Up-and-down Regulation of Skeletal Muscle Acetylcholine Receptors

Effects on Neuromuscular Blockers

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Key Words: Acetylcholine receptor; agonists; antagonists; bungarotoxin binding; down-regulation; up-regulation. Cholinesterase inhibitors: irreversible; reversible. Complications: denervation; immobilization; infection; myasthenia gravis; neuronal injury; organophosphorus poisoning. Injury: burns; muscle; neuronal. Neuromuscular relaxants: drug interaction; hyperkalemia; nondepolarizing; pharmacokinetics; pharmacodynamics; resistance; sensitivity; succinylcholine.
By the middle of this century, neuromuscular blockers had been introduced to clinical practice, as both depolarizing (decamethonium and succinylcholine [SCh]) and competitive or nondepolarizing muscle relaxants (NDMR). Shortly thereafter, it became apparent that certain pathologic states were associated with both hypersensitivity and hyposensitivity (resistance) to these drugs.1–4 Critically ill patients receiving multiple doses of analgesics and sedative drugs were seen to be resistant to the neuromuscular effects of NDMR, whereas myasthenic patients were noted to be extremely sensitive to the same drugs.1–4 Later, the puzzling problem of cardiac arrest following the use of SCh surfaced.5,6 New and sensitive techniques for assay of drugs and receptors, together with the use of sophisticated computer and electrophysiologic techniques examined and reexamined some of these phenomena and have increased our understanding of the mechanisms of these aberrant responses. These hypersensitivity and hyposensitivity responses can now be explained, at least in part, in terms of a cohesive theory of receptor–drug interaction featuring up-and-down regulation of skeletal muscle acetylcholine receptors (AChRs). This report focuses on these pathologic states and processes that induce quantitative and qualitative changes in AChRs at and around the neuromuscular junction (NMJ) and the mechanism whereby these receptor changes may play a role in the aberrant responses to neuromuscular blockers.

The classical theory regarding antagonist- or agonist-induced up-regulation (increased numbers) and down-regulation (decreased numbers) of receptors7–11 is used to explain the abnormal responses observed with muscle relaxants. The term “up-and-down regulation” generally refers to changes in availability or reactivity of receptors, but these changes usually do not involve or imply a change in amino acid composition. In the muscle membrane, however, there is a potential for two molecular species of AChRs to exist concomitantly, particularly during up-regulation (see sections II and III). These differences, nonetheless, do not appear to prevent the application of these classical dogmas to the responses observed with competitive antagonists (e.g., NDMR) and agonists (e.g., SCh) of AChR. (Although both SCh and decamethonium produce neuromuscular blockade, they are, like acetylcholine [ACh], AChR agonists because they initially stimulate the receptor.12,13) The receptor theory proposes that in conditions where there is a proliferation of AChRs, there will be increased sensitivity to agonists and decreased sensitivity to antagonists. The increased sensitivity to agonists in the extreme form results in a lethal hyperkalemic response to SCh5,6 (see section III.B). Clinical conditions in which the neuromuscular responses simulate an increased receptor number include denervation,5,6,14–16 di- suse muscle atrophy,17–19 thermal and direct muscle trauma,20–23 infection,24 and chronic treatment with antagonists of neuromuscular transmission.25–28 Typically, down-regulation of a receptor occurs during continuous agonist stimulation of that receptor; the surface receptors are decreased because of their internalization.7 Down-regulation is associated with decreased sensitivity to agonists (e.g., ACh and SCh) and increased sensitivity to antagonists (e.g., d-tubocurarine [dTC]). In the NMJ, although pharmacologic responses resembling a down-regulated AChR are seen in myasthenia gravis, conditioning exercise, and chronic cholinesterase inhibition (table 1), the pathophysiologic mechanisms of the decrease in AChR are different (see section VI).29–31

Although target organ or pharmacodynamic changes may play a principal etiologic role, pharmacokinetic and pharmacogenetic components also may contribute to these variations in drug response (table 2). The proposed arguments do not imply that the AChR changes are the only factors, but they emphasize what appears to be the principal mechanism. Factors other than AChR changes that may contribute to pharmacodynamic alterations include cholinesterase activity and terminal nerve sprouting (e.g., denervation or disuse) or pre- and postjunctional phenomena.15,32–36 In this review, the principally AChR-mediated altered responses to muscle relaxants and contributions by pharmacokinetic or pharmacogenetic factors are discussed, where relevant. Altered sensitivity to muscle relaxants due to prejunctional effects (e.g., antibiotics, magnesium, or calcium) may affect the responses of AChR.

### Table 1. Conditions Associated with Up-and-down Regulation of Acetylcholine Receptors

<table>
<thead>
<tr>
<th>Resistance to NDMR, Hyperkalemia to Agonist</th>
<th>Increased AChR</th>
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<tbody>
<tr>
<td>Any neurologic motor defect (see section IVa)</td>
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<td>Direct muscle trauma (see section IVa)</td>
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<td>Diuse atrophy (see section IVc)</td>
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<td>ICU—prolonged use of relaxants (see section IVd)</td>
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<td>Severe infection (see section IVf)</td>
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<tr>
<th>Resistance to NDMR; no hyperkalemia; AChR</th>
<th>Decreased AChR</th>
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<td>Cerebral palsy (see section IVa)</td>
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<td>Myelomeningocele (see section IVb)</td>
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<td>Chronic anticonvulsants (see section IVe)</td>
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<th>Sensitivity to NDMR, Resistance to Agonist</th>
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<td>ICU = intensive care unit; AChR = acetylcholine receptors.</td>
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tor events (e.g., second messengers) that are also pharmacodynamic are not discussed.

I. Physiology of Neuromuscular Transmission

To understand the pathophysiologic responses that occur at the NMJ, some knowledge of the basic events of neuromuscular transmission is necessary. In the majority of mammalian muscles, each muscle fiber has a single region of contact with the axon of its controlling motor neuron. This region constitutes the NMJ. Its function is to transfer the propagated nerve impulse from the motor nerve ending to the muscle fiber, resulting ultimately in muscular contraction. Although the nerve terminal and the postsynaptic membrane lie close together, they are separated by a gap that must be bridged by ACh to complete neurotransmission and to generate contraction of the muscle fiber. The surface of skeletal muscle is composed almost entirely of electrically sensitive membrane. It is similar to that of nerve membrane surfaces, in that it is depolarized by local electrical currents that are actively propagated along the surface of the membrane. The end plate area is different. Here there are receptor sites sensitive to chemical agents.

The sequence of events that results in muscular contraction may be summarized as follows. An action potential (initiated by an impulse to the nerve) is propagated along the membrane of the motor neuron to the nerve terminal. This impulse at the nerve terminal causes the release of quanta of ACh. Each quantum contains approximately 10⁴ molecules and is believed to be the contents of one synaptic vesicle. The liberated ACh rapidly diffuses across the synaptic cleft of about 20 nm and binds to AChRs on the postsynaptic muscle membrane. The binding of ACh to the AChR promotes a conformational change, associated with a brief (1 ms) opening of an ion channel. This allows passage of sodium and potassium ions down their electrochemical gradients. There is a net influx of sodium ions (and efflux of potassium ions), resulting in a reduction of membrane potential or depolarization of the muscle membrane from approximately -70 mV to a lower value. When sufficient quanta of ACh are released, the end plate potential at the NMJ reaches a threshold to activate the voltage-dependent sodium ion channels of the adjacent muscle membrane, thereby initiating a muscle action potential. The muscle action potential is actively propagated down the transverse tubules, thereby initiating a sequence of events that results in muscle contraction. The action of ACh is terminated by its dissociation from the AChR; subsequent reassociation is minimized by acetylcholinesterase activity. The resulting activation of the muscle fibers can be measured either mechanically using a force transducer (tension response) or electrically (evoked electromyogram).

