throughout the length of muscle fibers.[176] In mammals, acetylcholinesterase is encoded by a single gene. It has been localized to chromosome 7q22 in humans.[177] Much of the acetylcholinesterase at the NMJ occurs in the asymmetric or A12 form consisting of three tetramers of catalytic subunits covalently linked to a collagen-like tail. Asymmetric acetylcholinesterase is bound to the junctional BL.[178] The distribution of acetylcholinesterase molecules on the synaptic BL closely matches the distribution of nAChRs.[179]

Acetylcholinesterase is regulated, in part, by muscle activity and by the spontaneous or nerve-evoked depolarization of the plasma membrane.[180] Fast muscles express severalfold higher levels of acetylcholinesterase

activity than slow muscles, and this is correlated with the relative abundance of acetylcholinesterase mRNAs in these muscles. Drugs that block membrane depolarization, such as the sodium channel antagonist tetrodotoxin, decrease accumulation of acetylcholinesterase.[181] In contrast, sodium channel agonists such as veratridine dramatically increase acetylcholinesterase assembly.[182] After denervation, there is a large decrease in the density of acetylcholinesterase molecules at the NMJ that can be restored by electrical stimulation of the denervated muscles or by their reinnervation either at the original[183] or at ectopic sites.[184]

In addition to hydrolysis of acetylcholine, acetylcholinesterase has other functions such as nerve growth-promoting activities[185] and modulation of nAChRs.[186]

Clinical Implications

The importance of the enzyme is illustrated by the following conditions. Congenital acetylcholinesterase deficiency results in a disabling congenital myasthenic syndrome.[187] This subset of congenital myasthenic syndrome is caused by genetic defect in the collagenic tail of acetylcholinesterase that attaches the enzyme to the BL of the endplate.[187] On the other hand, inhibition of the enzyme, *e.g.*, by nerve gas, results in prolonged exposure of nAChR to acetylcholine, causing desensitization of nAChR and a depolarization block at physiologic rates of stimulation.[188] Chronic fatigue is a symptom of Gulf War syndrome, a disorder proposed to result from exposure to acetylcholinesterase inhibitors.[189] Partial inhibition of acetylcholinesterase, *e.g.*, by overexposure to insecticides, results in excessive influx of Ca^{2+} through the nAChRs ion channel, which leads to local necrotic myopathy and an endplate myopathy.[107] Oximes are clinically important reactivators of acetylcholinesterase that can prevent these degenerative effects of insecticide intoxication.[190] Nevertheless, acetylcholinesterase inhibitors are therapeutically useful for antagonism of residual neuromuscular block and for symptomatic treatment of patients with MG.

The active surface of the acetylcholinesterase is best viewed as having two subunits, the anionic site and esteratic site.[191] The anionic site is concerned with binding and orienting the substrate molecule.[191] The esteratic site is responsible for the hydrolytic process.[191] A second "anionic" site, which became known as the "peripheral" anionic site, was proposed based on binding of bis-quaternary ammonium compounds.[192] Binding of ligands to the peripheral anionic site causes inactivation of the enzyme, although the mechanism of inhibition is not clear. There is also evidence for a role of the peripheral anionic site of acetylcholinesterase in neurite regeneration and outgrowth and in the growth and differentiation of spinal motor neurons.[193]

Neostigmine and edrophonium are the most commonly used anticholinesterases in the operating room. Edrophonium is a prosthetic inhibitor that binds to the anionic site on the acetylcholinesterase by electrostatic attachment and to the esteratic subsite by hydrogen bonding. The dissociation half-life of this reaction is less than 0.5 min.[194] The *in vivo* activity of edrophonium is predicted to be rapid in onset, and, clinically, edrophonium has a more rapid onset of action than neostigmine.[195] Neostigmine and pyridostigmine are oxydiaphoretic (acid transferring) inhibitors of acetylcholinesterase. Neostigmine and pyridostigmine transfer a carbamate group to the acetylcholinesterase, which forms a covalent bond at the esteratic site. The dissociation half-life of the carbamate-enzyme bond of neostigmine is at least 7 min.[194] However, it should be noted that the pharmacologic actions of neostigmine and edrophonium are not limited to enzyme inhibition.[196,197] Evidence suggests that the direct influences of the acetylcholinesterase inhibitors on neuromuscular transmission independent of enzyme inhibition involve at least three distinct, although possibly interacting mechanisms: (1) a weak agonist action, (2) the formation of desensitized receptor-complex intermediates, and (3) the alteration of the conductance properties of active channels.

Aging

Aging is associated with progressive decrease in skeletal muscle mass and strength (sarcopenia) caused by reduction of anabolic hormone concentrations, decline in muscle protein turnover, and other neuromuscular alterations.[198] Between 20 and 80 yr of age, the cumulative decline in skeletal muscle mass amounts to 35–40%. The loss of muscle mass is not associated with weight loss because of a corresponding increase in fat.[199] Loss of muscle mass, particularly the preferential loss of type II fibers,[199] results in diminished strength and power-generating capacity.[200] This has been attributed to structural changes in myosin caused by protein oxidation.[201] The issue of whether skeletal muscle oxidative capacity declines with age remains controversial. The aging process also includes a slowing of time and rate of relaxation of skeletal muscle probably caused by decreased rates of maximal sarcoplasmic reticulum Ca^{2+} uptake and sarcoplasmic reticulum Ca^{2+}-ATPase activity.[202] Although the loss of muscle mass associated with aging may be of multifactorial etiology, it is modifiable through resistance training.

Age-related Compensatory Plasticity at the Neuromuscular Junction

In the soleus muscle of old mice, SV density declined to 32% of adult values.[203] However, no electromyographic decrements were seen at trains of 10 Hz stimulation.[204] The decreased SVs density was accompanied by an increase in the quantal content of transmitter release in the soleus (but not diaphragm or sternomas-

toid muscles) of old mice.[205] The rate constant of transmitter turnover in old mice was also found to be more than twice that in adults.[206] The increased transmitter turnover seems a compensation for diminished SVs. With increasing age, an increase of the number of RyR1 uncoupled from DHPR has been found in humans.[207] Uncoupling of DHPR-RyR1 leads to a significant reduction in the amount of releasable Ca^{2+} in skeletal muscles from old humans.

As mentioned previously (see Signals from the Nerve), the exchange of trophic factors by motor neurons and muscle fibers maintains the NMJ. Neurotrophic factors (muscle-derived trophic factors acting on motor neurons) and myotrophic factors (motor–neuron-derived trophic factors acting on muscle fibers) may play a role in the generation of secondary myotubes and the maturation of NMJs during development.[208] It has been suggested that the expression of the trophic factors and their receptors (*trk*B) might be altered with age, resulting in synaptic dysfunction and cell death.[209] TrkB is a family of transmembrane proteins composed of a tyrosine kinase that serve as receptors for brain-derived neurotrophic factor, neurotrophin-3, and neurotrophin-4/5. Knockout mice lacking brain-derived neurotrophic factor or neutrotrophin-3 did not exhibit significant motor neuron loss, but mice lacking *trk*B had significant reduced numbers of motor neurons.[210]

Age-related Morphologic Changes of the Neuromuscular Junction

Aging is associated with a reduction in total muscle fiber number. A substantial selective atrophy of fast, glycolytic type II fibers was observed with aging.[199] It is believed that type II fibers have a reduced reinnervation capacity compared with type I fibers. The specific force developed by both fast- and slow-twitch single intact muscle fibers declines with aging, and more significantly in the former.[211] In humans (aged $>$ 60 yr), reduction in number of α-motor neurons and their myelinated axons were observed in lumbar ventral roots.[212] Axonal atrophy is probably caused by a reduction in the expression and axonal transport of cytoskeletal proteins in the peripheral nerve. For NMJs of humans, aging is associated with a decrease in motor unit numbers.[213] In some junctions, motor neurons regenerate by sprouting and formation of new sites.[209] The newly formed synaptic sites appear to be unstable, with many disappearing within several weeks.[209] Greater complexity of terminal arborization is observed in the elderly NMJs than those in the adult.[214] Areas of axonal contact become progressively more scarce with advancing age, leading to a reduction of the effective area of synaptic contact in the NMJ. This can result in a decline in the trophic interaction of nerve and muscle and in impairment of stimulus transmission.[215] The repeated cycles of retraction and compensatory outgrowth probably represent the altered balance between degeneration and regeneration of nerve terminals.[204] The greater complexity of terminal arborization may reflect an adaptive and reactive response at the NMJ in an attempt to preserve synaptic area and to compensate for the loss of adhesion of nerve terminals to the synaptic matrix or surrounding Schwann cells.[19] The cellular mechanisms underlying these changes are unclear, although a deficiency of actin has been implicated by some investigators.[204]

Clinical Implications

The adaptive process to aging at the NMJ includes increase of transmitter release despite reduced supply of synaptic vesicles, functional reactive sprouting after partial denervation, and maintenance of nerve terminal integrity in the face of increased outgrowth and retraction.[204] Although function may be initially preserved, the increasing extent of adaptation means a progressively more fragile system. Increased fragmentation and loss of active synaptic areas can lead to deterioration of NMJ structure and function. Therefore, the capacity of skeletal muscle to generate force declines with age.[216] In the elderly, the diaphragm undergoes significant reduction in specific force.[217] This would increase the workload on the diaphragm.[218]

Not only the functional changes at the NMJ but also the multitude of physiologic changes that accompany the aging process (decreases in total body water, glomerular filtration and renal blood flow, liver mass and splanchnic blood flow, and serum albumin concentrations, and increases in fat) affect the action of neuromuscular blockers in the elderly. The onset of nondepolarizing neuromuscular blockers is delayed in the elderly compared with the young. This has been attributed to slower biophase equilibration.[219] However, there have been conflicting reports of the pharmacodynamics and pharmacokinetics of neuromuscular blockers in the elderly. Ornstein *et al.*[220] reported minor differences in the pharmacokinetics of cisatracurium in elderly patients that were not associated with alterations in recovery after a single dose of cisatracurium. Other investigators noted that the duration of action of mivacurium was prolonged in the elderly by approximately 30% as compared with young adults.[221] A decrease in butyrylcholinesterase activity may be the reason for the longer duration of action of mivacurium in the elderly.

Rupp *et al.*[222] noted that elderly patients had significantly decreased plasma clearances and volumes of distribution of vecuronium, whereas elimination half-life and recovery index were not different when compared with that of their younger counterparts. In contrast, other investigators reported that both spontaneous recovery[223,224] and elimination half-life of vecuronium were prolonged and plasma clearance of vecuronium was reduced in older *versus* younger patients.[224] Similar results were reported with rocuronium; however, there

was no difference in the elimination half-lives between the two groups.[225]

It appears that the prolonged duration of action of neuromuscular blockers in the elderly patients is secondary to altered pharmacokinetics. The pharmacokinetics and pharmacodynamics of compounds primarily dependent on spontaneous degradation *via* Hofmann elimination (for example, cisatracurium) are not markedly affected by advancing age. In contrast, the action of steroidal neuromuscular blockers, agents dependent on organ elimination, is prolonged in the elderly.

The duration of action of neostigmine and pyridostigmine is reported to be prolonged in the elderly, probably because of reduction in plasma clearance.[226,227] However, it has been shown that a greater dose of neostigmine is required in the elderly than in the young to produce adequate antagonism of neuromuscular blockade.[228] Aging is associated with a prolongation of the elimination half-life and a reduction of the plasma clearance that resulted in higher plasma concentration of edrophonium.[229] However, this was not reflected in an increased duration of antagonism in the elderly as compared with younger patients.

Neuromuscular Disorders

The physiology and pharmacology of the NMJ is pivotal to many aspects of the practice of anesthesiology, including intraoperative care, intensive care unit (ICU) treatment, and pain management. The complexities of normal neuromuscular transmission described above are altered in many pathologic states. The NMJ can be affected by reduced central neuronal activity in spinal cord trauma, stroke, and states of prolonged inactivity. Deficient primary motor neuron activity in Guillain-Barré syndrome (GBS) and amyotrophic lateral sclerosis (ALS) causes changes in the neuromuscular unit. Diseases such as the Lambert-Eaton syndrome, a myasthenic syndrome, as well as exogenously administered magnesium and certain antibiotics, result in reduced presynaptic release of acetylcholine. MG and rare congenital nicotinic channelopathies produce postsynaptic abnormalities of skeletal muscle receptor function. Ion channel dysfunction in skeletal muscle has emerged as pivotal in understanding the etiology of neuromuscular disorders. For instance, sodium and chloride channelopathies are now linked to myotonia and periodic paralysis. Mutations of Ca^{2+} channels at the sarcoplasmic reticulum have been identified in some cases of malignant hyperthermia.[230] The progress in the fields of molecular genetics and cellular electrophysiology has changed the traditional clinical classification of the large and heterogeneous group of neuromuscular disorders.

Table 1. Conditions Associated with Up- and Down-regulation of Acetylcholine Receptors

nAChR Up-regulation	nAChR Down-regulation
Spinal cord injury	Myasthenia gravis
Stroke	Anticholinesterase poisoning
Burns	Organophosphate poisoning
Prolonged immobility	
Prolonged exposure to neuromuscular blockers	
Multiple sclerosis	
Guillain-Barré syndrome	

nAChR = nicotinic acetylcholine receptor.

Up- and Down-regulation of Nicotinic Acetylcholine Receptors

This subject was reviewed in 1992 by Martyn *et al.*[231] A classic principle of pharmacology suggests that decreased exposure to an agonist results in postsynaptic receptor up-regulation (increases in number), whereas increased agonist exposure results in receptor down-regulation (decreases in number).[231] Therefore, diseases that cause reduced neuronal input will result in an up-regulation of nAChRs in skeletal muscle (table 1). In conditions of primary myopathy, including many of the muscular dystrophies, there is an increase in the number of postsynaptic nAChRs on the basis of chronic muscle regeneration. Nicotinic receptor up-regulation is complicated by the existence of two forms of nAChRs (mature and fetal nAChRs) in muscle tissue (as discussed in Development of the Neuromuscular Junction and The γ to ε Subunit Shift). Up-regulation of nAChRs, found in states of functional or surgical denervation, is characterized by the spreading of fetal type ($\alpha_2\beta\delta\gamma$) receptors at extrajunctional sites.[232] This is noted within 48 h after partial denervation.[233] Furthermore, after denervation, in addition to the usual mature isoform of the Na^+ channel, an immature isoform of the Na^+ channel is expressed on the muscle membrane.[234] The fetal-type nAChRs are resistant to nondepolarizing neuromuscular blockers and more sensitive to succinylcholine.[235] When depolarized, the immature isoform has a prolonged open channel time that exaggerates the K^+ efflux.[234] A positive correlation was found between the number of nAChRs and the intensity of the hyperkalemia after administration of succinylcholine.[236] In contrast, reduced expression of the postsynaptic nAChR results in resistance to depolarizing and sensitivity to nondepolarizing neuromuscular blockers.[237]

Nevertheless, there exist multiple reports in the clinical literature of "increased sensitivity" to nondepolarizing neuromuscular blockers in patients with actual or functional denervation.[238,239] The resolution to this apparent paradox likely lies in the well-studied margin of safety for neuromuscular transmission. The later is defined as the fraction of AChRs that could be pharmacologically blocked before action potential generation was

prevented.[240] Normally, the twitch response is not reduced unless more than 70% of the receptors are occupied by a nondepolarizing relaxant.[240] These "extra" receptors insure the remarkable fidelity of neuromuscular transmission. Unlike the healthy patient, the patient with functional denervation may have preexisting clinical or subclinical weakness and a reduction in the margin of safety for neuromuscular transmission. In such a patient, inhibition of even 10% of postsynaptic nAChRs by a small dose of a nondepolarizing muscle relaxant may result in clinically detected weakness. Thus, despite up-regulation of fetal-type nAChRs and an associated reduction in the potency of nondepolarizing neuromuscular blockers, complete reversal of neuromuscular blockade is required for adequate respiratory function in these patients.

Spinal Cord Injury and Stroke

Spinal cord trauma and stroke are associated with muscle weakness or paralysis based on the dysfunction of central motor neurons. Degeneration of the α-motor neuron results from central malfunction, most likely because of a loss of trophic factors.[241] Reduced exposure to acetylcholine results in up-regulation of the immature form of the nAChR. Up-regulation of extra junctional fetal nAChRs is associated with resistance to nondepolarizing neuromuscular blockers and increased sensitivity to succinylcholine and susceptibility to hyperkalemia.[242]

Clinical Implications. The period of vulnerability to succinylcholine-induced hyperkalemia has not been defined. Based on several case reports, the onset of the hyperkalemic response ranges from 1 week to several months.[243,244] Because nAChR up-regulation occurs within 48 h after partial denervation,[233] succinylcholine appears to be safe within the first 24 h after the insult. In one report, succinylcholine-induced hyperkalemia persisted for a period of up to 6 months in patients with upper motor neuron lesions.[245] With recovery after stroke or cord section, the exaggerated response to succinylcholine is not likely to diminish until after resistance to nondepolarizing relaxants has reverted to normal.[246] Up-regulation of the immature form of nAChRs may persist indefinitely, depending on the degree of denervation and renervation. Recent work demonstrated an increased hyperkalemic response to succinylcholine more than 1 yr after injury.[247] Because the safe period varies depending on the degree of abnormal nAChR expression and other factors, it is best to avoid succinylcholine in these patients if possible.

Immobility

Prolonged immobility, in which individuals are confined to wheelchairs or beds, is associated with muscle atrophy secondary to disuse. In contrast with upper or lower motor neuron disease, the nerves themselves are not damaged. The decreased protein synthesis, increased protein degradation, muscle atrophy, decreased glucose uptake, and apoptosis observed after muscle disuse or immobilization have been attributed to decreased insulin action and defective insulin signaling *via* phosphatidylinositol 3-kinase.[248] The latter is a key signaling molecule that is needed for the anabolic actions of insulin.