Random bursting of single vesicles also results in the release of ACh, which, when bound to AChRs, can cause a conformational change and spontaneous depolarization of the end plate. These potentials are about 1/100 the amplitude (0.5–1 mV) of the end plate potential that causes an action potential. These are called miniature end plate potentials. Many channels must open to allow enough flow of current to form miniature end plate potentials. Although there is a linear relationship between miniature end plate potential amplitude and number of AChRs, a 30% loss of AChRs is required to produce a significant reduction in miniature end-plate potentials.

Under normal circumstances, the amount of ACh released and the number of AChRs activated is much larger than the minimum required to initiate an action potential. That is, the pre- and postjunctional components constitute a system that is larger than that needed for depolarization. This excess reflects the margin of safety or excess voltage above that needed for depolarization of the muscle membrane, ensuring effective neuromuscular transmission. Any event that increases or reduces the probability of receptor opening, either due to altered release of ACh or AChR number, can change the sensitivity of the response to agonists or antagonists. For example, a decreased release of ACh (e.g., Lambert-Eaton myasthenic syndrome [LEMS]) or decreased number of functional AChRs (e.g., myasthenia gravis) decreases the margin of safety. The converse is true as well (see section III). This concept is important in understanding some of the
aberrant responses to neuromuscular blockers induced by changes at the NMJ.

II. Biology of the Neuromuscular Junction

Biochemical insight into the AChR is more advanced than for any other type of receptor. A combined effort by many workers has led to a detailed knowledge and understanding of the structure, function, and binding properties about this "archetypal" receptor protein. These advances are attributed to four factors:44-50

1. The discovery of an abundant source of AChRs in the elasmobranch and teleost fishes (Torpedo sp. and Electrophorus sp.).
2. The use of snake α-neurotoxins, principally α-bungarotoxin (α-BGT) as specific probes with high affinity for the AChR at the NMJ.
3. The use of detergents to solubilize the protein from its membrane environment without loss of biologic activity.
4. The more recent application of the sophisticated and powerful techniques of molecular biology, including the translation systems of the Xenopus oocyte, fibroblasts, and COS cells.

In normal adult muscle, AChRs are present only in the junctional area and are considered "mature" receptors. These receptors are glycoproteins that traverse the membrane region. A mature or junctional receptor is formed of five subunits, each approximately 400–500 amino acids long.53,59,67,68,69 Four proteins, termed α, β, ε, and δ subunits in order of increasing molecular weight, are present in a ratio of 2:1:1:1 (fig. 1). The binding sites for ACh and for some of the ligands (drugs) that bind to AChR are located in the α subunits and bind to a region at or near cysteine residues (unique to the α-chain) at amino acid positions 192–193 of the subunit (Torpedo numbering).52 α-BT has recently been demonstrated to bind to a heptapeptide region 189–197 in human and Torpedo α subunits.55,54 The two binding sites for ACh on the α-subunit are nonidentical and can be distinguished by differential binding of some competitive antagonists. Experiments using dTC have revealed that the sites are of high and low affinity. The source of this difference is suggested to be the direct contribution of subunits that flank the α subunit, specifically ε or γ and δ.51,55

Although the innervated NMJ synthesizes only the mature type of AChR, the muscle nuclei have the genes for synthesis of another receptor protein. These genes do not direct the synthesis of this receptor as long as there is muscle activity and active contact with the nerve.52,53,56-58 When there is deprivation of neural influence or activity, as in the fetus or after denervation, the "immature or denervated" new protein is synthesized, with a subunit composition of α, β, γ, and δ in a ratio of 2:1:1:1.52,53,56-58 Thus, the γ subunit substitutes for the ε subunit. The immature receptors are no longer localized to the end plate region but are inserted throughout the muscle membrane into the junctional and extrajunctional area.59-61

The nerve and activity dependent factors governing localization or spread of AChRs in muscle have not been fully characterized. Many neuropeptides released from the nerve are believed to be part of a nerve–muscle trophic interaction that stabilizes, localizes, and expresses the receptor; these neuropeptides include sciatin, calcitonin gene–related peptide, agrin, and arias.62-65 The ACh co-released may modulate or facilitate the action of these neuropeptides, because the absence of ACh synaptic vesicles results in deficient formation of synapses.66

α-BT binding alone does not differentiate between mature junctional and immature receptors,15,33,45-47 whereas electrophysiologic, molecular biologic, or immunologic (monoclonal antibody) techniques can distinguish them.33,59-60,67-69 Using these techniques, it is apparent that physiologic, pharmacologic, and metabolic characteristics of AChRs differ in the mature versus immature AChR. The transition of these properties during development or in denervation is probably related to changes in subunit composition.70,71 The mature (junctional) AChRs are metabolically stable, with a half-life approximating 2 weeks, whereas immature or denervated AChRs have a metabolic half-life of < 24 h.65,72 Calcium influx associated with muscle activity seems to play an important role in metabolic stability.73 Developing or denervated AChRs have a smaller single-channel conductance and a 2- to 10-fold longer mean channel open time than do AChRs at mature end plates.33,58,60 The subunit

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**Fig. 1.** AChR channels with the subunits (α, β, ε, and δ or α, β, γ, and δ) arranged around the central cation channel. Binding of acetylcholine to the two α-subunits induces the conformational change that converts the channel from closed to open, although the mean channel open times differ between the two types of AChRs depicted here.
changes and proliferation of AChRs occurring with
denervation may also alter the sensitivity and/or affinity of
AChR for specific ligands. Agonists such as ACh, deca-
methionium, and ScH depolarize immature receptors
more easily and result in cation fluxes; compared to the
innervated state, one tenth to one hundredth of normal
doses can effect depolarization.15,74 The potency of com-
petitive antagonists such as dTC may also be altered. Low
concentrations of dTC effectively antagonize the actions
of iontophoretically applied ACh in the mature receptor,
whereas the extrajunctional receptor’s response to ion-
tophoretically applied ACh is less sensitive to dTC.75-77
Similar differences were observed when cholinergic li-
gands such as dTC, decamethionium, and carbamylcholine
were used to prevent α-BT binding to junctional and ex-
trajunctional receptors; low concentrations of dTC were
more effective in decreasing the rate of toxin binding to
junctional than to extrajunctional receptors.78-81 The ap-
parent dissociation constant (Kd) for dTC binding to
junctional receptor was 4.5 × 10⁻⁸ M, whereas the value
for extrajunctional receptor was 5.5 × 10⁻⁷ M.78 These
differences, however, cannot be attributed to alterations
in affinity alone. Recent studies by Gu et al.,50 in which
mature and immature AChRs were transfected and ex-
pressed in COS cells, demonstrated identical association
rates for binding α-BT and identical curves for inhibition
of toxin binding by dTC. The altered sensitivities for cho-
linergic ligands may also result from changes in sarcople-
mal composition known to occur after denervation.82,83

III. Basic Receptor Pharmacology and Its
Application to the Acetylcholine Receptor

The term “up-regulation” generally refers to increased
numbers of a receptor ordinarily present in a given
amount of cell membrane or cytosol.7 This multiplication
of receptors typically occurs when there is chronic ex-
pose to antagonist (or conditions that decrease concen-
trations of transmitter).7,8,84 Down-regulation occurs with
a diminution in receptor number and classically occurs
with chronic exposure to agonist (or conditions that in-
crease synaptic concentrations of natural transmitter, such
as blockade of inactivation mechanism).10,84 In a typical
receptor system, an increase in receptor number is usually
associated with increased sensitivity to agonists and resis-
tance to competitive antagonists.7-9,84 Conversely, a de-
crease in receptor number is associated with hyposensi-
tivity to agonists and extreme sensitivity to competitive
agonists.7,10-11