Despite the presence of an intact motor neuron, extrasynaptic nAChRs develop with some of the characteristics of immature nAChRs.[249] There is resistance to nondepolarizing neuromuscular blockers and increased sensitivity to acetylcholine and succinylcholine.[250] Resistance to nondepolarizing neuromuscular blockers was noted 4 days after complete immobilization in dogs.[231] Administration of succinylcholine resulted in hyperkalemia and cardiac arrest and death.[251] After remobilization, changes at the NMJ revert to normal within 20–50 days.[231]

Studies on the effect of single-limb immobilization in animals showed that the increased response to nondepolarizing neuromuscular blockers was not only noticeable in the immobilized limb, but also in the other unaffected limbs.[252] The diaphragm, however, was not affected.[252] Another interesting finding was that the potassium release after succinylcholine was significantly increased in beagles who had one limb immobilized by casting.[253] This increase required 14–42 days to become apparent.[253]

Clinical Implications. In the case of total-body immobilization, the onset of vulnerability to succinylcholine-induced hyperkalemia has not been well defined. Death caused by hyperkalemic cardiac arrest after the administration of succinylcholine was reported in one patient 5 days after immobilization.[251] For this reason, it is probably best to avoid succinylcholine when total-body immobilization exceeds 24 h. Data regarding single-limb immobilization are less conclusive, and the reported response to succinylcholine in the literature should be interpreted in relation to both the etiology and duration of the immobilization.[253]

Weakness Syndromes in the Critically Ill (Critical Illness Polyneuropathy and Myopathy)

Syndromes of weakness in critically ill patients are relatively common and likely polymorphic in origin. In a retrospective study of 92 critically ill patients with clinically diagnosed weakness, electromyographic studies indicated that 43% of the patients suffered from myopathy, whereas 28% suffered from peripheral neuropathy.[254] Weakness can lead to prolonged weaning from the ventilator and increased time for rehabilitation.[238,254] Myopathy may be either caused by immobility discussed above or the catabolism associated with negative nitrogen balance.[238] In addition, myasthenia-like syndromes are also seen in critically ill patients. Evidence for local immune activation by cytokine ex-

pression in the skeletal muscle was reported in patients with critical illness polyneuropathy and myopathy.[255] Furthermore, the presence of antibodies to nAChR associated with decreased number of nAChRs and increased sensitivity to d-tubocurarine has been demonstrated in a rodent model of subacute or prolonged sepsis.[256] Three main types have been identified: critical illness myopathy, myopathy with selective loss of myosin filaments, and acute necrotizing myopathy of intensive care.

The polyneuropathy seen in the critically ill has been termed "critical illness polyneuropathy." Critical illness polyneuropathy is a diffuse axonal polyneuropathy and occurs in 50–70% of patients with multisystem organ failure and sepsis.[257] Recovery from critical illness polyneuropathy can be rapid and complete when the patient survives the critical illness.[238,257] There may be a role for humoral factors associated with multisystem organ failure, but the etiology is likely multifactorial.[238,257] Prolonged use of neuromuscular blocking agents singly and in association with glucocorticoids[258] may have toxic effects on motor axons, but the results of studies so far have been inconclusive.

Clinical Implications. It is likely that up-regulation of nAChRs induced by immobilization and chronic neuromuscular blockade contributed to the cardiac arrest associated with the use of succinylcholine in ICU patients.[251,259] As most critically ill patients are immobilized, it is impossible to determine whether weakness is caused by immobility, polyneuropathy, or myopathy of critical illness without pathologic diagnosis. Nevertheless, as succinylcholine can cause hyperkalemia in any of these syndromes, it is best to avoid succinylcholine in ICU patients in whom total-body immobilization exceeds 24 h.

Several reports have implicated nondepolarizing neuromuscular blocking drugs to cause generalized weakness after their long-term administration (to ICU patients) that required recovery periods from 2 days to 6 months.[260] However, it is not clear whether neuromuscular blockers were a precipitating factor since other possible contributing conditions were frequently present, *e.g.*, polyneuropathy of critical illness, disuse atrophy, renal failure, aminoglycoside, and steroid administration.[257,258,260,261] A clinical impression has been reported that prolonged recovery from neuromuscular block occurs more frequently when steroidal neuromuscular relaxants are used.[260] Prolonged neuromuscular block has been associated with renal failure and increased serum concentrations of the active metabolite of vecuronium, 3-desacetylvecuronium.[260] Although corticosteroids are not thought to be a risk factor for polyneuropathy of critical illness, when administered with vecuronium, both *in vivo* and *in vitro*, inhibition of nAChR activation is additive.[258] Recovery of neuromuscular function after discontinuation of neuromuscular blocking drug infusion in ICU patients was found to be faster with cisatracurium than with vecuronium despite equivalent reduction in train-of-four suppression at baseline.[262] Nevertheless, the variability in the time course and the etiology of alteration recovery of neuromuscular function demonstrates that routine neuromuscular monitoring alone is not sufficient in eliminating prolonged recovery and myopathy in ICU patients.[262]

Demyelinating Diseases

Multiple Sclerosis. Multiple sclerosis is a demyelinating disease resulting from an abnormal immune response to an antigen present in the myelin sheath within the central nervous system. It is common in young adults. Demyelination in multiple sclerosis follows a waxing and waning pattern and is thought to be inflammatory in origin. There is evidence for both genetic predisposition and previous exposure to an unknown causative agent.[263] Demyelinating lesions may occur in any part of the brain and spinal cord and can result in sensory, motor, autonomic, or neuropsychological disability. In multiple sclerosis, mean firing rates of the motor unit action potentials are reduced, and firing variability is increased.[264]

Clinical Implications. There is some evidence that the stress of intercurrent illness–surgery–anesthesia may increase the rate of relapse in multiple sclerosis, but the interplay between these factors is unclear. There have been several case series published that do not demonstrate any association between the use of general anesthesia and an increased rate of relapse.[265,266] The use of regional anesthesia in multiple sclerosis is more controversial. Both lumbar epidural and subarachnoid anesthesia have been reported in patients with multiple sclerosis without clear evidence for an increase in the relapse rate.[267] There is some suggestion that higher concentrations of local anesthetic may be neurotoxic. In one study in which patients received either 0.5 or 0.25% bupivacaine for epidural anesthesia, relapses only occurred in patients receiving the higher dose of local anesthetic.[265] Patients with multiple sclerosis may have exacerbations of their symptoms if they become hyperthermic.[266]

The use of neuromuscular blockers in patients with multiple sclerosis depends on the clinical syndrome. In patients with chronic motor weakness, central denervation is the probable cause. As with any patient with denervation or disuse, there may be up-regulation in nAChR numbers and increased sensitivity to depolarizing neuromuscular blockers. In this case, the patient is at risk for hyperkalemia after administration of succinylcholine. As discussed above, there are paradoxical reports of increased sensitivity to nondepolarizing neuromuscular blockers in patients with multiple sclerosis, probably because of reduced muscle mass or reduced margin of safety for neuromuscular transmission.[264] It is significant to note that muscle denervation of any origin will cause muscle depolarization.[268] As a consequence, the inactive state of sodium channels will be favored so that

endplate potentials fail to generate action potentials.[269] The denervation-induced decline of the resting potential will significantly contribute to muscle weakness.

Motor Neuron Diseases. The motor neuron diseases are a group of heterogenous disorders characterized by muscle weakness, atrophy, or spastic paralysis caused by involvement of lower or upper motor neurons, respectively. ALS is the most common motor neuron disease and involves both upper and lower motor neurons. Spinobulbar muscular atrophy (or Kennedy disease) affects lower motor neurons only. Hereditary spastic paraplegia, on the other hand, involves upper motor neurons. ALS, commonly known as Lou Gehrig disease, is a progressive disease characterized by degeneration of cortical, brainstem, and spinal motor neurons.[270] Motor neuron degeneration results in denervation, muscle wasting, and eventual paralysis and death. Cognitive and sensory systems are left intact. The incidence of ALS is 2–4 in 100,000. The etiology of ALS is not known, although a role for oxidative stress has been suggested since mutations in the gene for Cu^{2+}–Zn^{2+} superoxide dismutase (SOD1) have been identified in familial ALS. Knockout of the *SOD1* gene in mice results in a syndrome similar to ALS.[271] Experimental data also suggest the presence of antibodies to voltage-gated Ca^{2+} channels in ALS patients.[272] These antibodies cause an increase in quantal release at the NMJ probably secondary to increased function of the presynaptic Ca^{2+} channels.[273] Increased Ca^{2+} influx and intracellular Ca^{2+} concentration may contribute to pathologic changes seen at the NMJ.[274] In animals, long-term neuromuscular dysfunction is reproduced by passive transfer of ALS immunoglobulins.[275] There is currently no cure for ALS, and treatment is aimed at symptomatic support and comfort.[270]

Clinical Implications. As in other patients with muscle wasting from states of functional denervation (multiple sclerosis, GBS), there is compensatory up-regulation of nAChRs that may be extrasynaptic. These patients are at risk for hyperkalemia after administration of succinylcholine.[276] There may be a perceived hypersensitivity to nondepolarizing neuromuscular blockers because of weakness caused by muscle wasting. Patients, particularly in late stages of the disease, may be cachectic from inadequate nutrition and have reduced plasma protein binding for many of the anesthetic drugs. These patients have reduced respiratory muscle reserve, abnormal airway protective reflexes, and are at increased risk for respiratory depression and aspiration secondary to the use of sedative and anesthetic drugs. Epidural anesthesia has been used in ALS patients without untoward effects.[277]

Guillain-Barré Syndrome. Guillain-Barré syndrome is made up of a spectrum of diseases that commonly include a generalized polyradiculopathy, affecting the limbs proximally more than distally, and may also involve cranial and bulbar nerves.[278] GBS is relatively common, with an incidence of 4 in 10,000 throughout the world.[278] The diseases that underlie GBS have recently been reclassified.[278] Acute inflammatory polyradiculoneuropathy is common in the white populations of North America and Europe. Lymphocytic invasion in the peripheral nervous system and primary macrophage penetration of apparently normal myelin are typical of acute inflammatory polyradiculoneuropathy. In contrast, in Central America, China, Japan, and India, GBS is caused by an axonopathy that affects both motor and sensory neurons. These syndromes are called acute motor axonal neuropathy and acute motor and sensory neuropathy depending on the presence of sensory involvement. The Fisher syndrome is an additional variant of GBS in which the patients have ophthalmoplegia, ataxia, and loss of tendon reflexes but no limb weakness.

There is strong evidence for an association between certain infections and GBS. The most prevalent infections and events associated with GBS are *Campylobacter jejuni*, Cytomegalovirus, Epstein-Barr virus, *Mycoplasma pneumoniae*, rabies, and the "Swine Flu" vaccines. Undoubtedly, very few patients infected or vaccinated with the above agents will develop GBS. A predisposition for GBS possibly requires a particular genetic background or specific strains of infective organisms.[278]

Patients with GBS commonly have high autoantibody titer to antiganglioside antibodies directed at the ganglioside GQ1b. Gangliosides are present in high concentrations in peripheral nerve axons and myelin, and several studies indicated that different gangliosides are present at nodes of Ranvier and at the NMJ.[279] It is unclear whether these antibodies cause demyelination or are a secondary result of the disease.[278] Neuromuscular weakness in the acute stage of GBS has been attributed, in part, to circulating antibodies that can block both presynaptic voltage-gated calcium channels[280] and postsynaptic nAChR channels.[281] Patients with GBS commonly have symptomatic improvement after plasmapheresis.[282] The final common pathway in acute inflammatory polyradiculoneuropathy is invasion of the myelin sheath by macrophages. The macrophages displace and phagocytose the myelin from the axon, leaving cleanly demyelinated axons.[283] Demyelination produces functional denervation of the muscle and up-regulation of nAChRs at the postsynaptic membrane.

Clinical Implications. Patients with GBS present to the anesthesiologist in the ICU with motor weakness, at which time tracheal intubation and ventilation is often necessary because of insufficient ability to generate inspiratory force or because of concurrent infection. These patients may need anesthetic intervention for surgery or for assisted delivery in pregnancy. Succinylcholine is contraindicated because of the risk of hyperkalemic cardiac arrest secondary to the proliferation of postsynaptic nAChRs.[284,285] This risk may persist over a long period after recovering from the symptomatic neu-

rologic deficit.[286] Preexistent loss of motor units and presynaptic or postsynaptic nAChR channel blockade by antibodies[281] may result in sensitivity to nondepolarizing neuromuscular blockers.[285,287] Regional anesthesia is not contraindicated, although patients with GBS are sensitive to local anesthetics secondary to preexistent axonal conduction abnormalities.[285] Patients with GBS have a high incidence of autonomic instability, and the slower onset of an epidural block may be preferable to the rapid onset of subarachnoid anesthesia. GBS has been reported in four patients 1–2 weeks after epidural anesthesia. It was postulated that local trauma to nerve roots may initiate a cascade of immunologic events that result in demyelinating neuropathy in these patients.[288]

Charcot-Marie-Tooth Disease. Charcot-Marie-Tooth disease (CMTD; hereditary motor and sensory demyelinating polyneuropathy) is the most common genetic neuropathy, with an incidence of 1 in 2,600.[289] CMTD has heterogeneous genetic (autosomal dominant, X-linked, or autosomal recessive) and clinical presentations.[290,291] Three genes responsible for CMTD type 1 have been identified: peripheral myelin protein 22 and myelin protein zero for the autosomal dominant form and connexin 32 for the X-linked dominant variant.[292] The latter variant encodes a gap junction protein.[291]

Gap junctions are aggregations of intercellular channels that provide a direct pathway for the exchange of nutrients, metabolites, ions, and small molecules up to approximately 1,000 Da between closely apposed cells.[293] The channels are composed of connexins, a family of highly related proteins.[293] In the nervous system, gap junctional channels play a key role in the propagation of signals between electrically excitable cells.[294] Failure of the gap junctions may therefore lead to impaired Schwann cell function and subsequent demyelination. Electron microscopy shows gap junctions to be extremely rare between adjacent myelinating Schwann cells in genetic abnormalities in connexin 32 associated with CMTD.[292]

Charcot-Marie-Tooth disease can be divided into two distinct groups based on electrophysiologic studies.[295] CMTD type 1 exhibits moderately to severely reduced motor nerve conduction velocities.[296] The conduction deficit in CMTD type 1 is bilaterally symmetric, which suggests an intrinsic Schwann cell defect.[296] In contrast, CMTD type 2 results from neuronal atrophy and degeneration and exhibits normal or only mildly reduced motor nerve conduction velocities with decreased amplitudes.[295]

Peroneal nerve atrophy leading to weakness in the anterior and lateral compartments is the most common clinical pattern in CMTD, but considerable variability exists in the pattern of atrophy. Abnormalities of feet and toes, including pes cavus, are usually present. Intrinsic atrophy of the calf musculature is a common finding in CMTD. The sensory disturbance is milder than the motor disturbance. Autonomic disturbances such as orthostatic hypotension and hypohidrosis are occasionally reported.[297] Pregnancy may be associated with exacerbations of CMTD.[298] Respiratory insufficiency has also been described in patients with CMTD.[299]

Clinical Implications. Loss of motor units and the resultant muscle weakness in CMTD might result in sensitivity to nondepolarizing neuromuscular blocking drugs. However, there is no evidence of prolonged response to atracurium and mivacurium in patients with CMTD.[300] Succinylcholine and other malignant hyperthermia-triggering agents have been used in CMTD patients without untoward effects.[301] Although there is no clear evidence that CMTD predisposes patients to an increased risk of malignant hyperthermia, incidents of malignant hyperthermia in patients with CMTD have been reported.[302] Use of drugs known to trigger malignant hyperthermia must be carefully considered.

Table 2. Molecular Etiology of the Muscular Dystrophies

Diseases	Molecular Etiology	Reference No.
Duchenne	Absence of dystrophin	373
Becker	Reduced dystrophin	304
Limb-girdle	Sarcoglycan deficiency	374
Congenital	Laminin α2 chain	375–377
	Integrin α7 (laminin receptor)	
	Fukutin	
Facioscapulohumeral	4q35 rearrangements	378

Primary Muscle Diseases

Muscular Dystrophies. Muscular dystrophies are a group of heterogeneous, genetically determined disorders of skeletal muscle and, in some cases, cardiac muscle. These disorders have been classified on the basis of clinical symptomatology and genetic inheritance, but with the advent of molecular diagnosis, categories have shifted (table 2). Patients may present with symptoms of muscle weakness and atrophy at different stages of development. The time course and prognosis differs with each syndrome. Most symptomatology is a result of muscle weakness and related pulmonary complications and, in some cases, cardiac abnormalities.[303]

Duchenne muscular dystrophy is one of the most common genetic diseases in humans, with an incidence of 1 in 3,500 male births, whereas Becker muscular dystrophy is milder and affects 1 in 30,000 male births.[303] Duchenne–Becker dystrophy is caused by an X-linked recessive mutation resulting in abnormal or absent dystrophin or related glycoproteins that link the extracellular matrix to the cytoskeleton (see also Signals from the Nerve). In Duchenne muscular dystrophy, dystrophin is usually absent, whereas in Becker muscular dystrophy, the protein is present but qualitatively and quantitatively abnormal.[304] As a result of chronic muscle regeneration in patients with Duchenne dystrophy, there is coexpres-

sion of both fetal and adult nAChRs in the mature muscle membrane.[305] In Duchenne dystrophy, weakness leading to inability to ambulate generally occurs before puberty, and patients typically develop nocturnal hypoventilation by their late 20s.[303] Progressive cardiomyopathy develops in the midteens, and patients typically succumb to cardiac or pulmonary manifestations of their disease in their late teens or 20s.[303] Cognitive impairment is also observed and has been attributed to an abnormality in the neuronal membrane caused by a lack of dystrophin.[306]

Becker muscular dystrophy results from abnormalities in the same gene as Duchenne dystrophy with similar symptomatology. However, it is milder and has slower progression. Onset in childhood may occur as late as 16 yr. Cardiac problems may be more severe than the skeletal muscle weakness.[303] Limb-girdle dystrophy is similar to Duchenne dystrophy and is found most commonly in families in North Africa. Congenital muscular dystrophy has the worst prognosis. Affected infants present at birth with hypotonia, weakness, and respiratory and swallowing abnormalities. Mutations in the laminin $\alpha 2$ chain cause the most severe form of congenital muscular dystrophy. Muscle fiber deterioration in this disease is thought to be caused by impaired formation of the basement membrane and its inability to interact with the DGC or the integrins. Deficiency of laminin $\alpha 2$ is accompanied by up-regulation of the laminin $\alpha 4$ chain, giving rise to laminin-8, which binds poorly to DGC in the muscle fiber. Recently, it has been possible to rescue dystrophic symptoms in a mouse model for congenital muscular dystrophy by muscle-specific overexpression of an agrin minigene, which bound to laminin-8 and the DGC,[306A] replacing the missing link between the basement membrane and the muscle fiber.[306A] Therefore, overexpression of an engineered molecule may become an exciting novel approach to devising new therapeutic tools to restore muscle function in human muscular dystrophies. Facioscapulohumeral muscular dystrophy usually presents in late childhood with facial and scapulohumeral weakness. There may also be weakness of the pelvic girdle with a lordotic posture, but there is usually no cardiac involvement. Patients may develop retinal vasculopathy and sensorineural hearing loss.[303]

Clinical Implications. There have been many reports of succinylcholine-induced hyperkalemia and cardiac arrest in patients with undiagnosed muscular dystrophies.[307,308] This response has lead to a Food and Drug Administration–mandated warning against the use of succinylcholine in pediatric patients because of potential mortality in patients with clinically inapparent muscular dystrophies. Innervation is relatively normal in dystrophic muscle, but the postsynaptic nAChRs are expressed as a mixture of fetal- and mature-type receptors characteristic of chronic denervation.[305] The expression of the fetal nAChR in the dystrophic muscle is not a characteristic of dystrophy but a consequence of muscle regeneration.[305]

Resistance to nondepolarizing neuromuscular blockers would be expected on the basis of the reduced sensitivity of fetal nAChRs to competitive antagonists. However, clinically the reverse is seen.[250,309] Patients with myopathy are unusually sensitive to nondepolarizing neuromuscular blockers.[309] There is an increase in the incidence of malignant hyperthermia in patients with myopathies,[310] and there is an association of rhabdomyolysis with the use of volatile anesthetics.[310]

Myotonias. Myotonias are characterized by difficulty in initiating muscle movement with delayed muscle relaxation after voluntary contraction. Myotonic dystrophy is a progressive disease that manifests in late childhood or adulthood with muscle weakness and atrophy. There is associated frontal balding, cataracts, and testicular atrophy. Myotonic dystrophy occurs with an incidence of 1 in 8,000, making it one of the most common neuromuscular diseases. Myotonia may be precipitated by cold, shivering, diathermy, and succinylcholine. Mutations in the pore-forming subunits of sodium and chloride channels cause myotonia because of an alteration in the electrical excitability of the muscle fiber.[311,312] Myotonic dystrophy is an autosomal dominant disorder associated with an expanded trinucleotide sequence at the 3' untranslated end of the gene for myotonic dystrophy protein kinase (DMPK).[313] DMPK is a serine–threonine protein kinase highly expressed in heart, brain, and skeletal muscle.[313] In skeletal muscle, DMPK is located at the terminal cisternae of the sarcoplasmic reticulum, but its role in the pathophysiology of the disease is unclear.[314] There is some evidence that DMPK is involved in cellular Ca^{2+} homeostasis.[314] Maturational-related abnormality or altered modulatory mechanisms of sarcoplasmic reticulum Ca^{2+} transport have been noted in myotonic dystrophic slow-twitch muscle fibers.[315]

In normal muscle, depolarization of the postsynaptic membrane causes brief openings of sodium channels that occur within the first few milliseconds after membrane depolarization. The voltage-sensitive chloride channels then traffic chloride ion to return the muscle membrane potential to its resting level.[141] Sodium channels harboring mutations causing myotonia exhibit an abnormal tendency to open later or more persistently after membrane depolarization.[141,316] Residual sodium entry through these abnormal channels repeatedly reinitiates the cycle of membrane depolarization.[141] Chloride channel mutations associated with myotonia reduce the amount of chloride ion that can enter the cell to repolarize the membrane, leading to oscillations.[141] In patients with either abnormal sodium or chloride channels, the muscle becomes hyperexcitable.[146] The increased excitability in the muscle results in the generation of repetitive action potentials after voluntary contractions.

Patients with myotonic dystrophy have increased mortality from respiratory complications of their muscle weakness as well as cardiac disease. Cardiac abnormalities include conduction block distal to the His bundle, ventricular arrhythmias, and an increased incidence of sudden death. The severity of the symptoms is somewhat related to the number to trinucleotide repeats in DMPK.[317] The mechanism of muscle weakness is loss of contractile tissue, probably in combination with contractile dysfunction.[317]

Clinical Implications. Despite an apparently normal response to curare in a research setting,[239] patients with myotonia have been reported to require reduced doses of nondepolarizing neuromuscular blockers in a clinical setting.[318] This has been attributed to the underlying muscle wasting and reduced ability to produce contractile force.[319] Anticholinesterase agents may precipitate myotonia[320] because of increased sensitivity of the myotonic muscle to the effects of acetylcholine. The use of succinylcholine in patients with myotonic dystrophy, despite apparently normal nAChRs, is to be avoided. There are reports of extreme muscle rigidity and cardiac arrest after a dose of succinylcholine in patients with myotonic dystrophy.[319] The cardiac arrest was assumed to be caused by increased serum potassium concentration; however, potassium concentrations were not verified before cardiopulmonary resuscitation. The cardiac arrest might have been caused by intrinsic cardiac abnormalities that are associated with myotonic dystrophy. In contrast, trauma patients with undiagnosed severe myotonic dystrophy were given succinylcholine without side effects.[321] The association between myotonia and malignant hyperthermia is uncertain, and the difficulty in interpretation of the caffeine–halothane contracture test in myotonic patients further complicates the nature of the association.[322,323]

Patients with myotonic dystrophy may suffer respiratory compromise as a result of muscle weakness. There may be an increased risk of aspiration caused by velopalatal insufficiency. Children with myotonic dystrophy are at particular risk for the respiratory-depressant effects of general anesthetics and should be carefully monitored before discharge.[324] Clinical deterioration may occur in pregnancy, probably because of hormonal changes, with exacerbation of the muscle weakness, myotonia, and muscle wasting.[325]

Myasthenic Syndromes. Muscle weakness and fatigability are pathognomonic of the myasthenic syndromes. In recent years it has become clear that the myasthenias represent a group of diseases. MG and the LEMS are both caused by autoimmune disease. MG is caused by autoantibody targeting of an extracellular portion of the muscle receptor for acetylcholine.[237] Antibody targeting of this region results in cross-linking of two adjacent nAChRs, complement fixation, and focal lysis of the postsynaptic membrane.[326] Antigenic modulation also results in an increased rate of internalization and degradation of nAChR on the muscle membrane. There are, in addition, antigenic T-cell epitopes throughout the α subunit. Interestingly, antibodies from MG patients do not cross-react with the α3 nAChR subunit that is found principally in the autonomic nervous system or $\alpha_4\beta_2$ nAChRs that occur in the central nervous system, perhaps explaining the lack of autonomic and central nervous system symptoms in typical MG.[327] The net result of antigenic modulation and focal lysis is a reduced number and altered structure of the postsynaptic nAChRs, which impairs neuromuscular transmission and causes muscle weakness.[237] Electron microscopic studies show that the postsynaptic membrane has abnormally sparse, shallow folds with markedly simplified geometric patterns.[328]

The cause of the induction of the immune response in MG is not well known. It is clear, however, that immunization with nAChRs from *Electrophorus* electric organs can cause the induction of antibodies to the nAChR and a syndrome of muscular weakness that has become an animal model for MG.[329] A small percentage of MG patients develop autoantibodies as part of a paraneoplastic syndrome (12% have thymoma).[237] Thymic myoid cells express fetal nAChRs and other muscle proteins. Approximately 70% of MG patients have thymic lymphoid follicular hyperplasia and exhibit germinal centers that produce antibodies to nAChRs.[237] Antibodies to nAChRs must also be produced in other locations. This is based on the evidence that thymectomy may be beneficial to the clinical course of MG, but it may not be curative. Fetal-type nAChRs may be immunogenic, as indicated by the common involvement of extraocular muscles in MG that selectively express fetal nAChRs in adult life.[237] There is also some evidence to indicate that immune molecules created in response to microbial antigens may cross-react with nAChR. This may constitute initial triggers of MG in some patients.[330]

In chronic MG, the nAChR content is reduced to approximately 30%, and most of the remaining nAChRs are bound by antibody.[237] Acetylcholine sensitivity is reduced, and decrementing response to repetitive stimulation occurs. There is no specific immunotherapy for MG as there are abnormalities in all arms of the immune response. Nonspecific immunosuppression with steroids and other drugs and plasmapheresis are often combined with thymectomy and symptomatic treatment with anticholinesterases.

Congenital myasthenic syndromes are heterogenous disorders that do not occur because of autoantibodies, but are caused by inherited mutations in the SVs, acetylcholinesterase, or nAChRs.[114,187,237,331] These mutations result in a range of muscle weaknesses and fatigability that are characteristic of myasthenia. Mutations in the α, β, δ, and most frequently the ε subunit of nAChRs can cause congenital myasthenic syndromes (see also Sub-

unit Mutations and the Myasthenic Syndromes and Acetylcholinesterase at the Neuromuscular Junction). The inheritance of congenital myasthenic syndromes is either autosomal dominant or autosomal recessive. In contrast to neonatal MG that is caused by passive transfer of anti-AChR antibodies to the fetus by a myasthenic mother, the mother of congenital myasthenic syndromes has no myasthenia.

The LEMS is a presynaptic disorder of neuromuscular transmission in which patients exhibit profound muscle weakness in response to nerve stimuli. LEMS is an autoimmune disease that is known to occur with, or precede, a variety of malignancies. Approximately 60% of LEMS patients exhibit a paraneoplastic response, often in conjunction with small-cell carcinoma of the lung.[237] LEMS is caused by an autoantibody targeting the voltage-gated Ca^{2+} channels that mediate acetylcholine release at the motor neuron terminals.[332] Depolarization of the motor axon causes less Ca^{2+} influx, and less acetylcholine is released. The acetylcholine content and acetyltransferase activity in diseased nerve endings are normal. In contrast to MG, there is an increase in contractile force on sustained muscle stimulation in LEMS. In fact, repetitive stimulation causes summation of presynaptic Ca^{2+} signals and improved release.[333] Exercise or tetanic stimulation improves rather than reduces muscle strength in LEMS. As discussed in Vesicle Mobilization and Docking, the interaction between synaptotagmin and the voltage-gated Ca^{2+} channel plays an important role in docking synaptic vesicles at the plasma membrane before rapid neurotransmitter release. It has been suggested that an autoantibody binding to a synaptotagmin–Ca^{2+}-channel complex may be involved in the etiology of LEMS.[163] Assay of voltage-gated calcium channels antibody titer and electrophysiologic tests help to differentiate LEMS from other disorders of the NMJ. In contrast to MG, approximately 30% of patients with LEMS have autonomic dysfunction.

Treatment with 3,4-diaminopyridine results in significant improvement in symptoms and in the summated amplitude of compound muscle action potentials in patients with LEMS.[334] 3,4-Diaminopyridine selectively blocks potassium channels, preventing potassium efflux and causing increase in action potential duration. The latter results in prolonged activation of voltage-gated Ca^{2+} channels and increases intracellular Ca^{2+} concentrations in the nerve terminal with a concomitant increase in acetylcholine release.

Clinical Implications. Anesthesia for myasthenic patients has been reviewed by Baraka.[335] Because of the decreased number of nAChRs or their functional blockade by antibodies, myasthenic patients are resistant to succinylcholine.[336] On the other hand, butyrylcholinesterase activity may be decreased in myasthenic patients by preoperative plasmapheresis or administration of pyridostigmine, and this would result in potentiation of succinylcholine- (or mivacurium[337])-induced block. The interplay between these two factors (resistance to succinylcholine *vs.* reduction in butyrylcholinesterase activity) should be considered when administering succinylcholine to patients with MG. Progression to phase II block is not uncommon in these patients.[335] Succinylcholine should be avoided in patients with SCCMS because succinylcholine would be expected to worsen the existent state of excitotoxicity.

With the loss of 70–89% of the functional nAChRs and hence the margin of safety of neurotransmission, patients with MG are extremely sensitive to nondepolarizing neuromuscular blockers. The decrease in available nAChRs in MG means that even mildly symptomatic myasthenic patients are just at the border of the safety margin for neuromuscular transmission, as evidenced by their easy fatigability.[237] The effective dose of vecuronium is 250% greater in control patients than in MG patients.[338] Indeed, a case of congenital myasthenia with minor clinical signs has been diagnosed as the result of an exaggerated response to a small dose of a nondepolarizing neuromuscular blocker.[339] However, with careful titration and with adequate monitoring of neuromuscular function, nondepolarizing agents have been used safely in myasthenic patients undergoing thymectomy.[340]

On the other hand, patients with LEMS are sensitive to both depolarizing and nondepolarizing neuromuscular blockers.[341] In fact, patients with LEMS have a significantly greater sensitivity to nondepolarizing neuromuscular blockers when compared with those with MG.[239]

Mitochondrial Myopathies

The mitochondrial myopathies are a clinically and biochemically heterogeneous group of disorders characterized by abnormalities of mitochondrial structure. Mitochondrial myopathies are often associated with abnormal proliferation of mitochondria, which accumulate beneath the sarcolemma and between muscle fibers. The massive proliferation of giant mitochondria is probably caused by up-regulation of both mitochondrial DNA and nuclear DNA transcripts, presumably in an effort to compensate for the bioenergetic defect caused by a mitochondrial DNA mutation.[342] These collections of abnormal mitochondria stain purple or red with the modified Gomori trichrome stain, resulting in so called "ragged red fibers." However, ragged red fibers are not pathognomonic of a mitochondrial DNA mutation, as they also appear in aged muscle and in other myopathies.[343] In some cases, the fibers do not have a ragged appearance. Affected fibers also contain an excess of glycogen granules and increased numbers of fine neutral lipid droplets.

Mutations in mitochondrial DNA have been associated with mitochondrial myopathies.[344] These mutations will cause impaired electron transport chain function. This, in turn, results in decreased ATP production and forma-

tion of damaging free radicals. These toxic events produce further mitochondrial damage, including oxidation of mitochondrial DNA, proteins, and lipids. Reactive oxygen species have also been implicated in mitochondrial myopathies.[345] Normally, mammalian mitochondria generate most of the ATP for cells by the process of oxidative phosphorylation. During oxidative phosphorylation, between 0.4 and 4% of the oxygen consumed is reduced to form superoxide anion.[345] During normal circumstances, superoxide is reduced to H_2O_2 by the mitochondrial form of superoxide dismutase. Within the mitochondria, the H_2O_2 is either converted to water by mitochondrial glutathione peroxidase or can participate in Fenton type chemistry, giving rise to further reactive oxygen species such as the hydroxyl radical.[345]

Both isolated myopathies and several multisystem syndromes have been identified. The syndromes, which are defined through characteristic clinical manifestations in addition to mitochondrial myopathy, are chronic progressive external ophthalmoplegia, including Kearns-Sayre syndrome, MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes) syndrome, MERRF (myoclonus epilepsy and ragged red fibers) syndrome, MNGIE (myopathy, external ophthalmoplegia, neuropathy, and gastrointestinal encephalopathy) syndrome, and NARP (neuropathy, ataxia, and retinitis pigmentosa) syndrome. Acquired mitochondrial myopathy has been associated with the use of zidovudine, an antiretroviral drug that depletes muscle mitochondrial DNA.[346]

There is evidence to support mitochondrial involvement in posttetanic potentiation of synaptic transmission at the NMJ.[347] Electrophysiologic studies do not correlate with any specific biochemical or genetic defect, but are consistent with diagnosis in patients presenting with clinical signs of mitochondrial myopathy.[348]

Anesthetic Considerations. Although it has been suggested that mitochondrial myopathy does not involve the NMJ,[349] increased sensitivity to different nondepolarizing neuromuscular blockers has been demonstrated in patients with mitochondrial myopathies.[350] This enhanced sensitivity is of a magnitude similar to that observed in MG.[350] Increased sensitivity to succinylcholine was also noted in these patients.[351] The association between malignant hyperthermia and mitochondrial myopathies is not clear, but published reports indicate a possible association.[352]

Genetic Disorders That Affect Channels (Channelopathies)

Cell membranes are composed of two lipid layers that are not permeable to ions. The channels are macromolecular protein complexes within the lipid membrane and are opened by ligands or voltage changes. They regulate the traffic of ions in and out of the cell causing depolarization and hyperpolarization of the cell. The channel structure is determined by different genes that encode each protein subunit in the channel.[353] In skeletal muscle, disorders are associated with mutations in Na^+, K^+, Ca^{2+}, Cl^-, and nAChR channels. Some of these disorders (myotonia, CMTD, and congenital myasthenic syndromes) have been discussed previously (table 3). For more extensive accounts on ion channels and disease, see Ashcroft.[354]

Table 3. Channels Mutated in Human Neuromuscular Disease (Channelopathies)

Ion Channel Subunit	Disease	Reference No.
Voltage-sensitive	Hyperkalemic periodic paralysis	355
Na^+ channel α	Paramyotonia congenita	311
Voltage-gated Cl^- channel	Myotonia congenita	312
Voltage-gated Ca^{2+} channel	Hypokalemic periodic paralysis	358
Ligand-gated Ca^{2+} channel (RyR1)	Malignant hyperthermia Central core disease	230
nAChR channel	Congenital myasthenic syndromes	114
Connexin	Charcot-Marie-Tooth disease	292

nAChR = nicotinic acetylcholine receptor.