In skeletal muscle, the most extensively studied con-
dition of up-regulation is that occurring after motor nerve
denervation. As indicated previously, activation of skeletal
muscle by motor nerve seems to play an important, but
not exclusive, role in these alterations in AChR number.
This up-regulation not only involves increased receptor
number but also the appearance of a new receptor protein
that is similar to but not identical in structure and function
to the original receptor (see section II). Diseases that pro-
duce a greater degree of loss of nerve function result in
greater receptor changes. Diseases that cause up-regula-
tion include lower motor neuron lesion (motor nerve sec-
tion, polio, or Guillian-Barré syndrome), upper motor
nerve lesion (stroke or cord section), thermal trauma,
direct muscle trauma, muscle disuse due to enforced in-
activity or immobilization, and any cause of decreased
release or activity of ACh at the NMJ such as chronic
administration of NDMR or local anesthetic blockade of
motor nerve.5,6,14-28,85 Chronic therapy with anticonvuls-
ants or prolonged severe infections appear to induce
similar changes24,28,85 (see section IV for specific effects).
It is unknown if all of these conditions and pathologic
states result in conversion of ε to γ subunits. The altered
pharmacologic responses observed at the NMJ in these
denervated states comprise increased sensitivity to
cholinergic agonists (ScH) and/or resistance to competitive
cholinergic antagonists (NDMRs). The sensitivity to
agonists in the extreme form also results in a potentially
lethal hyperkalemic response (see below).5,6,86

Because of the pattern of development and the half-
lives of mature and immature AChRs, their incorporation
into the junctional and extrajunctional areas appears ini-
tially to involve resistance to NDMR, and later in time to
involve the hyperkalemic response to ScH.17 This time-
lag for the response is related to both quality and quantity
of AChRs. The new receptors initially incorporated in
junctional and perijunctional areas (the latter not present
previously) are less sensitive to the action of NDMR76-77
but can be depolarized by ACh.15,74 The increase in
AChRs in the perijunctional area, however, is insufficient
to cause severe hyperkalemia (see section III.B). Later,
when AChRs are spread throughout the muscle mem-
brane, the hyperkalemic response to ScH occurs. Clinical
conditions featuring only resistance without hyperkalemia
include chronic therapy with anticonvulsants24 and disuse
atrophy of a single limb.17,85

A decrease in AChR number, broadly referred to as
down-regulation in this review to include decreased syn-
thesis, internalization, or increased breakdown, is a more
restricted phenomenon. Conditions that have direct and/
or supportive evidence for down-regulation include
myasthenia gravis, chronic cholinesterase inhibition (e.g.,
organophosphorus poisoning) and, possibly, conditioning
exercise. Although there are no data examining receptor
changes during chronic conditioning exercise, clinical
and/or laboratory studies in all three conditions represent
what would be expected to occur if down-regulation had
actually occurred: increased sensitivity to NDMR and resistance to SCh and ACh.

A. PHARMACOLOGIC BASIS FOR RESISTANCE TO NONDEPOLARIZING MUSCLE RELAXANT

How does proliferation of AChRs induce resistance to neuromuscular relaxants? Although it may seem paradoxical that, for example, an atrophied muscle will show resistance (hyposenstivity) to the effects of a drug that further weakens the contractile properties, it does occur (see below). These observations can be explained on a pharmacodynamic basis and are analogous to pharmacologic responses observed in other receptor systems exposed to antagonist drugs during up-regulation of receptors.\textsuperscript{7,11}

Paton and Waud have described the margin of safety of neuromuscular transmission and have developed the concept of the relative excess of AChRs that needs to be blocked prior to twitch depression.\textsuperscript{97} They found in normal subjects that 75% receptor occupancy by the antagonist is necessary before any effect can be seen, and at least 95% receptor occupancy is necessary for complete suppression of twitch. The percent receptor occupancy, and not the absolute number blocked, varies with dose and therefore with concentrations reached at the NMJ. In other words, if the receptor number is increased, and if the same proportion of receptors is occupied by antagonist, the absolute number of receptors remaining unblocked is increased. Thus, this concentration or dose of NDMR will produce a smaller effect on twitch height,\textsuperscript{88,89} provided that other factors, such as ACh release, which also affect the margin of safety, are unaltered. Supportive evidence for this theory—that quantitative increases in AChRs are associated with tolerance to NDMR—is provided by Hogue et al.\textsuperscript{16} and by Kim et al.,\textsuperscript{32} who observed a significant positive correlation between AChR and effective dose of dTC for twitch inhibition (fig. 2).

Normally, only the junctional receptors are involved in neuromuscular transmission, because the number of extrajunctional receptors is insignificant. The pathologic process of "denervation" incorporates new immature receptors both in the junctional and extrajunctional areas. We do not know how these new AChRs located in the extrajunctional area modify junctional neurotransmission. The possibility exists that the perijunctional increase in AChRs may act as a "sink" for accumulating NDMR molecules. An alternative explanation is that the immature extrajunctional receptors (with altered subunit composition), incorporated in the junctional area with "denervation," have altered functional and pharmacologic characteristics that make NDMRs less effective. Voltage-clamp techniques indicate that ACh induced currents in these immature receptors are less easily blocked by dTC.\textsuperscript{75-77} Although decreased affinity of the receptor for competitive antagonists was believed to be the reason for this, recent evidence does not seem to support this.\textsuperscript{99} The leftward shift in the dose–response curve to ACh or the increased susceptibility of the muscle membrane to depolarization by ACh\textsuperscript{15,74} may also account for the higher concentrations of NDMR required to counteract depolarization. That NDMRs, specifically dTC, may act as a partial agonist in these new receptors may contribute to the altered sensitivity.\textsuperscript{95,91} An additional factor that may contribute to resistance induced by nerve injury is decreased acetylcholinesterase activity.\textsuperscript{15} Here, breakdown of ACh would be diminished, increasing levels of ACh at the NMJ; thus, more ACh would compete with antagonist drugs, shifting dose–response curves to the right.\textsuperscript{92} The contribution of this component to the altered response to neuromuscular relaxant following up-regulation is
probably small, because the efficacy of the enzyme is quite high.

B. PHARMACOLOGIC BASIS OF SENSITIVITY AND HYPERKALEMIA TO SUCCYNILCHOLINE

Tolmie et al., 95 documented that hyperkalemia was responsible for cardiac arrest with SCh. SCh consists of two ACh molecules joined by an ester bond. It is not surprising, therefore, that it has a depolarizing effect similar to that of ACh at the postsynaptic membrane. Direct and indirect evidence suggests that up-regulation of AChRs plays a significant role in this hyperkalemic response. In the normal NMJ, the conformational change in AChRs occurring with depolarization allows potassium to leave the cell only through end plate receptors. This causes serum potassium levels to increase about 0.5 meq/L, without any cardiovascular consequences. 17 With the appearance of AChRs in extrajunctional areas, the area of chemosensitiveness expands; there is a shift to the left in the dose–response curves of ACh and SCh. Depolarization and chemical transmission can occur through all of these junctional as well as extrajunctional receptors. 6,15,74 More importantly, more ion channels are now available to release potassium during depolarization with SCh. When many muscles are involved, this flooding of the extracellular fluid with potassium results in hyperkalemia and cardiac arrest. Altered channel properties of the muscle membrane, including a longer apparent mean channel open time during depolarization, 33,56,69 may play a role in the exaggerated release of potassium seen with SCh.

The hyperkalemic response to SCh in these denervation states is dose-dependent; the marked rise in potassium can be ameliorated by prior administration of large doses of NDMR 94 or completely ablated 95 by the use of smaller doses of SCh. The use of SCh in these situations is still clinically contraindicated because of unpredictability of the potassium response, even with small doses. Furthermore, the smaller dose may cause contracture of affected muscles and may be ineffective for relaxing unaffected muscles such as the diaphragm or upper airway muscles. Although some believe that SCh-induced release of catecholamines rather than potassium is the cause of the cardiac arrest, 96 there is no evidence to support this theory. Substantiation of the hypothesis that up-regulation of AChRs plays an important role in the aberrant response to SCh is provided by the report that a patient with rhabdomyosarcoma exhibited a hyperkalemic response to SCh. 97 Rhabdomyosarcoma cells, also referred to as the TE671 cell line (originally misidentified as medulloblastoma), are noted for the abundant presence of muscle type AChR throughout the muscle membrane. Immunologically and pharmacologically, these cells behave like denervated muscles. 98,99 The increased AChRs present in these rhabdomyosarcoma cells were likely responsible for the hyperkalemic response with SCh.