Voltage-sensitive Sodium Channelopathies. Hyperkalemic periodic paralysis is an autosomal dominant disorder characterized by episodes of muscle weakness associated with hyperkalemia. Mutations in the gene encoding the human skeletal muscle Na^+ channel α subunit have been identified in hyperkalemic periodic paralysis.[355] Muscle fibers from affected individuals exhibit sustained Na^+ currents that depolarize the sarcolemma and inactivate normal Na^+ channels. This inactivation disables the formation of action potentials during the attack of paralysis.[144,146,316] Attacks usually begin in the second decade and vary both in frequency and duration. Respiration is rarely affected, and the disorder is considered benign.[146] The attacks of paralysis are frequent, brief, and often precipitated by rest after exertion, stress, the ingestion of foods with high potassium content such as bananas, or the administration of potassium. Prophylactic treatment with potassium-wasting diuretics is often successful in reducing the frequency and severity of attacks by lowering serum potassium.

Anesthetic Considerations. Depletion of potassium before surgery, prevention of carbohydrate depletion, avoidance of potassium-releasing anesthetic drugs, and maintenance of normothermia are the key steps of anesthetic management.[356] Succinylcholine should be avoided because it will result in increases in serum potassium concentrations and can cause myotonic symptoms in these patients. The association between malignant hyperthermia and hyperkalemic periodic paralysis

to the adult skeletal muscle sodium channel gene has been established.[357] There is no evidence that patients with hyperkalemic periodic paralysis exhibit abnormal sensitivity to nondepolarizing neuromuscular relaxants.[356]

Voltage-gated Calcium Channelopathies. Hypokalemic periodic paralysis is an autosomal dominant muscle disease manifested by episodic weakness associated with hypokalemia during attacks. It is thought to also arise from the abnormal function of Ca^{2+} channels.[358] The causative gene was shown to encode the $\alpha 1$ subunit of the dihydropyridine receptor. Although it is the most common form of the periodic paralyzes in humans, it is still a rare disease, with a prevalence of only 1:100,000.[144] The hypokalemia has been attributed to the stimulation of the sodium-potassium pump by insulin. Low potassium concentration may cause electrical destabilization of the cell membrane because the potassium equilibrium becomes very negative, and the potassium conductance approaches zero.[144]

This disorder differs from hyperkalemic periodic paralysis in several additional aspects: the attacks can be very severe in certain patients, women with the same mutation are much less severely affected than men, attacks are often triggered by high carbohydrate intake or insulin challenge, and this condition can lead to a progressive disabling myopathy.[146] Symptomatic treatment of severe attacks entails ingestion of high levels of potassium. Prophylactic treatment with acetazolamide (a carbonic anhydrase inhibitor) is also successful, perhaps by producing metabolic acidosis. The latter decreases the urinary excretion of K^+.[359]

Anesthetic Considerations. Hypothermia, glucose and salt loading, or metabolic alkalosis can precipitate an attack. Therefore, careful intraoperative monitoring of body temperature, glucose, serum electrolytes, and acid-base status is important. Careful and frequent monitoring of plasma potassium concentrations is of greatest importance.

Despite the recommendation that relaxants be avoided, careful titration of short- or intermediate-acting nondepolarizing neuromuscular blockers with adequate monitoring of neuromuscular function is uneventful in patients not suffering from acute episodes of paralysis.[360] In a review of 21 anesthetics administered to members of a family with hypokalemic periodic paralysis, seven patients suffered from mild or severe postoperative paralysis.[361] Hypokalemia should be considered in the differential diagnosis of postoperative residual weakness. A normal response to succinylcholine was noted in these patients,[360] but the association between hypokalemic periodic paralysis and malignant hyperthermia has been reported.[362]

Spinal and epidural anesthesia was reported to be safe alternatives to general anesthesia in these patients.[363] It should be noted, however, that epidural nerve blocks lower serum potassium concentrations.[364] Administration of epinephrine with the local anesthetic accounts for a proportion of this decline, but another unknown mechanism appears to contribute to the reduction in serum potassium in patients not receiving β-adrenergic agonists.[364]

Ligand-gated Ca^{2+} Channelopathies (Ryanodine Receptors). Malignant hyperthermia (MH) is the genetic predisposition that responds to triggering agents such as inhalational anesthetics and depolarizing neuromuscular blockers with abnormalities in intracellular Ca^{2+} homeostasis. These abnormalities, more common in patients with muscle disease, result in tetany, increased metabolism, rhabdomyolysis, hyperkalemia, acidosis, and, if untreated, death.[322,365] Often inherited as an autosomal dominant trait, MH has linkage to 30 different mutations in the type-1 ryanodine receptor (*RyR1*) gene. The *RyR* gene encodes a channel that mediates the release of Ca^{2+} from the sarcoplasmic reticulum membrane to initiate contraction in skeletal muscle. The reverse of this is muscle relaxation, *via* both inactivation (closure) of the channel and ATP-dependent pumping of calcium back into the sarcoplasmic reticulum. *RyR1* mutations with linkage to MH are thought to cause an abnormal opening of the calcium-release channel, when it is exposed to certain anesthetic drugs. Mutations in this gene are considered to account for susceptibility to MH in more than 50% of cases.[230] MH is a heterogeneous disorder and may, in some pedigrees, be caused by mutations in genes on chromosomes other than 19q.[365] Another mutation in the Ca^{2+} channel $\alpha_2\delta$ subunit has also been linked to MH.[366] The molecular diagnosis of this disease is made more complicated because it is variably expressed, and there is incomplete penetrance of the clinical phenotype. Although multiple mutations likely exist, the final common result is abnormal Ca^{2+} homeostasis in response to triggering agents commonly used in anesthesia. For a recent account on MH, see the review by Hopkins.[367]

In North America and Europe, the overall frequency is 1 in 15,000 anesthetics. If adult patients are considered only, the occurrence may be as low as 1 in 50,000 anesthetics. Mortality is more than 60% in untreated patients. Early administration of dantrolene (a lipid-soluble hydantoin analog) is invaluable in the treatment of MH crises, presumably by preventing Ca^{2+} release from the sarcoplasmic reticulum. Although prompt recognition and appropriate treatment have markedly reduced the mortality rate in recent years, MH remains an important contributor to anesthetic-induced morbidity and mortality.

Central core disease (CCD) is also a dominantly inherited neuromuscular condition often associated with a susceptibility to malignant hyperthermia. CCD is linked to mutations in the gene encoding *RyR1* and is thought to arise from "leaky" or "uncoupled" sarcoplasmic reticulum Ca^{2+}-release channels.[368] It has been widely as-

sumed that CCD and a locus for MH may be allelic (*i.e.*, a single genetic defect is responsible for coinheritance of CCD and MH). However, not all individuals with CCD are susceptible to MH.[369] It has also been suggested that all patients with CCD should be tested for MH susceptibility.[369]

Histologic examination of CCD muscles shows the presence of central areas (cores) mainly in type 1 muscle fibers. The core regions consist of unstructured myofibrils and a general lack (or absence) of mitochondria and oxidative enzymatic activity.[370] Electron microscopic analysis of the central cores reveals a disintegration of the contractile apparatus and alterations in the structure and amount of sarcoplasmic reticulum and transverse tubule membranes.[370] Because expression of the CCD phenotype is variable, the CCD diagnosis is based on histologic signs as well as on clinical expression of the disease. The phenotype may include fetal hypotonia (floppy infant syndrome), delayed motor development, and proximal muscle weakness. Exercise-induced muscle cramps are frequently reported. However, because the clinical course of CCD is slow or nonprogressive, many patients are not diagnosed until later in life. A small number of patients may be severely affected. Muscle atrophy is a frequent finding. Musculoskeletal deformities, including kyphoscoliosis, congenital hip dislocation, foot deformities, and joint contractures, are not uncommon. Cardiac abnormalities have rarely been reported in association with CCD.[371]

Anesthetic Considerations. Surgical treatment may be required for some of the musculoskeletal deformities in patients with CCD. All patients with CCD should be considered at risk for MH unless *in vitro* contracture tests show that the particular patient is free of the trait.[369,371]

Perspectives

Considerable information about the molecular mechanisms regulating formation of the NMJ is available, but the complete picture remains fragmentary. The molecular mechanisms by which polyneuronal innervation of perinatal muscle is reduced to a single motor neuron are largely unknown. Other major questions remaining unresolved concern the mechanisms by which agrin activates MuSK, as well as the signaling pathways downstream of MuSK. Neural agrin activation of MuSK requires additional muscle-specific activities,[31,34] none of which are identified. In addition to aggregating AChRs and other components of the subsynaptic apparatus, phosphorylated MuSK also organizes a secondary NRG–ErbB receptor pathway activating the transcription of genes encoding AChR subunits and possibly other synaptic components. These two aspects of MuSK function are apparently mediated by different signaling pathways since agrin-induced AChR gene transcription but not AChR clustering depends on the binding of agrin to a substrate located in the BL.[63] The intracellular signals involved in either of these pathways are still largely unknown. As a first step toward their identification, recent experiments have aimed at mapping the intracellular MuSK domain for the tyrosine residues phosphorylated by and involved in agrin-induced signaling.

Another problem requiring further investigation is whether synapse-specific expression of AChR genes depends on NRG-1 derived from motor neurons, or whether subsynaptic differentiation is controlled by neural agrin alone. The development of conditional gene knockout technology allowing the deletion of NRG-1 selectively in either skeletal muscle or in motor neurons will help to resolve this problem.

Studies of the SCCMS provide a view of ongoing evolution of the nAChR. The impact of gain- or loss-of-function mutations in these syndromes is becoming clearer, but the physiologic role, if any, of subunit changes during development remains a mystery. The fact that developmental subunit changes also occur for other ligand-gated ion channels suggests an influence on brain function. Receptor subunit composition clearly influences the pharmacologic sensitivity of central synapses.

General anesthetics may interact with specific amino acid residues of both muscle and neuronal nAChRs,[372] but the modulatory role of lipids on these drug-receptor interactions is not clear. Understanding of such issues can be expected to lead to the development of drugs having a greater selectivity of action. Advances in research in the neurobiology of the NMJ are impressive. Future investigation will continue to impact on the practice of anesthesiology.

References

1. Sanes JR, Lichtman JW: Development of the vertebrate neuromuscular junction. Annu Rev Neurosci 1999; 22:389–442

2. Duclert A, Changeux JP: Acetylcholine receptor gene expression at the developing neuromuscular junction. Physiol Rev 1995; 75:339–68

3. Riethmacher D, Sonnenberg-Riethmacher E, Brinkmann V, Yamaai T, Lewin GR, Birchmeier C: Severe neuropathies in mice with targeted mutations in the ErbB3 receptor. Nature 1997; 389:725–30

4. Mishina M, Takai T, Imoto K, Noda M, Takahashi T, Numa S, Methfessel C, Sakmann B: Molecular distinction between fetal and adult forms of muscle acetylcholine receptor. Nature 1986; 321:406–11

5. Colquhoun D, Sakmann B: Fast events in single-channel currents activated by acetylcholine and its analogues at the frog muscle end-plate. J Physiol (Lond) 1985; 369:501–57

6. Jaramillo F, Vicini S, Schuetze SM: Embryonic acetylcholine receptors guarantee spontaneous contractions in rat developing muscle. Nature 1988; 335: 66–8

7. Redfern PA: Neuromuscular transmission in new-born rats. J Physiol (Lond) 1970; 209:701–9

8. Salpeter MM, Loring RH: Nicotinic acetylcholine receptors in vertebrate muscle: Properties, distribution and neural control. Prog Neurobiol 1985; 25: 297–325

9. Brenner HR, Witzemann V, Sakmann B: Imprinting of acetylcholine receptor messenger RNA accumulation in mammalian neuromuscular synapses. Nature 1990; 344:544–7

10. Sanes JR, Johnson YR, Kotzbauer PT, Mudd J, Hanley T, Martinou JC,

Merlie JP: Selective expression of an acetylcholine receptor-lacZ transgene in synaptic nuclei of adult muscle fibers. Development 1991; 113:1181–91

11. Villarroel A, Sakmann B: Calcium permeability increase of endplate channels in rat muscle during postnatal development. J Physiol (Lond) 1996; 496: 331–8

12. Witzemann V, Brenner HR, Sakmann B: Neural factors regulate AChR subunit mRNAs at rat neuromuscular synapses. J Cell Biol 1991; 114:125–41

13. Caroni P, Rotzler S, Britt JC, Brenner HR: Calcium influx and protein phosphorylation mediate the metabolic stabilization of synaptic acetylcholine receptors in muscle. J Neurosci 1993; 13:1315–25

14. Balice-Gordon RJ, Lichtman JW: Long-term synapse loss induced by focal blockade of postsynaptic receptors. Nature 1994; 372:519–24

15. Balice-Gordon RJ, Chua CK, Nelson CC, Lichtman JW: Gradual loss of synaptic cartels precedes axon withdrawal at developing neuromuscular junctions. Neuron 1993; 11:801–15

16. Colman H, Nabekura J, Lichtman JW: Alterations in synaptic strength preceding axon withdrawal. Science 1997; 275:356–61

17. Lomo T MR, Pockett S: Formation of neuromuscular junctions in adult rats: Role of postsynaptic impulse activity, Neuromuscular Diseases. Edited by Serratrice G CD, Desnuelle C, Gastaut JL, Pellissier JF, Pouget J, Schiano A. New York, Raven, 1984, pp 393–9

18. Busetto G, Buffelli M, Tognana E, Bellico F, Cangiano A: Hebbian mechanisms revealed by electrical stimulation at developing rat neuromuscular junctions. J Neurosci 2000; 20:685–95

19. Sanes JR, Marshall LM, McMahan UJ: Reinnervation of muscle fiber basal lamina after removal of myofibers: Differentiation of regenerating axons at original synaptic sites. J Cell Biol 1978; 78:176–98

20. Burden SJ, Sargent PB, McMahan UJ: Acetylcholine receptors in regenerating muscle accumulate at original synaptic sites in the absence of the nerve. J Cell Biol 1979; 82:412–25

21. Nitkin RM, Smith MA, Magill C, Fallon JR, Yao YM, Wallace BG, McMahan UJ: Identification of agrin, a synaptic organizing protein from Torpedo electric organ. J Cell Biol 1987; 105:2471–8

22. McMahan UJ: The agrin hypothesis. Cold Spring Harb Symp Quant Biol 1990; 55:407–18

23. Rupp F, Payan DG, Magill-Solc C, Cowan DM, Scheller RH: Structure and expression of a rat agrin. Neuron 1991; 6:811–23

24. Tsim KW, Ruegg MA, Escher G, Kroger S, McMahan UJ: cDNA that encodes active agrin [published erratum appears in Neuron 1992; 9:381]. Neuron 1992; 8:677–89

25. Denzer AJ, Gesemann M, Schumacher B, Ruegg MA: An amino-terminal extension is required for the secretion of chick agrin and its binding to extracellular matrix. J Cell Biol 1995; 131:1547–60

26. Ruegg MA, Tsim KW, Horton SE, Kroger S, Escher G, Gensch EM, McMahan UJ: The agrin gene codes for a family of basal lamina proteins that differ in function and distribution. Neuron 1992; 8:691–9

27. Ferns M, Hoch W, Campanelli JT, Rupp F, Hall ZW, Scheller RH: RNA splicing regulates agrin-mediated acetylcholine receptor clustering activity on cultured myotubes. Neuron 1992; 8:1079–86

28. Gesemann M, Denzer AJ, Ruegg MA: Acetylcholine receptor-aggregating activity of agrin isoforms and mapping of the active site. J Cell Biol 1995; 128:625–36

29. Denzer AJ, Brandenberger R, Gesemann M, Chiquet M, Ruegg MA: Agrin binds to the nerve-muscle basal lamina via laminin. J Cell Biol 1997; 137:671–83

30. Burgess RW, Skarnes WC, Sanes JR: Agrin isoforms with distinct amino termini: Differential expression, localization, and function. J Cell Biol 2000; 151:41–52

31. Glass DJ, Bowen DC, Stitt TN, Radziejewski C, Bruno J, Ryan TE, Gies DR, Shah S, Mattsson K, Burden SJ, DiStefano PS, Valenzuela DM, DeChiara TM, Yancopoulos GD: Agrin acts via a MuSK receptor complex. Cell 1996; 85:513–23

32. Gautam M, Noakes PG, Moscoso L, Rupp F, Scheller RH, Merlie JP, Sanes JR: Defective neuromuscular synaptogenesis in agrin-deficient mutant mice. Cell 1996; 85:525–35

33. DeChiara TM, Bowen DC, Valenzuela DM, Simmons MV, Poueymirou WT, Thomas S, Kinetz E, Compton DL, Rojas E, Park JS, Smith C, DiStefano PS, Glass DJ, Burden SJ, Yancopoulos GD: The receptor tyrosine kinase MuSK is required for neuromuscular junction formation in vivo. Cell 1996; 85:501–12

34. Glass DJ, Apel ED, Shah S, Bowen DC, DeChiara TM, Stitt TN, Sanes JR, Yancopoulos GD: Kinase domain of the muscle-specific receptor tyrosine kinase (MuSK) is sufficient for phosphorylation but not clustering of acetylcholine receptors: Required role for the MuSK ectodomain? Proc Natl Acad Sci U S A 1997; 94:8848–53

35. Froehner SC: The submembrane machinery for nicotinic acetylcholine receptor clustering. J Cell Biol 1991; 114:1–7

36. Gautam M, Noakes PG, Mudd J, Nichol M, Chu GC, Sanes JR, Merlie JP: Failure of postsynaptic specialization to develop at neuromuscular junctions of rapsyn-deficient mice. Nature 1995; 377:232–6

37. Apel ED, Glass DJ, Moscoso LM, Yancopoulos GD, Sanes JR: Rapsyn is required for MuSK signaling and recruits synaptic components to a MuSK-containing scaffold. Neuron 1997; 18:623–35

38. Straub V, Campbell KP: Muscular dystrophies and the dystrophin-glycoprotein complex. Curr Opin Neurol 1997; 10:168–75

39. Patton BL, Miner JH, Chiu AY, Sanes JR: Distribution and function of laminins in the neuromuscular system of developing, adult, and mutant mice. J Cell Biol 1997; 139:1507–21

40. Grady RM, Zhou H, Cunningham JM, Henry MD, Campbell KP, Sanes JR: Maturation and maintenance of the neuromuscular synapse: Genetic evidence for roles of the dystrophin–glycoprotein complex. Neuron 2000; 25:279–93

41. Kim S, Nelson PG: Transcriptional regulation of the prothrombin gene in muscle. J Biol Chem 1998; 273:11923–9

42. Jia M, Li M, Dunlap V, Nelson PG: The thrombin receptor mediates functional activity-dependent neuromuscular synapse reduction via protein kinase C activation in vitro. J Neurobiol 1999; 38:369–81

43. Zoubine MN, Ma JY, Smirnova IV, Citron BA, Festoff BW: A molecular mechanism for synapse elimination: Novel inhibition of locally generated thrombin delays synapse loss in neonatal mouse muscle. Dev Biol 1996; 179:447–57

44. Liu Y, Fields RD, Festoff BW, Nelson PG: Proteolytic action of thrombin is required for electrical activity- dependent synapse reduction. Proc Natl Acad Sci U S A 1994; 91:10300–4

45. Mbebi C, Rohn T, Doyennette MA, Chevessier F, Jandrot-Perrus M, Hantai D, Verdiere-Sahuque M: Thrombin receptor induction by injury-related factors in human skeletal muscle cells. Exp Cell Res 2001; 263:77–87

46. Lanuza MA, Li MX, Jia M, Kim S, Davenport R, Dunlap V, Nelson PG: Protein kinase C-mediated changes in synaptic efficacy at the neuromuscular junction in vitro: The role of postsynaptic acetylcholine receptors. J Neurosci Res 2000; 61:616–25

47. Kim S, Nelson PG: Involvement of calpains in the destabilization of the acetylcholine receptor clusters in rat myotubes. J Neurobiol 2000; 42:22–32

48. Brenner HR, Herczeg A, Slater CR: Synapse-specific expression of acetylcholine receptor genes and their products at original synaptic sites in rat soleus muscle fibres regenerating in the absence of innervation. Development 1992; 116:41–53

49. Jo SA, Burden SJ: Synaptic basal lamina contains a signal for synapse-specific transcription. Development 1992; 115:673–80

50. Fischbach GD, Rosen KM: ARIA: A neuromuscular junction neuregulin. Annu Rev Neurosci 1997; 20:429–58

51. Zhu X, Lai C, Thomas S, Burden SJ: Neuregulin receptors, erbB3 and erbB4, are localized at neuromuscular synapses. Embo J 1995; 14:5842–8

52. Loeb JA, Khurana TS, Robbins JT, Yee AG, Fischbach GD: Expression patterns of transmembrane and released forms of neuregulin during spinal cord and neuromuscular synapse development. Development 1999; 126:781–91

53. Meier T, Masciulli F, Moore C, Schoumacher F, Eppenberger U, Denzer AJ, Jones G, Brenner HR: Agrin can mediate acetylcholine receptor gene expression in muscle by aggregation of muscle-derived neuregulins. J Cell Biol 1998; 141: 715–26

54. Loeb JA, Fischbach GD: ARIA can be released from extracellular matrix through cleavage of a heparin-binding domain. J Cell Biol 1995; 130:127–35

55. Tansey MG, Chu GC, Merlie JP: ARIA/HRG regulates AChR epsilon subunit gene expression at the neuromuscular synapse via activation of phosphatidylinositol 3-kinase and Ras/MAPK pathway. J Cell Biol 1996; 134:465–76

56. Altiok N, Altiok S, Changeux JP: Heregulin-stimulated acetylcholine receptor gene expression in muscle: Requirement for MAP kinase and evidence for a parallel inhibitory pathway independent of electrical activity. Embo J 1997; 16:717–25

57. Koike S, Schaeffer L, Changeux JP: Identification of a DNA element determining synaptic expression of the mouse acetylcholine receptor delta-subunit gene. Proc Natl Acad Sci U S A 1995; 92:10624–8

58. Duclert A, Savatier N, Schaeffer L, Changeux JP: Identification of an element crucial for the sub-synaptic expression of the acetylcholine receptor epsilon-subunit gene. J Biol Chem 1996; 271:17433–8

59. Si J, Miller DS, Mei L: Identification of an element required for acetylcholine receptor-inducing activity (ARIA)-induced expression of the acetylcholine receptor epsilon subunit gene. J Biol Chem 1997; 272:10367–71

60. Schaeffer L, Duclert N, Huchet-Dymanus M, Changeux JP: Implication of a multisubunit Ets-related transcription factor in synaptic expression of the nicotinic acetylcholine receptor. Embo J 1998; 17:3078–90

61. Briguet A, Ruegg MA: The Ets transcription factor GABP is required for postsynaptic differentiation in vivo. J Neurosci 2000; 20:5989–96

62. Corfas G, Fischbach GD: The number of Na+ channels in cultured chick muscle is increased by ARIA, an acetylcholine receptor-inducing activity. J Neurosci 1993; 13:2118–25

63. Jones G, Herczeg A, Ruegg MA, Lichtsteiner M, Kroger S, Brenner HR: Substrate-bound agrin induces expression of acetylcholine receptor epsilon-subunit gene in cultured mammalian muscle cells. Proc Natl Acad Sci U S A 1996; 93:5985–90

64. Jones G, Meier T, Lichtsteiner M, Witzemann V, Sakmann B, Brenner HR: Induction by agrin of ectopic and functional postsynaptic-like membrane in innervated muscle. Proc Natl Acad Sci U S A 1997; 94:2654–9

65. Meier T, Hauser DM, Chiquet M, Landmann L, Ruegg MA, Brenner HR: Neural agrin induces ectopic postsynaptic specializations in innervated muscle fibers. J Neurosci 1997; 17:6534–44

66. Cohen I, Rimer M, Lomo T, McMahan UJ: Agrin-induced postsynaptic-like apparatus in skeletal muscle fibers in vivo [published erratum appears in Mol Cell Neurosci 1997; 10:208]. Mol Cell Neurosci 1997; 9:237–53

67. Rimer M, Cohen I, Lomo T, Burden SJ, McMahan UJ: Neuregulins and erbB

receptors at neuromuscular junctions and at agrin- induced postsynaptic-like apparatus in skeletal muscle. Mol Cell Neurosci 1998; 12:1–15

68. Jones G, Moore C, Hashemolhosseini S, Brenner HR: Constitutively active MuSK is clustered in the absence of agrin and induces ectopic postsynaptic-like membranes in skeletal muscle fibers. J Neurosci 1999; 19:3376–83

69. Dai Z, Peng HB: Presynaptic differentiation induced in cultured neurons by local application of basic fibroblast growth factor. J Neurosci 1995; 15:5466–75

70. Campagna JA, Ruegg MA, Bixby JL: Agrin is a differentiation-inducing "stop signal" for motoneurons in vitro. Neuron 1995; 15:1365–74

71. Campagna JA, Ruegg MA, Bixby JL: Evidence that agrin directly influences presynaptic differentiation at neuromuscular junctions in vitro. Eur J Neurosci 1997; 9:2269–83

72. Burgess RW, Nguyen QT, Son YJ, Lichtman JW, Sanes JR: Alternatively spliced isoforms of nerve- and muscle-derived agrin: Their roles at the neuromuscular junction. Neuron 1999; 23:33–44

73. Patton BL, Chiu AY, Sanes JR: Synaptic laminin prevents glial entry into the synaptic cleft. Nature 1998; 393:698–701

74. Gonzalez M, Ruggiero FP, Chang Q, Shi YJ, Rich MM, Kraner S, Balice-Gordon RJ: Disruption of Trkb-mediated signaling induces disassembly of postsynaptic receptor clusters at neuromuscular junctions. Neuron 1999; 24: 567–83

75. Trachtenberg JT, Thompson WJ: Nerve terminal withdrawal from rat neuromuscular junctions induced by neuregulin and Schwann cells. J Neurosci 1997; 17:6243–55

76. van Mier P, Lichtman JW: Regenerating muscle fibers induce directional sprouting from nearby nerve terminals: Studies in living mice. J Neurosci 1994; 14:5672–86

77. Son YJ, Thompson WJ: Nerve sprouting in muscle is induced and guided by processes extended by Schwann cells. Neuron 1995; 14:133–41

78. Devillers-Thiery A, Giraudat J, Bentaboulet M, Changeux JP: Complete mRNA coding sequence of the acetylcholine binding alpha- subunit of Torpedo marmorata acetylcholine receptor: A model for the transmembrane organization of the polypeptide chain. Proc Natl Acad Sci U S A 1983; 80:2067–71

79. Noda M, Takahashi H, Tanabe T, Toyosato M, Kikyotani S, Furutani Y, Hirose T, Takashima H, Inayama S, Miyata T, Numa S: Structural homology of Torpedo californica acetylcholine receptor subunits. Nature 1983; 302:528–32

80. Takai T, Noda M, Mishina M, Shimizu S, Furutani Y, Kayano T, Ikeda T, Kubo T, Takahashi H, Takahashi T, Kuno M, Numa S: Cloning, sequencing and expression of cDNA for a novel subunit of acetylcholine receptor from calf muscle. Nature 1985; 315:761–4

81. Hucho F, Oberthur W, Lottspeich F: The ion channel of the nicotinic acetylcholine receptor is formed by the homologous helices M II of the receptor subunits. FEBS Lett 1986; 205:137–42

82. Pedersen SE, Cohen JB: d-Tubocurarine binding sites are located at alpha-gamma and alpha-delta subunit interfaces of the nicotinic acetylcholine receptor. Proc Natl Acad Sci U S A 1990; 87:2785–9

83. Sine SM: Identification of equivalent residues in the gamma, delta, and epsilon subunits of the nicotinic receptor that contribute to alpha- bungarotoxin binding. J Biol Chem 1997; 272:23521–7

84. Jackson MB, Imoto K, Mishina M, Konno T, Numa S, Sakmann B: Spontaneous and agonist-induced openings of an acetylcholine receptor channel composed of bovine muscle alpha-, beta- and delta-subunits. Pflugers Arch 1990; 417:129–35

85. Sine SM, Claudio T, Sigworth FJ: Activation of Torpedo acetylcholine receptors expressed in mouse fibroblasts: Single channel current kinetics reveal distinct agonist binding affinities. J Gen Physiol 1990; 96:395–437

86. Grosman C, Zhou M, Auerbach A: Mapping the conformational wave of acetylcholine receptor channel gating. Nature 2000; 403:773–6

87. Grosman C, Salamone FN, Sine SM, Auerbach A: The extracellular linker of muscle acetylcholine receptor channels is a gating control element. J Gen Physiol 2000; 116:327–40

88. Ziskind L, Dennis MJ: Depolarising effect of curare on embryonic rat muscles. Nature 1978; 276:622–3

89. Trautmann A: Curare can open and block ionic channels associated with cholinergic receptors. Nature 1982; 298:272–5

90. Ohno K, Anlar B, Engel AG: Congenital myasthenic syndrome caused by a mutation in the Ets-binding site of the promoter region of the acetylcholine receptor epsilon subunit gene. Neuromuscul Disord 1999; 9:131–5

91. Witzemann V, Schwarz H, Koenen M, Berberich C, Villarroel A, Wernig A, Brenner HR, Sakmann B: Acetylcholine receptor epsilon-subunit deletion causes muscle weakness and atrophy in juvenile and adult mice. Proc Natl Acad Sci U S A 1996; 93:13286–91

92. Missias AC, Mudd J, Cunningham JM, Steinbach JH, Merlie JP, Sanes JR: Deficient development and maintenance of postsynaptic specializations in mutant mice lacking an 'adult' acetylcholine receptor subunit. Development 1997; 124:5075–86

93. Schwarz H, Giese G, Muller H, Koenen M, Witzemann V: Different functions of fetal and adult AChR subtypes for the formation and maintenance of neuromuscular synapses revealed in epsilon-subunit- deficient mice. Eur J Neurosci 2000; 12:3107–16

94. Stroud RM, McCarthy MP, Shuster M: Nicotinic acetylcholine receptor superfamily of ligand-gated ion channels. Biochemistry 1990; 29:11009–23

95. Karlin A, Akabas MH: Toward a structural basis for the function of nicotinic acetylcholine receptors and their cousins. Neuron 1995; 15:1231–44

96. Lobos EA: Five subunit genes of the human muscle nicotinic acetylcholine receptor are mapped to two linkage groups on chromosomes 2 and 17. Genomics 1993; 17:642–50

97. Kues WA, Sakmann B, Witzemann V: Differential expression patterns of five acetylcholine receptor subunit genes in rat muscle during development. Eur J Neurosci 1995; 7:1376–85

98. Witzemann V, Barg B, Criado M, Stein E, Sakmann B: Developmental regulation of five subunit specific mRNAs encoding acetylcholine receptor subtypes in rat muscle. FEBS Lett 1989; 242:419–24

99. Altiok N, Bessereau JL, Changeux JP: ErbB3 and ErbB2/neu mediate the effect of heregulin on acetylcholine receptor gene expression in muscle: Differential expression at the endplate. Embo J 1995; 14:4258–66

100. Moscoso LM, Chu GC, Gautam M, Noakes PG, Merlie JP, Sanes JR: Synapse-associated expression of an acetylcholine receptor-inducing protein, ARIA/heregulin, and its putative receptors, ErbB2 and ErbB3, in developing mammalian muscle. Dev Biol 1995; 172:158–69

101. Vicini S, Schuetze SM: Gating properties of acetylcholine receptors at developing rat endplates. J Neurosci 1985; 5:2212–24

102. Witzemann V, Barg B, Nishikawa Y, Sakmann B, Numa S: Differential regulation of muscle acetylcholine receptor gamma- and epsilon-subunit mRNAs. FEBS Lett 1987; 223:104–12

103. Missias AC, Chu GC, Klocke BJ, Sanes JR, Merlie JP: Maturation of the acetylcholine receptor in skeletal muscle: Regulation of the AChR gamma-to-epsilon switch. Dev Biol 1996; 179:223–38

104. Bouzat C, Bren N, Sine SM: Structural basis of the different gating kinetics of fetal and adult acetylcholine receptors. Neuron 1994; 13:1395–402

105. Decker ER, Dani JA: Calcium permeability of the nicotinic acetylcholine receptor: The single-channel calcium influx is significant. J Neurosci 1990; 10:3413–20

106. Cash S, Dan Y, Poo MM, Zucker R: Postsynaptic elevation of calcium induces persistent depression of developing neuromuscular synapses. Neuron 1996; 16:745–54

107. Leonard JP, Salpeter MM: Agonist-induced myopathy at the neuromuscular junction is mediated by calcium. J Cell Biol 1979; 82:811–9

108. Choi DW: Glutamate neurotoxicity in cortical cell culture is calcium dependent. Neurosci Lett 1985; 58:293–7

109. Leonard JP, Salpeter MM: Calcium-mediated myopathy at neuromuscular junctions of normal and dystrophic muscle. Exp Neurol 1982; 76:121–38

110. Ebihara T, Saffen D: Muscarinic acetylcholine receptor-mediated induction of zif268 mRNA in PC12D cells requires protein kinase C and the influx of extracellular calcium. J Neurochem 1997; 68:1001–10

111. Dykens JA: Isolated cerebral and cerebellar mitochondria produce free radicals when exposed to elevated CA2+ and Na+: Implications for neurodegeneration. J Neurochem 1994; 63:584–91

112. Engel AG, Ohno K, Milone M, Wang HL, Nakano S, Bouzat C, Pruitt JN 2nd, Hutchinson DO, Brengman JM, Bren N, Sieb JP, Sine SM: New mutations in acetylcholine receptor subunit genes reveal heterogeneity in the slow-channel congenital myasthenic syndrome. Hum Mol Genet 1996; 5:1217–27

113. Ohno K, Wang HL, Milone M, Bren N, Brengman JM, Nakano S, Quiram P, Pruitt JN, Sine SM, Engel AG: Congenital myasthenic syndrome caused by decreased agonist binding affinity due to a mutation in the acetylcholine receptor epsilon subunit. Neuron 1996; 17:157–70

114. Sine SM, Ohno K, Bouzat C, Auerbach A, Milone M, Pruitt JN, Engel AG: Mutation of the acetylcholine receptor alpha subunit causes a slow- channel myasthenic syndrome by enhancing agonist binding affinity. Neuron 1995; 15: 229–39

115. Fukudome T, Ohno K, Brengman JM, Engel AG: Quinidine normalizes the open duration of slow-channel mutants of the acetylcholine receptor. Neuroreport 1998; 9:1907–11

116. Milone M, Wang HL, Ohno K, Prince R, Fukudome T, Shen XM, Brengman JM, Griggs RC, Sine SM, Engel AG: Mode switching kinetics produced by a naturally occurring mutation in the cytoplasmic loop of the human acetylcholine receptor epsilon subunit. Neuron 1998; 20:575–88

117. Guyon T, Wakkach A, Poea S, Mouly V, Klingel-Schmitt I, Levasseur P, Beeson D, Asher O, Tzartos S, Berrih-Aknin S: Regulation of acetylcholine receptor gene expression in human myasthenia gravis muscles: Evidences for a compensatory mechanism triggered by receptor loss. J Clin Invest 1998; 102:249–63