IV. SPECIFIC LESIONS OF UP-REGULATION OF ACETYLCOLINE RECEPTOR

A. DENERVATION SYNDROMES

Lower Motor Neuron and Direct Muscle Injury

Complete transection of a motor nerve results in Wallerian degeneration of the nerve. For this reason, the response to NDMR following complete motor nerve destruction has not been studied. The hypothesis that lower motor neuron injury with its associated proliferation of AChRs induces resistance to NDMR has been tested following partial denervation. 16 After a 75–80% lesion of the sciatic nerve, the effective dose for dTC was approximately twice as great on the denervated side as on the contralateral side or in control, non-denervated animals. Twitch tension recovered at a significantly higher plasma dTC concentration in the denervated leg than in the contralateral leg. In addition, the AChR number was significantly increased in the denervated leg, with a positive correlation (R² = 0.73) between effective dose of dTC and AChR number. 16 Future studies should address whether the quality or the quantity of the receptors is more important in the altered pharmacodynamics to NDMR.

The potential for SCh-induced hyperkalemia following lower motor neuron injury has been well established. 5,6,94 The time of onset of increased sensitivity to agonists depends on the length of the nerve distal to the point of section; the shorter the distal segment, the faster the denervation changes. 100 Based on human data, sensitivity to SCh following denervation probably begins at about 3–4 days and reaches dangerous levels at 7–8 days. 94,101–103 Direct muscle injury, irradiation, or irritation of muscle by a foreign substance results in similar supersensitivity responses to agonists. 20,21,104,105 Even a polyneuropathy, in the absence of complete transection of nerve, can cause a high potassium response to SCh. 106–109 In some neurologic and traumatic states the pathophysiologic process affecting nerve and muscle can continue for prolonged periods. 110,111 Whether these neuromuscular abnormalities could cause an exaggerated potassium response to SCh is unknown, but some data suggests that it persists for many years. 14

Upper Motor Neuron Injury

There is more extensive documentation of the interaction between NDMR and the pathologic state of upper
motor neuron denervation. The studies of Graham and Moorthy and Hilgenberg documented that, following stroke, the paretic side was resistant to the neuromuscular effects of pancuronium. Subsequent study by Iwasaki et al. confirmed the resistance and noted that some patients demonstrated resistance as early as 4–8 h after stroke. Electromyographic findings of denervation syndrome (fibrillation potentials and positive sharp waves) are present for an indefinite period following an upper motor neuron lesion. Nerve conduction velocity is also decreased. These electromyographic changes following central paresis first occur 2–3 weeks after stroke and are most frequently observed in the distal arm and hand muscles. The appearance of denervation changes in the presence of an anatomically intact motor nerve supports the concept that, even following central denervation, there is transynaptic degeneration of the α-motor neurons because of deprivation of trophic factors or inputs that are normally received from descending motor pathways. In other words, lower motor neuron type changes can be present even with upper motor neuron lesions. The frequency of denervation activity decreases in parallel with the development of spasticity, the relationship of this to the response of neuromuscular blockers is unknown.

An interesting but not readily explicable finding is that decreased sensitivity to metocurine was observed in patients with upper motor neuron disease, both on the affected (stroken) side and the unaffected (normal) side. These patients were studied as early as 40 h after stroke. Cerebral, thrombotic, and hemorrhagic events, though localized, are surrounded by an area of edema and inflammation. Although symptoms and signs of stroke may not be present on the opposite side, all of these diseases may be diffuse, causing undetectable changes or clinical symptoms on the opposite side. It is therefore possible that stroke patients, although affected mostly on one side, may have transient or permanent but minimal clinical affliction on the opposite side. This possibility has been confirmed in two studies in which the affected and the supposedly unaffected limbs of hemiplegic patients were observed to have a bilateral decrease in motor units and a decrease in conduction velocity. Despite the global nature of the affliction of the motor neurons following upper motor neuron lesions, preliminary observations indicate that the response of the diaphragm is unaltered. It is possible that the phrenic nerve was not affected by the pathologic process. If the pathologic state is obviously global or bilateral (e.g., head trauma), then one might anticipate resistance to NDMR on both sides, as shown for multiple sclerosis, syringomyelia, and/or quadriplegia, and the upper limb muscles relative to the lower appear more sensitive to NDMR. In multiple sclerosis, resistance to atracurium was seen in association with increased AChRs. Increased sensitivity to ACh develops within 3–5 days after cordotomy. Thus, the potential for hyperkalemia is present as early as in lower motor neuron injury. Upper motor neuron lesions associated with a hyperkalemic response include paraplegia, cerebral hemorrhage, or ruptured aneurysms (although this could not be confirmed) and closed head injury with or without paresis. Although one case report suggests a hyperkalemic response to SCH in Parkinson’s disease, a subsequent report in seven patients does not confirm an association. In contrast to the acquired neurologic diseases discussed above, cerebral palsy and meningocele are upper and lower motor neuron lesions, respectively, that are congenital and do not exhibit hyperkalemia to SCH. The response to NDMR, in contrast, may show increased or decreased sensitivity (resistance). The resistance to NDMR in the latter report may be related to the anticonvulsants these patients were receiving (see below). Another contradictory report is the observation that patients with spastic paraplegia can be hypersensitive to dTCG when studied by regional technique. There are other contradictory findings: although pathologic and electromyographic changes in chronic poliomyelitis include findings ordinarily observed with up-regulation, such as terminal nerve sprouting, denervation, disintegration of nerve fibers, and loss of motor neurons, sensitivity to NDMR can be increased. Similarly, despite electromyographic evidence of a denervation-like state (fibrillation and positive sharp waves) for polymyositis, the recovery from vecuronium can be prolonged. Increased sensitivity may be due in part to loss of functioning motor units.

**Clinical Use of Relaxants in Upper and Lower Motor Neuron Denervation**

Upper motor neuron lesions often lead to bilateral changes, whereas lower motor neuron injury causes changes only in the affected nerves. Polynuropathy results in the affliction of multiple nerves. The AChR changes result in resistance to NDMR and sensitivity to SCH as early as 3–7 days after injury. The duration of these aberrant responses is unknown, although modest increases in extrajunctional AChRs are observed even 3 yr after cord injury, and SCH-induced hyperkalemia has been noted as late as 7–10 yr with progressive neurologic disease. The risk of hyperkalemia following denervation is probably absent once resistance to NDMR disappears, but no studies have defined this. The inferences that can be made from these studies are that deviation from expected responses can occur in some of these complex neurologic disorders, and therefore mon-
itoring of neuromuscular function, especially of the dia-
phragm and muscles protecting the airway, is always im-
portant during administration and reversal of NDMR.

B. BURNS

Pharmacologic and Functional Changes

Burned and denervated patients share a supersensitivity
response to ACh or SCh \textsuperscript{5-6,15,142-144} and hyposensitivity
to the effects of NDMR. \textsuperscript{25,144-148} Thus, denervation-like
changes may be the basis of the altered response to agonist
and antagonist types of relaxants; clinical evidence sup-
ports this explanation. Denervation-like changes include
fibrillation potentials and positive sharp waves, \textsuperscript{**} peripheral
neuropathies, and reduced motor nerve conduc-
tion, \textsuperscript{149} with or without muscle weakness. \textsuperscript{150} The best
indirect evidence for denervation in burns has been dem-
onstrated by Aulick and co-workers. \textsuperscript{151} Measurement of
blood flow in burned and nonburned limbs demonstrated
that flow in burned limbs was unaffected by variation in
temperature. They concluded that physical or chemical
denervation is associated with thermal injury, accounting
for the lack of vasomotor response to temperature. These
neuromuscular abnormalities may play an important role
in the AChR changes described below.