118. Zhou M, Engel AG, Auerbach A: Serum choline activates mutant acetylcholine receptors that cause slow channel congenital myasthenic syndromes. Proc Natl Acad Sci U S A 1999; 96:10466–71

119. Jeyarasasingam G, Yeluashvili M, Quik M: Nitric oxide is involved in acetylcholinesterase inhibitor-induced myopathy in rats. J Pharmacol Exp Ther 2000; 295:314–20

120. Heidmann T, Sobel A, Popot JL, Changeux JP: Reconstitution of a functional acetylcholine receptor: Conservation of the conformational and allosteric transitions and recovery of the permeability response; role of lipids. Eur J Biochem 1980; 110:35–55

121. Barrantes FJ: Structural-functional correlates of the nicotinic acetylcholine receptor and its lipid microenvironment. Faseb J 1993; 7:1460–7

122. Jones OT, McNamee MG: Annular and nonannular binding sites for

cholesterol associated with the nicotinic acetylcholine receptor. Biochemistry 1988; 27:2364–74

123. Dalziel AW, Rollins ES, McNamee MG: The effect of cholesterol on agonist-induced flux in reconstituted acetylcholine receptor vesicles. FEBS Lett 1980; 122:193–6

124. Gonzalez-Ros JM, Llanillo M, Paraschos A, Martinez-Carrion M: Lipid environment of acetylcholine receptor from Torpedo californica. Biochemistry 1982; 21:3467–74

125. Rankin SE, Addona GH, Kloczewiak MA, Bugge B, Miller KW: The cholesterol dependence of activation and fast desensitization of the nicotinic acetylcholine receptor. Biophys J 1997; 73:2446–55

126. Roccamo AM, Pediconi MF, Aztiria E, Zanello L, Wolstenholme A, Barrantes FJ: Cells defective in sphingolipids biosynthesis express low amounts of muscle nicotinic acetylcholine receptor. Eur J Neurosci 1999; 11:1615–23

127. D'Alonzo AJ, McArdle JJ: Effects of 20,25-diazacholesterol treatment on the decay of end-plate currents. Exp Neurol 1982; 76:681–3

128. McArdle JJ: Overview of the physiology of the neuromuscular junction., The Neuromuscular Junction. Edited by Brumback RA GJ. New York, Futura Publishing Company, 1984, pp 65–111

129. Sunshine C, McNamee MG: Lipid modulation of nicotinic acetylcholine receptor function: The role of neutral and negatively charged lipids. Biochim Biophys Acta 1992; 1108:240–6

130. Antollini SS, Barrantes FJ: Disclosure of discrete sites for phospholipid and sterols at the protein-lipid interface in native acetylcholine receptor-rich membrane. Biochemistry 1998; 37:16653–62

131. Corbin J, Wang HH, Blanton MP: Identifying the cholesterol binding domain in the nicotinic acetylcholine receptor with [125I]azido-cholesterol. Biochim Biophys Acta 1998; 1414:65–74

132. Blanton MP, Xie Y, Dangott LJ, Cohen JB: The steroid promegestone is a noncompetitive antagonist of the Torpedo nicotinic acetylcholine receptor that interacts with the lipid-protein interface. Mol Pharmacol 1999; 55:269–78

133. Massol RH, Antollini SS, Barrantes FJ: Effect of organochlorine insecticides on nicotinic acetylcholine receptor-rich membranes. Neuropharmacology 2000; 39:1095–106

134. Ortiz-Miranda SI, Lasalde JA, Pappone PA, McNamee MG: Mutations in the M4 domain of the Torpedo californica nicotinic acetylcholine receptor alter channel opening and closing. J Membr Biol 1997; 158:17–30

135. Kilian PL, Dunlap CR, Mueller P, Schell MA, Huganir RL, Racker E: Reconstitution of acetylcholine receptor from Torpedo Californica with highly purified phospholipids: effect of alpha-tocopherol, phylloquinone, and other terpenoid quinones. Biochem Biophys Res Commun 1980; 93:409–14

136. Parsons SM, Bahr BA, Gracz LM, Kaufman R, Kornreich WD, Nilsson L, Rogers GA: Acetylcholine transport: Fundamental properties and effects of pharmacologic agents. Ann N Y Acad Sci 1987; 493:220–33

137. Smith DO: Sources of adenosine released during neuromuscular transmission in the rat. J Physiol (Lond) 1991; 432:343–54

138. Volknandt W, Zimmermann H: Acetylcholine, ATP, and proteoglycan are common to synaptic vesicles isolated from the electric organs of electric eel and electric catfish as well as from rat diaphragm. J Neurochem 1986; 47:1449–62

139. Silinsky EM, Hunt JM, Solsona CS, Hirsh JK: Prejunctional adenosine and ATP receptors. Ann N Y Acad Sci 1990; 603:324–33

140. Ribeiro JA, Walker J: The effects of adenosine triphosphate and adenosine diphosphate on transmission at the rat and frog neuromuscular junctions. Br J Pharmacol 1975; 54:213–8

141. Cooper EC, Jan LY: Ion channel genes and human neurological disease: Recent progress, prospects, and challenges. Proc Natl Acad Sci U S A 1999; 96:4759–66

142. Rios E, Pizarro G: Voltage sensor of excitation-contraction coupling in skeletal muscle. Physiol Rev 1991; 71:849–908

143. Franzini-Armstrong C, Protasi F: Ryanodine receptors of striated muscles: A complex channel capable of multiple interactions. Physiol Rev 1997; 77:699–729

144. Lehmann-Horn F, Jurkat-Rott K: Voltage-gated ion channels and hereditary disease. Physiol Rev 1999; 79:1317–72

145. Stamler JS, Meissner G: Physiology of nitric oxide in skeletal muscle. Physiol Rev 2001; 81:209–37

146. Hoffman EP: Voltage-gated ion channelopathies: Inherited disorders caused by abnormal sodium, chloride, and calcium regulation in skeletal muscle. Annu Rev Med 1995; 46:431–41

147. Vyskocil F, Nikolsky EE, Zemkova H, Krusek J: The role of non-quantal release of acetylcholine in regulation of postsynaptic membrane electrogenesis. J Physiol Paris 1995; 89:157–62

148. Rosahl TW, Spillane D, Missler M, Herz J, Selig DK, Wolff JR, Hammer RE, Malenka RC, Sudhof TC: Essential functions of synapsins I and II in synaptic vesicle regulation. Nature 1995; 375:488–93

149. Augustine GJ, Burns ME, DeBello WM, Hilfiker S, Morgan JR, Schweizer FE, Tokumaru H, Umayahara K: Proteins involved in synaptic vesicle trafficking. J Physiol (Lond) 1999; 520:33–41

150. Rosahl TW, Geppert M, Spillane D, Herz J, Hammer RE, Malenka RC, Sudhof TC: Short-term synaptic plasticity is altered in mice lacking synapsin I. Cell 1993; 75:661–70

151. Sudhof TC: The synaptic vesicle cycle: A cascade of protein-protein interactions. Nature 1995; 375:645–53

152. Mundigl O, De Camilli P: Formation of synaptic vesicles. Curr Opin Cell Biol 1994; 6:561–7

153. Llinas R, Gruner JA, Sugimori M, McGuinness TL, Greengard P: Regulation by synapsin I and Ca(2+)-calmodulin-dependent protein kinase II of the transmitter release in squid giant synapse. J Physiol (Lond) 1991; 436:257–82

154. Robitaille R, Adler EM, Charlton MP: Strategic location of calcium channels at transmitter release sites of frog neuromuscular synapses. Neuron 1990; 5:773–9

155. Geppert M, Goda Y, Hammer RE, Li C, Rosahl TW, Stevens CF, Sudhof TC: Synaptotagmin I: A major Ca2+ sensor for transmitter release at a central synapse. Cell 1994; 79:717–27

156. Seagar M, Takahashi M: Interactions between presynaptic calcium channels and proteins implicated in synaptic vesicle trafficking and exocytosis. J Bioenerg Biomembr 1998; 30:347–56

157. Reist NE, Buchanan J, Li J, DiAntonio A, Buxton EM, Schwarz TL: Morphologically docked synaptic vesicles are reduced in synaptotagmin mutants of Drosophila. J Neurosci 1998; 18:7662–73

158. Sollner T, Whiteheart SW, Brunner M, Erdjument-Bromage H, Geromanos S, Tempst P, Rothman JE: SNAP receptors implicated in vesicle targeting and fusion. Nature 1993; 362:318–24

159. Schiavo G, Matteoli M, Montecucco C: Neurotoxins affecting neuroexocytosis. Physiol Rev 2000; 80:717–66

160. Bennett MK, Calakos N, Scheller RH: Syntaxin: A synaptic protein implicated in docking of synaptic vesicles at presynaptic active zones. Science 1992; 257:255–9

161. Hata Y, Davletov B, Petrenko AG, Jahn R, Sudhof TC: Interaction of synaptotagmin with the cytoplasmic domains of neurexins. Neuron 1993; 10:307–15

162. Johnson EA: Clostridial toxins as therapeutic agents: benefits of nature's most toxic proteins. Annu Rev Microbiol 1999; 53:551–75

163. Leveque C, Hoshino T, David P, Shoji-Kasai Y, Leys K, Omori A, Lang B, el Far O, Sato K, Martin-Moutot N, Newsonm-Davis J, Takahashi M, Seagar MJ: The synaptic vesicle protein synaptotagmin associates with calcium channels and is a putative Lambert-Eaton myasthenic syndrome antigen. Proc Natl Acad Sci U S A 1992; 89:3625–9

164. Geppert M, Bolshakov VY, Siegelbaum SA, Takei K, De Camilli P, Hammer RE, Sudhof TC: The role of Rab3A in neurotransmitter release. Nature 1994; 369:493–7

165. Smith SJ, Augustine GJ: Calcium ions, active zones and synaptic transmitter release. Trends Neurosci 1988; 11:458–64

166. Hilfiker S, Greengard P, Augustine GJ: Coupling calcium to SNARE-mediated synaptic vesicle fusion. Nat Neurosci 1999; 2:104–6

167. Heuser JE, Reese TS: Evidence for recycling of synaptic vesicle membrane during transmitter release at the frog neuromuscular junction. J Cell Biol 1973; 57:315–44

168. Marsh M, McMahon HT: The structural era of endocytosis. Science 1999; 285:215–20

169. Betz WJ, Wu LG: Synaptic transmission: Kinetics of synaptic-vesicle recycling. Curr Biol 1995; 5:1098–101

170. Fesce R, Valtorta F, Meldolesi J: The membrane fusion machine and neurotransmitter release. Neurochem Int 1996; 28:15–21

171. Valtorta F, Meldolesi J, Fesce R: Synaptic vesicles: Is kissing a matter of competence? Trends Cell Biol 2001; 11:324–8

172. Van der Kloot W, Colasante C, Cameron R, Molgo J: Recycling and refilling of transmitter quanta at the frog neuromuscular junction. J Physiol (Lond) 2000; 523:247–58

173. Betz WJ, Bewick GS: Optical analysis of synaptic vesicle recycling at the frog neuromuscular junction. Science 1992; 255:200–3

174. Rosenberry TL: Acetylcholinesterase. Adv Enzymol Relat Areas Mol Biol 1975; 43:103–218

175. Zhou HX, Wlodek ST, McCammon JA: Conformation gating as a mechanism for enzyme specificity. Proc Natl Acad Sci U S A 1998; 95:9280–3

176. Cresnar B, Crne-Finderle N, Breskvar K, Sketelj J: Neural regulation of muscle acetylcholinesterase is exerted on the level of its mRNA. J Neurosci Res 1994; 38:294–9

177. Ehrlich G, Viegas-Pequignot E, Ginzberg D, Sindel L, Soreq H, Zakut H: Mapping the human acetylcholinesterase gene to chromosome 7q22 by fluorescent in situ hybridization coupled with selective PCR amplification from a somatic hybrid cell panel and chromosome-sorted DNA libraries. Genomics 1992; 13:1192–7

178. McMahan UJ, Sanes JR, Marshall LM: Cholinesterase is associated with the basal lamina at the neuromuscular junction. Nature 1978; 271:172–4

179. Hall ZW, Sanes JR: Synaptic structure and development: The neuromuscular junction. Cell 1993; 72(suppl):99–121

180. Rossi SG, Vazquez AE, Rotundo RL: Local control of acetylcholinesterase gene expression in multinucleated skeletal muscle fibers: Individual nuclei respond to signals from the overlying plasma membrane. J Neurosci 2000; 20:919–28

181. Fernandez-Valle C, Rotundo RL: Regulation of acetylcholinesterase synthesis and assembly by muscle activity: Effects of tetrodotoxin. J Biol Chem 1989; 264:14043–9

182. De La Porte S, Vigny M, Massoulie J, Koenig J: Action of veratridine on acetylcholinesterase in cultures of rat muscle cells. Dev Biol 1984; 106:450–6

183. Lomo T, Slater CR: Control of junctional acetylcholinesterase by neural and muscular influences in the rat. J Physiol (Lond) 1980; 303:191–202

184. Weinberg CB, Hall ZW: Junctional form of acetylcholinesterase restored at nerve-free endplates. Dev Biol 1979; 68:631–5

185. Sternfeld M, Ming G, Song H, Sela K, Timberg R, Poo M, Soreq H: Acetylcholinesterase enhances neurite growth and synapse development through alternative contributions of its hydrolytic capacity, core protein, and variable C termini. J Neurosci 1998; 18:1240–9

186. Sung JJ, Kim SJ, Lee HB, Chung JM, Choi YM, Cha CI, Suh YH, Lee KW: Anticholinesterase induces nicotinic receptor modulation. Muscle Nerve 1998; 21:1135–44

187. Engel AG, Lambert EH, Gomez MR: A new myasthenic syndrome with end-plate acetylcholinesterase deficiency, small nerve terminals, and reduced acetylcholine release. Ann Neurol 1977; 1:315–30

188. Maselli RA, Soliven BC: Analysis of the organophosphate-induced electromyographic response to repetitive nerve stimulation: Paradoxical response to edrophonium and D- tubocurarine. Muscle Nerve 1991; 14:1182–8

189. Sapolsky RM: The stress of Gulf War syndrome. Nature 1998; 393:308–9

190. Sellin LC, McArdle JJ: Multiple effects of 2,3-butanedione monoxime. Pharmacol Toxicol 1994; 74:305–13

191. Wilson IB, Bergmann F: Studies on cholinesterase. VII. The active surface of acetylcholine esterase derived from effects of pH on inhibitors. J Biol Chem 1950; 185:479–89

192. Bergmann F, Wilson IB, Nachmansohn D: The inhibitory effect of stilbamidine, curare and related compounds and its relationship to the active groups of acetylcholine esterase. Biochim Biophys Acta 1950; 6:217–24

193. Layer PG, Weikert T, Alber R: Cholinesterases regulate neurite growth of chick nerve cells in vitro by means of a non-enzymatic mechanism. Cell Tissue Res 1993; 273:219–26

194. Wilson IB: The interaction of tensilon and neostigmine with acetylcholinesterase. Arch Int Pharmacodyn 1955; 54:204–13

195. Naguib M, Abdulatif M, al-Ghamdi A: Dose-response relationships for edrophonium and neostigmine antagonism of rocuronium bromide (ORG 9426)-induced neuromuscular blockade. ANESTHESIOLOGY 1993; 79:739–45

196. Fiekers JF: Interactions of edrophonium, physostigmine and methanesulfonyl fluoride with the snake end-plate acetylcholine receptor-channel complex. J Pharmacol Exp Ther 1985; 234:539–49

197. Akaike A, Ikeda SR, Brookes N, Pascuzzo GJ, Rickett DL, Albuquerque EX: The nature of the interactions of pyridostigmine with the nicotinic acetylcholine receptor-ionic channel complex. II. Patch clamp studies. Mol Pharmacol 1984; 25:102–12

198. Bross R, Storer T, Bhasin S: Aging and muscle loss. Trends Endocrinol Metab 1999; 10:194–8

199. Proctor DN, Balagopal P, Nair KS: Age-related sarcopenia in humans is associated with reduced synthetic rates of specific muscle proteins. J Nutr 1998; 128:351S-5S

200. Skelton DA, Young A, Greig CA, Malbut KE: Effects of resistance training on strength, power, and selected functional abilities of women aged 75 and older. J Am Geriatr Soc 1995; 43:1081–7

201. Lowe DA, Surek JT, Thomas DD, Thompson LV: Electron paramagnetic resonance reveals age-related myosin structural changes in rat skeletal muscle fibers. Am J Physiol Cell Physiol 2001; 280:C540–7

202. Hunter SK, Thompson MW, Ruell PA, Harmer AR, Thom JM, Gwinn TH, Adams RD: Human skeletal sarcoplasmic reticulum Ca2+ uptake and muscle function with aging and strength training. J Appl Physiol 1999; 86:1858–65

203. Fahim MA, Robbins N: Ultrastructural studies of young and old mouse neuromuscular junctions. J Neurocytol 1982; 11:641–56

204. Robbins N: Compensatory plasticity of aging at the neuromuscular junction. Exp Gerontol 1992; 27:75–81

205. Kelly SS, Robbins N: Progression of age changes in synaptic transmission at mouse neuromuscular junctions. J Physiol (Lond) 1983; 343:375–83

206. Kelly SS, Robbins N: Sustained transmitter output by increased transmitter turnover in limb muscles of old mice. J Neurosci 1986; 6:2900–7

207. Delbono O, O'Rourke KS, Ettinger WH: Excitation-calcium release uncoupling in aged single human skeletal muscle fibers. J Membr Biol 1995; 148:211–22

208. Lindsay RM, Wiegand SJ, Altar CA, DiStefano PS: Neurotrophic factors: from molecule to man. Trends Neurosci 1994; 17:182–90