Acetylcholine Receptor Changes

The hypothesis that a denervation-like syndrome with
increased nicotinic AChRs occurs at the NMJ at sites dis-
tant from the burn has been tested in the rat after burn
injury to 45% of the body surface area. \textsuperscript{152} At 10, 14, and
21 days postburn, the animals did not gain weight and
had significant increases in AChR concentrations in the
diaphragm. At 28 days, however, the size of the original
burn area was significantly reduced to less than 20% of
body surface area; body weight was increased over pre-
burn weight; and the number of AChRs returned to con-
trol levels. Subsequent studies have confirmed an asso-
ciation (R \textsuperscript{2} = 0.65, r = 0.81) between the effective dose
for dTC and changes in AChR concentrations following
burns (fig. 2). \textsuperscript{22} In addition, the slopes of the dose–response
curves to dTC were flatter (smaller) in burn groups.
The nonparallel slope in the burn group compared to
that of controls is indirect evidence for an altered inter-
action, or affinity, between drug and receptor. \textsuperscript{7,22}

The hypothesis that AChR number is increased follow-
ing burns was not confirmed in one study in rats. \textsuperscript{153} In
that study the neuromuscular effects were investigated in
rats with a 30% surface area burn. Resistance to atrac-

** Mills A, Schreiber T, Martyn JA: Electromyographic studies of
patients with thermal injury (abstract). Anesthesiology 65:A294,
1986.

rium was observed only 40 days postburn with no changes
in AChRs. \textsuperscript{153} This discrepancy in rodent studies might be
related to the burn size, \textsuperscript{154} because burn injury to 30% or
less of body surface area does not consistently cause
resistance to NDMR. \textsuperscript{148,155-157} However, increased pro-
tein binding (see below) and altered affinity of the receptor
to atracurium may have played a role in the resistance to
atracurium at 40 days. Preliminary studies in humans have
confirmed the presence of increased AChRs at the muscle
membrane following burns. \textsuperscript{150} Further studies need to
address the importance of burn size and its effects on
quantitative and qualitative changes in receptor.

Although previous studies \textsuperscript{159} have indicated that loss of
drug through burn wound is significant following
burns, the contribution by this component to resistance
to NDMR is minimal since the volume of distribution
of NDMR was not different between control and burned
patients. \textsuperscript{160,161} Enhanced metabolic disposition does not
occur, since the elimination half-life and clearance were
similar in burned and unburned patients. \textsuperscript{160,161} Increased
protein binding is a factor in the resistance of burned
patients to NDMR. Metocurine, dTC, atracurium, and
numerous other drugs bind more to plasma protein from
burned patients. \textsuperscript{161-165} However, the increase in protein
binding cannot entirely account for the shifts to the right
in the dose–response curves to NDMR. \textsuperscript{162} The increased
protein binding of these drugs is due to the rise in blood
levels of an acute phase reactant protein called \textalpha-1-acid
glycoprotein. \textsuperscript{164} It is important to note that pharmacoki-
tetic factors, including protein binding of drug in
plasma, cannot account for the hypersensitivity to SCh,
which is purely related to changes at the NMJ, or to phar-
macodynamic factors.

Circulating mediators may play a role, since incubation
of normal rat diaphragm with plasma from burned pa-
tients results in hyposensitivity to the neuromuscular
effects of pancuronium and dTC. \textsuperscript{165} Potential mediators
that may play a role in altering neuromuscular responses
include catecholamines, prostaglandins, and hydrolytic
enzymes. \textsuperscript{166,167} Whether these circulating mediators effect
these changes by prejunctional or postjunctional mecha-
nisms is unknown. Immobilization due to bed rest (hu-
mans) or burn-wound–induced contracture (humans and
animals) may also increase AChRs and cause altered sen-
sitivity to muscle relaxants (see below). Immobilization
is not the only factor, however, since spread of AChRs was
observed in the diaphragm of spontaneously breathing rats,
a site distant from the burn area. \textsuperscript{162} Thus, neural or hu-
moral mechanisms probably are involved.

Clinical Implications

The relationship between changes in AChR number and
response to SCh has not been studied relative to

Clinical Implications

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ACETYLCHOLINE RECEPTOR INTERACTION WITH RELAXANTS

IMMobilization and Atrophy

Pathologic Changes

Several models of immobilization or inactivity have been studied. These include application of a plaster cast to one or more limbs, application of anesthetics or toxins to the nerve or muscle, and pinning of the joints. The physiologic state of immobilization contrasts with denervation syndromes in that there is no direct damage to cord or to nerve roots; the muscle fibers remain innervated, judged by morphologic criteria and the presence of miniature end plate potentials; and the distal segment of the nerves and muscle fibers function normally, since stimulation of the nerve results in brisk contraction of the muscle although the tension developed is less because of atrophy. Immobilization is comparable but lesser in magnitude to denervation syndromes in that there is muscle atrophy, enlargement of the ACh-sensitive area, a shift to the left in the dose–response curves to Ach or SCh, proliferation of AchR, decreased cholinesterase activity, and sprouting of terminal branches. All of these to a greater or lesser extent may account for the abnormal neuromuscular responses. Animal experiments indicate that all of these changes at the NMJ revert to normal within 20–50 days of the termination of immobilization.

Neuromuscular Responses

Receptor proliferation associated with immobilization results in increased sensitivity to Ach or SCh on the affected muscle. As expected, both dose–concentration–response curves to NDMR were shifted to the right. A pseudo-steady-state model demonstrates a 10-fold shift to the right in the dose requirement for paralysis of the immobilized muscle compared to controls. Resistance to NDMR can be seen in dogs as early as 4 days after immobilization, and therefore immobilization may play an important role in the resistance to NDMR in patients subjected to bed rest. The effects of single-limb immobilization on the immobilized and distant muscles were studied by Waud et al. in the guinea pig. Immobilization increased the effective dose of the drug, not only in the muscles from the casted hindlimb, but also in those from the contralateral limb and forelimbs. Resistance to NDMR was observed even in the absence of changes in muscle size or in maximal tension developed. The diaphragm, however, was unaffected by immobilization. Thus, a systemic effect does not appear to be present. Immobilization of one limb may have affected the mobility of the other limb muscles but not of the diaphragm. Complementing these observations is the opposite finding, that conditioning exercise increases sensitivity to the competitive antagonist metocurine (see section VI.C). These disuse studies concluded that the resistance observed after immobilization did not reflect a circulatory or pharmacokinetic artifact but rather some change in the neuromuscular apparatus itself that represents pharmacodynamic alterations. The parallel
shift of the dose–response curves observed by Fung et al. and the absence of change in the receptor dissociation constant documented by Waud et al. suggest that the quality of the receptors themselves did not change after immobilization. Molecular biologic studies are not available to document whether after immobilization, the subunit structure is changed from ε to γ.

Clinical Implications

Because immobilization of one extremity may produce sensitivity changes in other muscles, the anesthesiologist must not assume that monitoring from any limb will yield normal values in a patient with partial immobility. SCh is unlikely to cause hazardous acute hyperkalemia, if only a single limb is involved in immobilization or disuse. In contrast, atrophy, due to vascular insufficiency of a single limb results in denervation-like changes in muscle and can indeed cause a hyperkalemic response. The application of a total body spica or total bed rest as in intensive care patients may result in relative immobilization of several muscles. The administration of SCh in these situations may result in the lethal potassium response, as shown for intensive care patients although other factors probably contributed (see sections IV,E and IV,F). The hyperkalemic response to SCh seems to be dependent on the degree and duration of immobilization, because an exaggerated potassium response was not seen during challenges with SCh in the early period, but only after > 10 days of immobilization.