209. Balice-Gordon RJ: Age-related changes in neuromuscular innervation. Muscle Nerve Suppl 1997; 5:S83–7

210. Klein R, Smeyne RJ, Wurst W, Long LK, Auerbach BA, Joyner AL, Barbacid M: Targeted disruption of the trkB neurotrophin receptor gene results in nervous system lesions and neonatal death. Cell 1993; 75:113–22

211. Gonzalez E, Messi ML, Delbono O: The specific force of single intact extensor digitorum longus and soleus mouse muscle fibers declines with aging. J Membr Biol 2000; 178:175–83

212. Tomlinson BE, Irving D: The numbers of limb motor neurons in the human lumbosacral cord throughout life. J Neurol Sci 1977; 34:213–9

213. Doherty TJ, Brown WF: The estimated numbers and relative sizes of thenar motor units as selected by multiple point stimulation in young and older adults. Muscle Nerve 1993; 16:355–66

214. Oda K: Age changes of motor innervation and acetylcholine receptor distribution on human skeletal muscle fibres. J Neurol Sci 1984; 66:327–38

215. Frolkis VV, Martynenko OA, Zamostyan VP: Aging of the neuromuscular apparatus. Gerontology 1976; 22:244–79

216. Hurley BF: Age, gender, and muscular strength. J Gerontol A Biol Sci Med Sci 1995; 50:41–4

217. Gosselin LE, Johnson BD, Sieck GC: Age-related changes in diaphragm muscle contractile properties and myosin heavy chain isoforms [published erratum appears in Am J Respir Crit Care Med 1994; 150:879]. Am J Respir Crit Care Med 1994; 150:174–8

218. Prakash YS, Sieck GC: Age-related remodeling of neuromuscular junctions on type-identified diaphragm fibers. Muscle Nerve 1998; 21:887–95

219. Sorooshian SS, Stafford MA, Eastwood NB, Boyd AH, Hull CJ, Wright PM: Pharmacokinetics and pharmacodynamics of cisatracurium in young and elderly adult patients. ANESTHESIOLOGY 1996; 84:1083–91

220. Ornstein E, Lien CA, Matteo RS, Ostapkovich ND, Diaz J, Wolf KB: Pharmacodynamics and pharmacokinetics of cisatracurium in geriatric surgical patients. ANESTHESIOLOGY 1996; 84:520–5

221. Maddineni VR, Mirakhur RK, McCoy EP, Sharpe TD: Neuromuscular and haemodynamic effects of mivacurium in elderly and young adult patients. Br J Anaesth 1994; 73:608–12

222. Rupp SM, Castagnoli KP, Fisher DM, Miller RD: Pancuronium and vecuronium pharmacokinetics and pharmacodynamics in younger and elderly adults. ANESTHESIOLOGY 1987; 67:45–9

223. d'Hollander A, Massaux F, Nevelsteen M, Agoston S: Age-dependent dose-response relationship of ORG NC 45 in anaesthetized patients. Br J Anaesth 1982; 54:653–7

224. Lien CA, Matteo RS, Ornstein E, Schwartz AE, Diaz J: Distribution, elimination, and action of vecuronium in the elderly. Anesth Analg 1991; 73:39–42

225. Matteo RS, Ornstein E, Schwartz AE, Ostapkovich N, Stone JG: Pharmacokinetics and pharmacodynamics of rocuronium (Org 9426) in elderly surgical patients. Anesth Analg 1993; 77:1193–7

226. Young WL, Matteo RS, Ornstein E: Duration of action of neostigmine and pyridostigmine in the elderly. Anesth Analg 1988; 67:775–8

227. Stone JG, Matteo RS, Ornstein E, Schwartz AE, Ostapkovich N, Jamdar SC, Diaz J: Aging alters the pharmacokinetics of pyridostigmine. Anesth Analg 1995; 81:773–6

228. McCarthy GJ, Cooper R, Stanley JC, Mirakhur RK: Dose-response relationships for neostigmine antagonism of vecuronium-induced neuromuscular block in adults and the elderly. Br J Anaesth 1992; 69:281–3

229. Matteo RS, Young WL, Ornstein E, Schwartz AE, Silverberg PA, Diaz J: Pharmacokinetics and pharmacodynamics of edrophonium in elderly surgical patients. Anesth Analg 1990; 71:334–9

230. Quane KA, Healy JM, Keating KE, Manning BM, Couch FJ, Palmucci LM, Doriguzzi C, Fagerlund TH, Berg K, Ording H, Bendixen D, Mortier W, Linz J, Muller CR, McCarthy T: Mutations in the ryanodine receptor gene in central core disease and malignant hyperthermia. Nat Genet 1993; 5:51–5

231. Martyn JA, White DA, Gronert GA, Jaffe RS, Ward JM: Up-and-down regulation of skeletal muscle acetylcholine receptors: Effects on neuromuscular blockers. ANESTHESIOLOGY 1992; 76:822–43

232. Schuetze SM, Role LW: Developmental regulation of nicotinic acetylcholine receptors. Annu Rev Neurosci 1987; 10:403–57

233. Einsiedel LJ, Luff AR: Effect of partial denervation on motor units in the ageing rat medial gastrocnemius. J Neurol Sci 1992; 112:178–84

234. Kallen RG, Sheng ZH, Yang J, Chen LQ, Rogart RB, Barchi RL: Primary structure and expression of a sodium channel characteristic of denervated and immature rat skeletal muscle. Neuron 1990; 4:233–42

235. Martyn JA: Basic and clinical pharmacology of the acetylcholine receptor: Implications for the use of neuromuscular relaxants. Keio J Med 1995; 44:1–8

236. Yanez P, Martyn JA: Prolonged d-tubocurarine infusion and/or immobilization cause upregulation of acetylcholine receptors and hyperkalemia to succinylcholine in rats. ANESTHESIOLOGY 1996; 84:384–91

237. Lindstrom JM: Acetylcholine receptors and myasthenia. Muscle Nerve 2000; 23:453–77

238. Hund EF: Neuromuscular complications in the ICU: The spectrum of critical illness-related conditions causing muscular weakness and weaning failure. J Neurol Sci 1996; 136:10–6

239. Brown JC, Charlton JE: A study of sensitivity to curare in myasthenic disorders using a regional technique. J Neurol Neurosurg Psychiatry 1975; 38:27–33

240. Waud BE, Waud DR: The relation between tetanic fade and receptor occlusion in the presence of competitive neuromuscular block. ANESTHESIOLOGY 1971; 35:456–64

241. King VR, Bradbury EJ, McMahon SB, Priestley JV: Changes in truncated trkB and p75 receptor expression in the rat spinal cord following spinal cord hemisection and spinal cord hemisection plus neurotrophin treatment. Exp Neurol 2000; 165:327–41

242. Snow JC, Kripke BJ, Sessions GP, Finck AJ: Cardiovascular collapse following succinylcholine in a paraplegic patient. Paraplegia 1973; 11:199–204

243. Iwatsuki N, Kuroda N, Amaha K, Iwatsuki K: Succinylcholine-induced hyperkalemia in patients with ruptured cerebral aneurysms. ANESTHESIOLOGY 1980; 53:64–7

244. Smith RB, Grenvik A: Cardiac arrest following succinylcholine in patients with central nervous system injuries. ANESTHESIOLOGY 1970; 33:558–60

245. Gronert GA, Theye RA: Pathophysiology of hyperkalemia induced by succinylcholine. ANESTHESIOLOGY 1975; 43:89–99
246. Gronert GA: Succinylcholine hyperkalemia after burns (letter). ANESTHESIOLOGY 1999; 91:320–2
247. Yoo KY, Lee JU, Kim HS: Succinylcholine-induced hyperkalemia in patients with complete spinal cord injuries (abstract). ANESTHESIOLOGY 2000; 93: A1032
248. Hirose M, Kaneki M, Sugita H, Yasuhara S, Martyn JA: Immobilization depresses insulin signaling in skeletal muscle. Am J Physiol Endocrinol Metab 2000; 279:E1235–41
249. Ibebunjo C, Nosek MT, Itani MS, Martyn JA: Mechanisms for the paradoxical resistance to d-tubocurarine during immobilization-induced muscle atrophy. J Pharmacol Exp Ther 1997; 283:443–51
250. Gronert GA: Disuse atrophy with resistance to pancuronium. ANESTHESIOLOGY 1981; 55:547–9
251. Hansen D: Suxamethonium-induced cardiac arrest and death following 5 days of immobilization. Eur J Anaesthesiol 1998; 15:240–1
252. Waud BE, Waud DR: Tubocurarine sensitivity of the diaphragm after limb immobilization. Anesth Analg 1986; 65:493–5
253. Fung DL, White DA, Jones BR, Gronert GA: The onset of disuse-related potassium efflux to succinylcholine. ANESTHESIOLOGY 1991; 75:650–3
254. Lacomis D, Petrella JT, Giuliani MJ: Causes of neuromuscular weakness in the intensive care unit: A study of ninety-two patients. Muscle Nerve 1998; 21:610–7
255. De Letter MA, van Doorn PA, Savelkoul HF, Laman JD, Schmitz PI, Op de Coul AA, Visser LH, Kros JM, Teepen JL, van der Meche FG: Critical illness polyneuropathy and myopathy (CIPNM): Evidence for local immune activation by cytokine-expression in the muscle tissue. J Neuroimmunol 2000; 106:206–13
256. Tsukagoshi H, Morita T, Takahashi K, Kunimoto F, Goto F: Cecal ligation and puncture peritonitis model shows decreased nicotinic acetylcholine receptor numbers in rat muscle: Immunopathologic mechanisms? ANESTHESIOLOGY 1999; 91:448–60
257. Bolton CF, Breuer AC: Critical illness polyneuropathy. Muscle Nerve 1999; 22:419–24
258. Kindler CH, Verotta D, Gray AT, Gropper MA, Yost CS: Additive inhibition of nicotinic acetylcholine receptors by corticosteroids and the neuromuscular blocking drug vecuronium. ANESTHESIOLOGY 2000; 92:821–32
259. Dodson BA, Kelly BJ, Braswell LM, Cohen NH: Changes in acetylcholine receptor number in muscle from critically ill patients receiving muscle relaxants: An investigation of the molecular mechanism of prolonged paralysis. Crit Care Med 1995; 23:815–21
260. Segredo V, Caldwell JE, Matthay MA, Sharma ML, Gruenke LD, Miller RD: Persistent paralysis in critically ill patients after long-term administration of vecuronium. N Engl J Med 1992; 327:524–8
261. Sanders WE Jr, Sanders CC: Toxicity of antibacterial agents: Mechanism of action on mammalian cells. Annu Rev Pharmacol Toxicol 1979; 19:53–83
262. Prielipp RC, Coursin DB, Scuderi PE, Bowton DL, Ford SR, Cardenas VJ Jr, Vender J, Howard D, Casale EJ, Murray MJ: Comparison of the infusion requirements and recovery profiles of vecuronium and cisatracurium 51W89 in intensive care unit patients. Anesth Analg 1995; 81:3–12
263. Oksenberg JR, Barcellos LF: The complex genetic aetiology of multiple sclerosis. J Neurovirol 2000; 6(suppl 2):S10–4
264. Patten BM, Hart A, Lovelace R: Multiple sclerosis associated with defects in neuromuscular transmission. J Neurol Neurosurg Psychiatry 1972; 35:385–94
265. Bader AM, Hunt CO, Datta S, Naulty JS, Ostheimer GW: Anesthesia for the obstetric patient with multiple sclerosis. J Clin Anesth 1988; 1:21–4
266. Siemkowicz E: Multiple sclerosis and surgery. Anaesthesia 1976; 31: 1211–6
267. Warren TM, Datta S, Ostheimer GW: Lumbar epidural anesthesia in a patient with multiple sclerosis. Anesth Analg 1982; 61:1022–3
268. Albuquerque EX, McIsaac RJ: Fast and slow mammalian muscles after denervation. Exp Neurol 1970; 26:183–202
269. McArdle JJ, Michelson L, D'Alonzo AJ: Action potentials in fast- and slow-twitch mammalian muscles during reinnervation and development. J Gen Physiol 1980; 75:655–72
270. Martin LJ, Price AC, Kaiser A, Shaikh AY, Liu Z: Mechanisms for neuronal degeneration in amyotrophic lateral sclerosis and in models of motor neuron death. Int J Mol Med 2000; 5:3–13
271. Robberecht W: Oxidative stress in amyotrophic lateral sclerosis. J Neurol 2000; 247(suppl 1):I1–6
272. Llinas R, Sugimori M, Cherksey BD, Smith RG, Delbono O, Stefani E, Appel S: IgG from amyotrophic lateral sclerosis patients increases current through P-type calcium channels in mammalian cerebellar Purkinje cells and in isolated channel protein in lipid bilayer. Proc Natl Acad Sci U S A 1993; 90: 11743–7
273. Uchitel OD, Appel SH, Crawford F, Sczcupak L: Immunoglobulins from amyotrophic lateral sclerosis patients enhance spontaneous transmitter release from motor-nerve terminals. Proc Natl Acad Sci U S A 1988; 85:7371–4
274. O'Shaughnessy TJ, Yan H, Kim J, Middlekauff EH, Lee KW, Phillips LH, Kim YI: Amyotrophic lateral sclerosis: Serum factors enhance spontaneous and evoked transmitter release at the neuromuscular junction. Muscle Nerve 1998; 21:81–90
275. Uchitel OD, Scornik F, Protti DA, Fumberg CG, Alvarez V, Appel SH: Long-term neuromuscular dysfunction produced by passive transfer of amyotrophic lateral sclerosis immunoglobulins [published erratum appears in Neurology 1993; 43:1053]. Neurology 1992; 42:2175–80
276. Beach TP, Stone WA, Hamelberg W: Circulatory collapse following succinylcholine: Report of a patient with diffuse lower motor neuron disease. Anesth Analg 1971; 50:431–7
277. Kochi T, Oka T, Mizuguchi T: Epidural anesthesia for patients with amyotrophic lateral sclerosis. Anesth Analg 1989; 68:410–2
278. Hughes RA, Hadden RD, Gregson NA, Smith KJ: Pathogenesis of Guillain-Barre syndrome. J Neuroimmunol 1999; 100:74–97
279. Corbo M, Quattrini A, Latov N, Hays AP: Localization of GM1 and Gal(beta 1–3)GalNAc antigenic determinants in peripheral nerve. Neurology 1993; 43:809–14
280. Buchwald B, Toyka KV, Zielasek J, Weishaupt A, Schweiger S, Dudel J: Neuromuscular blockade by IgG antibodies from patients with Guillain-Barre syndrome: A macro-patch-clamp study. Ann Neurol 1998; 44:913–22
281. Buchwald B, Bufler J, Carpo M, Heidenreich F, Pitz R, Dudel J, Nobile-Orazio E, Toyka KV: Combined pre- and postsynaptic action of IgG antibodies in Miller Fisher syndrome. Neurology 2001; 56:67–74
282. Rudnicki S, Vriesendorp F, Koski CL, Mayer RF: Electrophysiologic studies in the Guillain-Barre syndrome: Effects of plasma exchange and antibody rebound. Muscle Nerve 1992; 15:57–62
283. Koski CL: Characterization of complement-fixing antibodies to peripheral nerve myelin in Guillain-Barre syndrome. Ann Neurol 1990; 27:S44–7
284. Dalman JE, Verhagen WI: Cardiac arrest in Guillain-Barre syndrome and the use of suxamethonium. Acta Neurol Belg 1994; 94:259–61
285. Brooks H, Christian AS, May AE: Pregnancy, anaesthesia and Guillain Barre syndrome. Anaesthesia 2000; 55:894–8
286. Feldman JM: Cardiac arrest after succinylcholine administration in a pregnant patient recovered from Guillain-Barre syndrome. ANESTHESIOLOGY 1990; 72:942–4
287. Fiacchino F, Gemma M, Bricchi M, Giudici D, Ciano C: Hypo- and hypersensitivity to vecuronium in a patient with Guillain-Barre syndrome. Anesth Analg 1994; 78:187–9
288. Steiner I, Argov Z, Cahan C, Abramsky O: Guillain-Barre syndrome after epidural anesthesia: Direct nerve root damage may trigger disease. Neurology 1985; 35:1473–5
289. Skre H: Genetic and clinical aspects of Charcot-Marie-Tooth's disease. Clin Genet 1974; 6:98–118
290. Su Y, Brooks DG, Li L, Lepercq J, Trofatter JA, Ravetch JV, Lebo RV: Myelin protein zero gene mutated in Charcot-Marie-tooth type 1B patients. Proc Natl Acad Sci U S A 1993; 90:10856–60
291. Hahn AF, Ainsworth PJ, Naus CC, Mao J, Bolton CF: Clinical and pathological observations in men lacking the gap junction protein connexin 32. Muscle Nerve 2000; 999:S39–48
292. Bergoffen J, Scherer SS, Wang S, Scott MO, Bone LJ, Paul DL, Chen K, Lensch MW, Chance PF, Fischbeck KH: Connexin mutations in X-linked Charcot-Marie-Tooth disease. Science 1993; 262:2039–42
293. Paul DL: New functions for gap junctions. Curr Opin Cell Biol 1995; 7:665–72
294. Dermietzel R: Gap junction wiring: A 'new' principle in cell-to-cell communication in the nervous system? Brain Res Brain Res Rev 1998; 26:176–83
295. Warner LE, Garcia CA, Lupski JR: Hereditary peripheral neuropathies: Clinical forms, genetics, and molecular mechanisms. Annu Rev Med 1999; 50: 263–75
296. Kaku DA, Parry GJ, Malamut R, Lupski JR, Garcia CA: Uniform slowing of conduction velocities in Charcot-Marie-Tooth polyneuropathy type 1. Neurology 1993; 43:2664–7
297. Kotani N, Hirota K, Anzawa N, Takamura K, Sakai T, Matsuki A: Motor and sensory disability has a strong relationship to induction dose of thiopental in patients with the hypertropic variety of Charcot-Marie-Tooth syndrome. Anesth Analg 1996; 82:182–6
298. Brian JE Jr, Boyles GD, Quirk JG Jr, Clark RB: Anesthetic management for cesarean section of a patient with Charcot- Marie-Tooth disease. ANESTHESIOLOGY 1987; 66:410–2
299. Nathanson BN, Yu DG, Chan CK: Respiratory muscle weakness in Charcot-Marie-Tooth disease: A field study. Arch Intern Med 1989; 149:1389–91
300. Naguib M, Samarkandi AH: Response to atracurium and mivacurium in a patient with Charcot-Marie- Tooth disease. Can J Anaesth 1998; 45:56–9
301. Antognini JF: Anaesthesia for Charcot-Marie-Tooth disease: A review of 86 cases. Can J Anaesth 1992; 39:398–400
302. Ducart A, Adnet P, Renaud B, Riou B, Krivosic-Horber R: Malignant hyperthermia during sevoflurane administration. Anesth Analg 1995; 80:609–11
303. Roland EH: Muscular dystrophy. Pediatr Rev 2000; 21:233–7
304. Hoffman EP, Fischbeck KH, Brown RH, Johnson M, Medori R, Loike JD, Harris JB, Waterston R, Brooke M, Specht L, Kupsky W, Chamberlain J, Caskey CT, Shapiro K, Kunkel L: Characterization of dystrophin in muscle-biopsy specimens from patients with Duchenne's or Becker's muscular dystrophy. N Engl J Med 1988; 318:1363–8
305. Koltgen D, Franke C: The coexistence of embryonic and adult acetylcholine receptors in sarcolemma of mdx dystrophic mouse muscle: An effect of regeneration or muscular dystrophy? Neurosci Lett 1994; 173:79–82