D. INCREASE OF ACETYLCHOLINE RECEPTORS BY NONDEPOLARIZING MUSCLE RELAXANTS

Pathophysiology

Regulation of receptor number by endogenous or exogenous ligands constitutes a homeostatic regulatory mechanism. The interaction between agonist and antagonist drugs during their chronic administration has been exhaustively characterized, particularly for the β-adrenergic receptor. Chronic competitive β-adrenergic receptor blockade, for example, causes up-regulation of receptor number. The latter receptor alteration is associated with resistance or tolerance to antagonist drugs (e.g., propranolol) and sensitivity to agonist drugs. These changes in receptor number and induced by exogenous and endogenous ligands, are an important determinant in the development of drug tolerance or sensitivity, and may play a significant role in the withdrawal symptoms that accompany cessation of drug usage.

Attempts have been made to follow changes in the number of AChRs on end plate as well as non–end plate areas during chronic treatment with drugs that interfere with release (β-bungarotoxin [β-BT] and hemicholinium) or action (dTC and α-BT) of ACh. The effects were achieved in vivo by local or systemic administration of drug without disruption of impulses in the nerve. Continued treatment with these drugs increased the binding of α-BT in the extrajunctional regions of the muscles. The resting potential and input resistance of the muscle fibers that had been paralyzed were similar to those in denervated muscle. These studies, however, were confounded by the fact that the competitive antagonists simultaneously caused complete or incomplete paralysis of the muscles. These investigators, in fact, concluded that immobilization was the cause of the spread of AChRs.

The hypothesis that continuous subliminal (subparalytic) competitive antagonism of AChRs (i.e., antagonism in the absence of immobilization) induces a proliferation of AChRs has been recently tested. The attendant pharmacodynamic alterations to dTC were also studied. Chronic subparalytic antagonism of the AChR was achieved in rats by an infusion of dTC through osmotic minipumps. After 2 weeks of infusion of dTC or saline, the animals were reanesthetized, and effective doses for dTC for twitch suppression of the gastrocnemius were determined. No difference in weight gain or mobility was observed between the groups at the end of 2 weeks. The slow continuous infusion of dTC resulted in a mean plasma concentration of 0.4 μg/ml at 2 weeks. The experimental group was able to develop a baseline tension similar to that of controls despite the presence of plasma dTC concentrations that normally would depress twitch 60%. Chronic constant presence of the antagonist altered dTC concentration–responses so that significantly higher plasma levels were required for twitch depression in the experimental group. These pharmacodynamic alterations were associated with increased extrajunctional AChRs. The diaphragmatic AChRs were not altered. This study, therefore, confirms the hypothesis that chronic competitive antagonism of the NMJ induces an up-regulation of AChR and resistance to antagonists, even in the absence of immobilization or paralysis. The absence of AChR changes in the diaphragm may be due to the higher margin of safety for muscle relaxants in the diaphragm than in peripheral muscles.

Clinical Implications

NDMR are extensively used in intensive care unit patients to facilitate mechanical ventilation. There have been numerous reports of resistance to the effects of NDMR in critically ill intensive care patients or after prolonged use. Co-administered drugs such as theophylline and azathioprine and immobilization have been implicated as etiologic factors. It is now clear that the administration of a competitive antag-
onist itself can be a significant factor in the development of tolerance to these drugs and the development of extrajunctional AChRs. Despite the pharmacologic basis explaining the need for increased amounts of muscle relaxants, anecdotal reports underscore the fact that the continued use of NDMR might cause paralysis lasting from days to months.192,198–200 Polyneuropathy of chronic disease also may have complicated the picture by causing changes in the NMJ.201–206 Because these reports concern critically ill patients with multiple pathology, it is difficult to establish the role of NDMR in these myopathies. A more important clinical conclusion is the use of SCh following chronic neuromuscular blockade. The use of SCh for changing endotracheal tubes or for reintubation is not uncommon in the intensive care unit because of its rapid action and short duration. Cardiac arrest with lethal potassium levels has been reported in some of these instances.105,180–183 It is likely that up-regulation of AChR induced by immobilization and chronic neuromuscular blockade contributed to the cardiac arrest associated with the use of SCh. For this reason, it is wise to avoid depolarizing relaxants in patients previously exposed to extended use of NDMR. The onset and duration of this effect is unknown.

E. Chronic Anticonvulsants

Pathophysiology

Several studies have demonstrated resistance to NDMR after prolonged therapy with phenytoin or carbamazepine.203–207 Although previously atracurium potency was said to be unaffected,205 more recent studies indicate that its dose–response is also altered by anticonvulsants.205 The acute pre- and postjunctional effects of phenytoin and other anticonvulsants are somewhat similar to the effects of small nonparalytic doses of NDMR.208–212 Carbamazepine and phenytoin acutely suppress posttetanic repetition in nerve terminals, with a resultant decrease in posttetanic potentiation of muscle and a reduction in end plate potential. These changes are due to the presynaptic inhibitory action on ACh quanta release at the nerve terminal. The similarity between pre- and postjunctional effects of anticonvulsants and nondepolarizing muscle relaxants suggests that chronic phenytoin therapy might lead to antagonism of ACh at pre- and postjunctional areas. It is conceivable, therefore, that anticonvulsants administered over a period of time simulate chronic chemical denervation resulting in proliferation of AChR and resistance to competitive antagonists, as previously described for dTC.27 In addition, numerous studies in humans have, in fact, confirmed varying forms of sensory, motor, and NMJ disorders in patients on long-term anticonvulsant therapies.213–215 Whether these disorders in nerve and muscle function additionally contribute to the denervation-like syndrome is unknown. Preliminary studies in the rat confirm a very modest elevation of AChR number and resistance to metocurine following phenytoin therapy for two weeks.28 Consistent with the finding of only a modest increase of AChRs is the absence of any reports of SCh-induced hyperkalemia with chronic anticonvulsant therapy. That is, the AChR changes are sufficient to result in NDMR resistance, but not in SCh hyperkalemia.

Additional explanations for resistance may be related to other systemic effects of anticonvulsants. The anticonvulsants presently in clinical use are potent inducers of liver metabolizing enzymes216–217 and may, therefore, enhance the metabolic clearance of NDMR. Supportive evidence to substantiate this speculation is not present. The anticonvulsants also release acute phase reactant proteins, including α1-acid glycoprotein, that bind to many drugs.28,164,217,218 Rats treated with chronic phenytoin had significant elevations of α1-acid glycoprotein, and there was a significant negative correlation between free (unbound) metocurine and α1-acid glycoprotein levels in plasma.28 Thus, significantly increased protein binding may partly explain the twitch recovery at higher plasma NDMR concentrations in both patients207 and rats28 receiving chronic phenytoin. The induction of hepatic enzymes, including release of α1-acid glycoprotein, by anticonvulsants takes approximately 2 weeks for peak effect218–219 and is consistent with observations of onset of anticonvulsant-induced resistance to NDMR.28,207 It seems, therefore, that the resistance to NDMR following chronic anticonvulsants may have pharmacodynamic and pharmacokinetic bases, particularly for drugs cleared by the liver.

Clinical Implications

The increase in AChRs following anticonvulsants is modest (as in disuse) and would explain the lack of reports of SCh hyperkalemia following anticonvulsants. Thus, it is probably safe to administer SCh to patients on anticonvulsants. The administration of NDMR following acute phenytoin therapy results in potentiation of neuromuscular effects.208–212 Onset of resistance to NDMR following chronic anticonvulsant therapy probably occurs after approximately 2 weeks.28,207

F. Infection

Pathophysiology

Inflammation or infection alters the NMJ and responses to SCh and NDMR. Bacterial toxins, such as those released by the Clostridium genus, inhibit the release of ACh which, if prolonged, can increase AChRs.220–224 The macromo-
lecular structures of clostridial toxins are similar. Botulinum toxin derived from Clostridium botulinum binds strongly to motor nerve terminals, where the toxin is internalized, and ultimately decreases the release of ACh. Tetanus toxin derived from Clostridium tetani is taken up in the periphery by motor nerve endings and is transported within peripheral nerves to the spinal cord, where it produces spasticity and convulsions. This central action is so prominent that peripheral effects usually are overshadowed. Tetanus toxin blocks ACh release both centrally and peripherally; the central inhibitory effect on the synapses are 2,000 times more potent than at peripheral synapses. The muscles affected by clostridial toxins become atrophic and functionally denervated and develop extrajunctional AChRs. This denervation phenomenon can result in hyperkalemic cardiac arrest with SCh. Resistance to NDMR during tetanus has not been documented by pharmacodynamic data, but patients needing prolonged mechanical ventilation were observed to require high infusion rates and high plasma concentrations of pancuronium. This may have been due to a combination of tetanus and NDMR-induced denervation syndrome. The inflammatory response associated with infection resulting in release of acute phase-reactant proteins that bind NDMR may also have played a role in the high dose requirement. Other infections, likewise, alter neuromuscular responses. Infection of Escherichia coli produces a 3–5-fold rightward shift in DTC dose–response curves. Weight loss and atrophy secondary to the effects of the toxin do not appear to be factors, because similar atrophy and weight loss due to malnutrition did not reproduce these alterations in response to NDMR. Reinforcement of these observations relative to NDMR are reports of an exaggerated potassium response to SCh in patients with serious infections lasting 1 or more weeks.

Clinical Implications

Anesthesiologists should expect resistance to NDMR in patients suffering from a severe chronic infection that has persisted for 3 or 4 days or longer. After 1 week, the risk of hyperkalemia after SCh becomes real. Patients with clostridial infections need these same precautions. Botulinum toxin is being used locally as therapy for blepharospasm, torticollis, and spasmodic dysphonia. Systemic denervation-like effects have been observed following local injection of botulinum toxin.

V. Period of Risk for Succinylcholine Hyperkalemia

A recent paper documents the confusion and inconsistencies in various anesthesia textbooks and publications concerning the hazardous period for SCh in disorders that involve up-regulation. Based on turnover and properties of extrajunctional type receptors and on degree of up-regulation, we believe that these inconsistencies can be resolved. If there is a total loss of ACh activity upon the end plate, there is a rapid spread of receptors. Disorders involving this total loss of effect of ACh include acute denervation or a lower motor neuron lesion, the neuronal shock period of an upper motor neuron lesion such as stroke or cord section, and direct muscle trauma. A hyperkalemic response would be evident by 3–5 days and hazardous by 7 days if sufficient muscle mass is affected. In disorders involving a less-than-complete loss of ACh activity, the onset appears to be about 7–10 days, although human data do not comprehensively examine this question. These disorders include thermal trauma, prolonged severe infection, and possibly prolonged total body immobilization. Also included here may be NDMR-induced suppression of reflex movement during prolonged care in the intensive care unit. In all of these situations, relaxant resistance probably occurs as soon as, or sooner than, SCh sensitivity.

The duration of risk is more difficult to pinpoint. Once resistance to competitive relaxants disappears, then SCh sensitivity is most likely absent also. Findings suggest healing, reinnervation, or total atrophy of muscle for denervation, and healing and mobility for burn trauma, muscle trauma, and infection. In the case of burn trauma, normal response to NDMR has been observed 3 yr after burn injury. Normal neurologic examination, however, does not completely rule out the potential for hyperkalemia, as shown in some patients (Guillain-Barré and ischemic leg). Thus, despite normal neurologic examination, it is apparent that extrajunctional AChRs may be present. For patients with progressive upper motor neuron lesions, the risk is always present, even as long as 10 yr. For those with nonprogressive lesions, hyperkalemia has been observed even after 7 yr. Thus, the duration of risk is sometimes years and may be estimated by NDMR response. More data concerning relaxant resistance in upper motor lesions are needed, because not all of their variations have been quantified. We caution that survival after use of SCh does not mean that its use was safe; measurement of potassium values is necessary to document that. Patients with serious hyperkalemia may show unaffected cardiovascular indices. Pretreatment with NDMR does not guarantee the ablation of SCh hyperkalemia.

VI. Down-regulation of Acetylcholine Receptors

Decrease of AChRs is a less common phenomenon, although it is seen in certain clinical and/or physiologic states, including myasthenia gravis, chronic cholinesterase inhibition, and possibly conditioning exercise. As indicated previously, down-regulation in the classical sense
ACETYLCOLINE RECEPTOR INTERACTION WITH RELAXANTS

A. MYASTHENIA GRAVIS

Pathophysiology

Myasthenia gravis is a disorder causing muscle weakness that becomes worse with repeated voluntary effort but that can be improved by rest or anticholinesterase drugs. Antibodies against AChRs are detectable in about 80% of patients, but antibody titers vary greatly between patients and do not always correlate well with clinical severity. The anti-AChR antibodies lead to a reduction in the number of functional AChRs by three mechanisms: 1) immunopharmacologic blockade of AChR function; 2) antibody-induced accelerated turnover of AChRs; and 3) complement-dependent lysis of the postsynaptic membrane. The majority of the antibodies directed against the AChR seem to bind to a distinct region on the surface of the α-subunit of AChR, designated the main immunogenic region. The pathophysiology of myasthenia gravis should be contrasted with another autoimmune-mediated disease of the NMJ, namely LEMS. LEMS has been seen with small cell carcinoma of multiple tissue origins. LEMS differs from myasthenia gravis in that it is a prejunctional phenomenon affecting the voltage-gated calcium channel of the nerve terminal. The disease is characterized by reduced quantal release of ACh from motor nerve terminals due to autoimmune antibodies against the voltage-gated calcium channels. The miniature end plate potential and the AChR number in LEMS are normal. The amplitude of the evoked muscle action potential is small and shows a decremental response at slow rates of stimulation and an incremental response with high-frequency stimulation or after voluntary effort. These findings contrast with those from myasthenia gravis, in which the quantal content is normal whereas the miniature end-plate potential and AChRs number are reduced.

Clinical Implications

Sensitivity to nondepolarizing and depolarizing relaxants has been observed in LEMS. The myasthenia gravis patient, in contrast, is resistant to the effects of SCh and decamethonium. Resistance to SCh and decamethonium has been confirmed in the in vitro nerve muscle preparation in experimental autoimmune myasthenia gravis. It is of interest that Wainwright and Brodridge noted resistance to SCh with 0.5-mg/kg doses but not with 1-mg/kg doses. The absence of resistance with a higher dose may be related to the development of phase II or dual block.

The extreme sensitivity to NDMR of patients with myasthenia gravis has been confirmed in a number of studies in animals and humans and by in vivo, in vitro, or isolated-nerve techniques. In an elegant study, Nilsson and Meretoja examined the effects of vecuronium dose requirements by electromyography and mechanomyography and correlated the neuromuscular sensitivity with clinical and immunologic parameters. The effective dose of vecuronium was 250% greater in control patients than in myasthenia gravis patients. The hourly dose of vecuronium required to maintain an 80–90% neuromuscular blockade was approximately one third in myasthenia gravis patients compared to controls. The effective dose requirement of vecuronium was significantly related to the patient’s AChRs antibody titer, which invariably relates to clinical severity or the down-regulation of AChRs (fig. 9).

The studies of Eisenkraft et al. relative to SCh and that of Nilsson and Meretoja with vecuronium indicated that the slopes of dose–response curves may be different in these patients compared to controls. This is suggestive evidence that the affinity of the receptor for both depolarizing and NDMR is altered by the pathologic process. As previously discussed, resistance to an agonist and extreme sensitivity to an antagonist in the presence of decreased AChR number can be explained by a pharmacologic analysis of drug–receptor interaction.