306. Blake DJ, Kroger S: The neurobiology of duchenne muscular dystrophy: Learning lessons from muscle? Trends Neurosci 2000; 23:92–9

306A. Moll J, Barzaghi P, Lin S, Bezakova G, Lochmuller H, Engvall E, Muller U, Ruegg MA: An agrin minigene rescues dystrophic symptoms in a mouse model for congenital muscular dystrophy. Nature 2001; 413:302–7

307. Genever EE: Suxamethonium-induced cardiac arrest in unsuspected pseudohypertrophic muscular dystrophy: Case report. Br J Anaesth 1971; 43: 984–6

308. Henderson WA: Succinylcholine-induced cardiac arrest in unsuspected Duchenne muscular dystrophy. Can Anaesth Soc J 1984; 31:444–6

309. Ririe DG, Shapiro F, Sethna NF: The response of patients with Duchenne's muscular dystrophy to neuromuscular blockade with vecuronium. ANESTHESIOLOGY 1998; 88:351–4

310. Ohkoshi N, Yoshizawa T, Mizusawa H, Shoji S, Toyama M, Iida K, Sugishita Y, Hamano K, Takagi A, Goto K, Arahata K: Malignant hyperthermia in a patient with Becker muscular dystrophy: Dystrophin analysis and caffeine contracture study. Neuromuscul Disord 1995; 5:53–8

311. Lerche H, Heine R, Pika U, George AL Jr, Mitrovic N, Browatzki M, Weiss T, Rivet-Bastide M, Franke C, Lomonaco M: Human sodium channel myotonia: Slowed channel inactivation due to substitutions for a glycine within the III-IV linker. J Physiol (Lond) 1993; 470:13–22

312. Steinmeyer K, Lorenz C, Pusch M, Koch MC, Jentsch TJ: Multimeric structure of ClC-1 chloride channel revealed by mutations in dominant myotonia congenita (Thomsen). Embo J 1994; 13:737–43

313. Aslanidis C, Jansen G, Amemiya C, Shutler G, Mahadevan M, Tsilfidis C, Chen C, Alleman J, Wormskamp NG, Vooijs M, Buxton J, Johnson K, Smeets HJM, Lennon GG, Carrano AV, Korneluk RG, Wieringa B, de Jong PJ: Cloning of the essential myotonic dystrophy region and mapping of the putative defect. Nature 1992; 355:548–51

314. Shimokawa M, Ishiura S, Kameda N, Yamamoto M, Sasagawa N, Saitoh N, Sorimachi H, Ueda H, Ohno S, Suzuki K, Kobayashi T: Novel isoform of myotonin protein kinase: Gene product of myotonic dystrophy is localized in the sarcoplasmic reticulum of skeletal muscle. Am J Pathol 1997; 150:1285–95

315. Damiani E, Angelini C, Pelosi M, Sacchetto R, Bortoloso E, Margreth A: Skeletal muscle sarcoplasmic reticulum phenotype in myotonic dystrophy. Neuromuscul Disord 1996; 6:33–47

316. Cannon SC: Sodium channel defects in myotonia and periodic paralysis. Annu Rev Neurosci 1996; 19:141–64

317. Krivickas LS, Ansved T, Suh D, Frontera WR: Contractile properties of single muscle fibers in myotonic dystrophy. Muscle Nerve 2000; 23:529–37

318. Diefenbach C, Lynch J, Abel M, Buzello W: Vecuronium for muscle relaxation in patients with dystrophia myotonica. Anesth Analg 1993; 76:872–4

319. Kaufman L: Dystrophia myotonica and succinylcholine (letter). Anaesthesia 2000; 55:929

320. Buzello W, Krieg N, Schlickewei A: Hazards of neostigmine in patients with neuromuscular disorders: Report of two cases. Br J Anaesth 1982; 54: 529–34

321. Tomlinson S, Macartney I, Lam S: Dystrophica myotonia and suxamethonium (letter). Anaesthesia 1999; 54:1234

322. Heiman-Patterson T, Martino C, Rosenberg H, Fletcher J, Tahmoush A: Malignant hyperthermia in myotonia congenita. Neurology 1988; 38:810–2

323. Lehmann-Horn F, Iaizzo PA: Are myotonias and periodic paralyses associated with susceptibility to malignant hyperthermia? Br J Anaesth 1990; 65: 692–7

324. Anderson BJ, Brown TC: Congenital myotonic dystrophy in children: A review of ten years' experience. Anaesth Intensive Care 1989; 17:320–4

325. Hopkins A, Wray S: The effect of pregnancy on dystrophia myotonica. Neurology 1967; 17:166–8

326. Tzartos SJ, Lindstrom JM: Monoclonal antibodies used to probe acetylcholine receptor structure: Localization of the main immunogenic region and detection of similarities between subunits. Proc Natl Acad Sci U S A 1980; 77:755–9

327. Vernino S, Adamski J, Kryzer TJ, Fealey RD, Lennon VA: Neuronal nicotinic ACh receptor antibody in subacute autonomic neuropathy and cancer-related syndromes. Neurology 1998; 50:1806–13

328. Woolf AL: Morphology of the myasthenic neuromuscular junction. Ann N Y Acad Sci 1966; 135:35–59

329. Infante AJ, Kraig E: Myasthenia gravis and its animal model: T cell receptor expression in an antibody mediated autoimmune disease. Int Rev Immunol 1999; 18:83–109

330. Deitiker P, Ashizawa T, Atassi MZ: Antigen mimicry in autoimmune disease: Can immune responses to microbial antigens that mimic acetylcholine receptor act as initial triggers of Myasthenia gravis? Hum Immunol 2000; 61: 255–65

331. Engel AG, Walls TJ, Nagel A, Uchitel O: Newly recognized congenital myasthenic syndromes: I. Congenital paucity of synaptic vesicles and reduced quantal release. II. High-conductance fast-channel syndrome. III. Abnormal acetylcholine receptor (AChR) interaction with acetylcholine. IV. AChR deficiency and short channel-open time. Prog Brain Res 1990; 84:125–37

332. Takamori M, Maruta T, Komai K: Lambert-Eaton myasthenic syndrome as an autoimmune calcium-channelopathy. Neurosci Res 2000; 36:183–91

333. Hewett SJ, Atchison WD: Serum and plasma from patients with Lambert-Eaton myasthenic syndrome reduce depolarization-dependent uptake of 45Ca2+ into rat cortical synaptosomes. Brain Res 1991; 566:320–4

334. Sanders DB, Massey JM, Sanders LL, Edwards LJ: A randomized trial of 3,4-diaminopyridine in Lambert-Eaton myasthenic syndrome. Neurology 2000; 54:603–7

335. Baraka A: Anaesthesia and myasthenia gravis. Can J Anaesth 1992; 39: 476–86

336. Eisenkraft JB, Book WJ, Mann SM, Papatestas AE, Hubbard M: Resistance to succinylcholine in myasthenia gravis: A dose-response study. ANESTHESIOLOGY 1988; 69:760–3

337. Seigne RD, Scott RP: Mivacurium chloride and myasthenia gravis. Br J Anaesth 1994; 72:468–9

338. Nilsson E, Meretoja OA: Vecuronium dose-response and maintenance requirements in patients with myasthenia gravis. ANESTHESIOLOGY 1990; 73:28–32

339. Engel AG, Ohno K, Bouzat C, Sine SM, Griggs RC: End-plate acetylcholine receptor deficiency due to nonsense mutations in the epsilon subunit. Ann Neurol 1996; 40:810–7

340. Naguib M, el Dawlatly AA, Ashour M, Bamgboye EA: Multivariate determinants of the need for postoperative ventilation in myasthenia gravis. Can J Anaesth 1996; 43:1006–13

341. Small S, Ali HH, Lennon VA, Brown RH, Jr, Carr DB, de Armendi A: Anesthesia for an unsuspected Lambert-Eaton myasthenic syndrome with autoantibodies and occult small cell lung carcinoma. ANESTHESIOLOGY 1992; 76:142–5

342. Heddi A, Lestienne P, Wallace DC, Stepien G: Mitochondrial DNA expression in mitochondrial myopathies and coordinated expression of nuclear genes involved in ATP production. J Biol Chem 1993; 268:12156–63

343. Rifai Z, Welle S, Kamp C, Thornton CA: Ragged red fibers in normal aging and inflammatory myopathy. Ann Neurol 1995; 37:24–9

344. Simon DK, Johns DR: Mitochondrial disorders: Clinical and genetic features. Annu Rev Med 1999; 50:111–27

345. Melov S, Coskun P, Patel M, Tuinstra R, Cottrell B, Jun AS, Zastawny TH, Dizdaroglu M, Goodman SI, Huang TT, Miziorko H, Epstein CJ, Wallace DC: Mitochondrial disease in superoxide dismutase 2 mutant mice. Proc Natl Acad Sci U S A 1999; 96:846–51

346. Dalakas MC Illa I, Pezeshkpour GH, Laukaitis JP, Cohen B, Griffin JL: Mitochondrial myopathy caused by long-term zidovudine therapy. N Engl J Med 1990; 322:1098–105

347. Tang Y, Zucker RS: Mitochondrial involvement in post-tetanic potentiation of synaptic transmission. Neuron 1997; 18:483–91

348. Girlanda P, Toscano A, Nicolosi C, Sinicropi S, Picciolo G, Macaione V, Quartarone A, Messina C: Electrophysiological study of neuromuscular system involvement in mitochondrial cytopathy. Clin Neurophysiol 1999; 110:1284–9

349. D'Ambra MN, Dedrick D, Savarese JJ: Kearns-Sayer syndrome and pancuronium–succinylcholine-induced neuromuscular blockade. ANESTHESIOLOGY 1979; 51:343–5

350. Naguib M, el Dawlatly AA, Ashour M, al-Bunyan M: Sensitivity to mivacurium in a patient with mitochondrial myopathy. ANESTHESIOLOGY 1996; 84: 1506–9

351. Lessell S, Kuwabara T, Feldman RG: Myopathy and succinylcholine sensitivity. Am J Ophthalmol 1969; 68:789–96

352. Etcharry-Bouyx F, Sangla I, Serratrice G: [Chronic rhabdomyolysis disclosing mitochondriopathy and malignant hyperthermia susceptibility]. Rev Neurol (Paris) 1995; 151:589–92

353. Mody I: Ion channels in epilepsy. Int Rev Neurobiol 1998; 42:199–226

354. Ashcroft FM: Ion Channels and Disease. London, Academic Press, 2000

355. Fontaine B, Khurana TS, Hoffman EP, Bruns GA, Haines JL, Trofatter JA, Hanson MP, Rich J, McFarlane H, Yasek DM, Romano D, Gusella JF, Brown RH Jr: Hyperkalemic periodic paralysis and the adult muscle sodium channel alpha-subunit gene. Science 1990; 250:1000–2

356. Ashwood EM, Russell WJ, Burrow DD: Hyperkalaemic periodic paralysis and anaesthesia. Anaesthesia 1992; 47:579–84

357. Moslehi R, Langlois S, Yam I, Friedman JM: Linkage of malignant hyperthermia and hyperkalemic periodic paralysis to the adult skeletal muscle sodium channel (SCN4A) gene in a large pedigree. Am J Med Genet 1998; 76:21–7

358. Fontaine B, Vale-Santos J, Jurkat-Rott K, Reboul J, Plassart E, Rime CS, Elbaz A, Heine R, Guimaraes J, Weissenbach J, Baumann M, Fardeau M, Lehmann-Horn F: Mapping of the hypokalaemic periodic paralysis (HypoPP) locus to chromosome 1q31–32 in three European families. Nat Genet 1994; 6:267–72

359. Lehmann-Horn F, Kuther G, Ricker K, Grafe P, Ballanyi K, Rudel R: Adynamia episodica hereditaria with myotonia: A non-inactivating sodium current and the effect of extracellular pH. Muscle Nerve 1987; 10:363–74

360. Siler JN, Discavage WJ: Anesthetic management of hypokalemic periodic paralysis. ANESTHESIOLOGY 1975; 43:489–90

361. Horton B: Anesthetic experiences in a family with hypokalemic familial periodic paralysis. ANESTHESIOLOGY 1977; 47:308–10

362. Lambert C, Blanloeil Y, Horber RK, Berard L, Reyford H, Pinaud M: Malignant hyperthermia in a patient with hypokalemic periodic paralysis. Anesth Analg 1994; 79:1012–4

363. Viscomi CM, Ptacek LJ, Dudley D: Anesthetic management of familial hypokalemic periodic paralysis during parturition. Anesth Analg 1999; 88:1081–2

364. Lofgren A, Hahn RG: Serum potassium levels after induction of epidural anaesthesia using mepivacaine with and without adrenaline. Acta Anaesthesiol Scand 1991; 35:170–4

365. MacLennan DH: The genetic basis of malignant hyperthermia. Trends Pharmacol Sci 1992; 13:330–4

366. Iles DE, Lehmann-Horn F, Scherer SW, Tsui LC, Olde Weghuis D, Suijkerbuijk RF, Heytens L, Mikala G, Schwartz A, Ellis FR: Localization of the gene encoding the alpha 2/delta-subunits of the L- type voltage-dependent calcium channel to chromosome 7q and analysis of the segregation of flanking markers in malignant hyperthermia susceptible families. Hum Mol Genet 1994; 3:969–75

367. Hopkins PM: Malignant hyperthermia: Advances in clinical management and diagnosis. Br J Anaesth 2000; 85:118–28

368. Avila G, O'Brien JJ, Dirksen RT: Excitation–contraction uncoupling by a human central core disease mutation in the ryanodine receptor. Proc Natl Acad Sci U S A 2001; 98:4215–20

369. Curran JL, Hall WJ, Halsall PJ, Hopkins PM, Iles DE, Markham AF, McCall SH, Robinson RL, West SP, Bridges LR, Ellis FR: Segregation of malignant hyperthermia, central core disease and chromosome 19 markers. Br J Anaesth 1999; 83:217–22

370. Hayashi K, Miller RG, Brownell AK: Central core disease: Ultrastructure of the sarcoplasmic reticulum and T-tubules. Muscle Nerve 1989; 12:95–102

371. Shuaib A, Paasuke RT, Brownell KW: Central core disease: Clinical features in 13 patients. Medicine (Baltimore) 1987; 66:389–96

372. Cardoso RA, Yamakura T, Brozowski SJ, Chavez-Noriega LE, Harris RA: Human neuronal nicotinic acetylcholine receptors expressed in Xenopus oocytes predict efficacy of halogenated compounds that disobey the Meyer-Overton rule. ANESTHESIOLOGY 1999; 91:1370–7

373. Culligan KG, Mackey AJ, Finn DM, Maguire PB, Ohlendieck K: Role of dystrophin isoforms and associated proteins in muscular dystrophy. Int J Mol Med 1998; 2:639–48

374. Moreira ES, Wiltshire TJ, Faulkner G, Nilforoushan A, Vainzof M, Suzuki OT, Valle G, Reeves R, Zatz M, Passos-Bueno MR, Jenne DE: Limb-girdle muscular dystrophy type 2G is caused by mutations in the gene encoding the sarcomeric protein telethonin. Nat Genet 2000; 24:163–6

375. Tsao CY, Mendell JR: The childhood muscular dystrophies: Making order out of chaos. Semin Neurol 1999; 19:9–23

376. Naom I, D'Alessandro M, Sewry CA, Jardine P, Ferlini A, Moss T, Dubowitz V, Muntoni F: Mutations in the laminin alpha2-chain gene in two children with early-onset muscular dystrophy. Brain 2000; 123:31–41

377. Saito Y, Mizuguchi M, Oka A, Takashima S: Fukutin protein is expressed in neurons of the normal developing human brain but is reduced in Fukuyama-type congenital muscular dystrophy brain. Ann Neurol 2000; 47:756–64

378. Orrell RW, Tawil R, Forrester J, Kissel JT, Mendell JR, Figlewicz DA: Definitive molecular diagnosis of facioscapulohumeral dystrophy. Neurology 1999; 52:1822–6

379. Sanes JR: Genetic analysis of postsynaptic differentiation at the vertebrate neuromuscular junction. Curr Opin Neurobiol 1997; 7:93–100