B. CHRONIC AGONISM OF ACETYLCOLINCHLINE RECEPTOR

Pathophysiology

The hypothesis that chronic decreased levels of ACh can result in the proliferation of AChR has been confirmed (see sections IV.D and IV.F). The test drugs were
botulinum toxin, β-BT, and hemicholinium, which, unlike dTC and α-BT, have a predominant prejunctional effect, including decreased spontaneous and evoked release of ACh. The complementary hypothesis that chronic agonism of the AChR results in down-regulation of the receptor has also been tested (see below). These responses are, therefore, akin to that observed in the adrenergic receptor, where the continued presence of adrenergic agonist (e.g., norepinephrine) results in down-regulation of β-adrenergic receptor number and an attenuated response to inotropic and chronotropic effects of the drug. In cultured myotubes and mouse muscle cell lines probably consisting mostly of immature AChRs, nicotinic AChRs are down-regulated by agonists, e.g., carbamylcholine, and nicotine. The effects of continued high concentrations of ACh have been studied in vivo by chronic administration of reversible (neostigmine) and irreversible (organophosphorus) cholinesterase inhibitors. The effects of cholinesterase inhibition include decreased release of neurotransmitter, myonecrosis, and an attenuated response to directly and indirectly elicited muscle contraction, direct effects on the AChR ion channel, increased functional sensitivity to ACh, and a down-regulation of AChR number on the end plate. The associated myonecrosis may or may not account for the decrease in AChR with chronic cholinesterase inhibition. Voltage clamp electrophysiologic techniques using noise analysis confirmed an adaptive reduction in the number of functional AChRs at the NMJ without affecting single channel properties. The decrease in AChR number may be related to increases in intracellular calcium levels. As expected, dose-response curves to ACh or ScH in this situation will shift to the right; i.e., there is a loss of a cholinergic agonist.

Clinical situations in which pathologic elevations of ACh activity could occur include an overdose of cholinesterase inhibitors in the treatment of myasthenia gravis, chronic administration of reversible cholinesterase inhibitors as prophylaxis during threat of chemical (nerve gas) warfare, and acute and chronic exposure to organophosphorus insecticide compounds (nerve gas). With myasthenia gravis, in which the disease process itself causes decreases in AChRs, the contribution of elevated levels of ACh by chronic use of cholinesterase inhibitors will be difficult to ascertain. More appropriate clinical examples would be those related to organophosphorus poisoning or to chronic use of cholinesterase inhibitors as prophylaxis in chemical warfare. In 1974, the World Health Organization, using data from 19 countries, estimated that approximately 500,000 cases of acute pesticide poisoning occur annually. Modes of intoxication include absorption via the gastrointestinal system, respiratory tract, or skin and deliberate or accidental ingestion. Although acute toxicity occurs because of inhibition of all esterase enzymes, chronic toxicity is related to phosphorylation of specific esteratic enzyme (neurotoxic esterase) in the nervous tissue. A number of other enzymes, including lipases, trypsin and chymotrypsin, also are phosphorylated by organophosphates.

**Clinical Implications**

The duration of action of organophosphorus compounds varies from 60 min to several weeks. A triphasic clinical picture may be seen with organophosphorus poisoning: acute cholinergic crisis, an intermediate syndrome, and a late neurotoxic polynuropathy. The inhibition of acetylcholinesterase results in acute elevations of ACh activity. During the first 24 h, such acute elevations in ACh do not alter AChR number. However, during this time, the dose-response curve to NDMR is shifted to the right (resistance) because of competition for the AChR by ACh and NDMR. The response to ScH is shifted to the left during acute intoxication and, in addition, prolonged paralysis occurs because of the decreased metabolism of ScH due to the inhibition of esterases. During the intermediate phase (> 24 h of poisoning), some of the electromyographic features resemble those of myasthenia gravis or a postsynaptic defect. The continued presence of high concentrations of ACh during this time results in a decreased number of AChRs. Although the response to neuromuscular blockers has not been quantified, one would anticipate sensitivity to NDMR. The myonecrosis that occurs during this phase may also contribute to increased sensitivity to NDMR, and this aspect of altered sensitivity is unrelated to the AChR number. ScH is rel-
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C. CONDITIONING EXERCISE

Neuromuscular Physiology

Stimulation of motor nerve results in the release of large quantities of ACh with resultant contraction of the muscle. Conditioning exercise could be considered an instance in which repeated release of ACh occurs. This should be contrasted with that of organophosphorus poisoning in that the duration of exposure to ACh is short-lived, depending on the duration of the exercise. Moreover, all of the ACh released during exercise would be efficiently and rapidly metabolized by the acetylcholinesterase enzyme. Whether exercise elicits a down-regulation of AChRs is unknown, but the response to NDMR is suggestive of such a change.

Clinical Implications

The response to SCh following conditioning exercise is unknown. With the NDMR metocurine, a response converse to that seen with immobilization was observed: a shift to the left in the dose–response curves (fig. 4). It is interesting that some of the biochemical changes in muscle after exercise are also opposite to those in immobilization. Thus, in physically conditioned patients, the response to normal doses of NDMR may be associated with a greater degree of paralysis in the conditioned muscles. Furthermore, monitoring the blockade of conditioned muscles need not necessarily reflect the surgical needs or the status of diaphragmatic recovery.

VII. Summary

Multiple factors alter the interaction of muscle relaxants with the NMJ. This review has focused on the aberrant responses caused principally by alterations in AChRs (table 1). Many pathologic states increase (up-regulate) AChR number. These include upper and lower motor neuron lesions, muscle trauma, burns, and immobilization. Pre- or postjunctional inhibition of neurotransmission by drugs or toxins also up-regulate AChRs. These include α- and β-BT, NDMR, anticonvulsants, and clostridial toxins. We speculate that other bacterial toxins also up-regulate AChR. With proliferation of AChRs, agonist drug dose–response curves are shifted to the left. The exaggerated release of potassium when depolarization occurs with the use of agonists such as SCh and decamethonium can be attributed to the increased number of AChR. Thus, SCh should be avoided in patients who are in the susceptible phase (see section V). In the presence of increased AChR, the requirement for NDMR is markedly increased. Thus, the response to NDMR may be used as an indirect estimator of increased sensitivity to SCh (table 1).

The most extensively studied pathologic state in which there is a decrease in AChRs is myasthenia gravis; there is immunologically mediated destruction and/or functional blockade of AChRs. The pathophysologic and pharmacologic changes in LEMS are quite distinct from those of myasthenia gravis. Decreased AChRs in myasthenia gravis result in resistance to agonists and increased sensitivity to competitive antagonists. In conditioning exercise, the perturbed muscles show sensitivity to NDMR that may be due to decreased AChRs. Chronic elevations of ACh observed with organophosphorus poisoning or chronic use of reversible cholinesterase inhibitors results in down-regulation of AChRs. In this condition, SCh should be avoided because its metabolic breakdown would be impaired; the requirement for NDMR may be decreased. All of the varied responses to SCh and NDMR, which are associated with concomitant changes in AChRs, are analogous to drug–receptor interactions observed in other biologic systems.

References


Fig. 4. Percent block versus effect compartment concentration of metocurine with twitch stimulation of the gastrocnemius muscle in dogs with exercise (running) and normal (kennel) activity. Muscle in exercised dogs is more sensitive to the effects of metocurine than is muscle from normal dogs, P < 0.05. (Reproduced from ref. 177, with permission.)


12. Burns BD, Paton WDM: Depolarization of the motor end plate by decamethonium and acetylcholine. J Physiol (Lond) 115:41–73, 1951


16. Hogue CW Jr, Imani MS, Martyn JA: Resistance to d-tubocurarine in lower motor neuron injury is related to increased acetylcholine receptors at the neuromuscular junction. ANESTHESIOLOGY 73: 703–709, 1990


28. Martyn JA, Kim CS: Decreased sensitivity to metocurine during chronic phenothiazine may be due to protein binding and receptor changes (abstract). ANESTHESIOLOGY 75:A640, 1991


55. Pedersen SE, Cohen JB: d-Tubocurarine binding sites are located at the $\alpha$- and $\beta$-subunit interfaces of the nicotinic acetylcholine receptor. Proc Natl Acad Sci USA 87:2785–2789, 1990
57. Goldman D, Brenner HR, Heinemann S: Acetylcholine receptor $\alpha$, $\beta$, $\gamma$, and $\delta$-subunit mRNA levels are regulated by muscle activity. Neuron 1:329–333, 1988
66. Busis NA, Daniels MP, Bauer HG, Pudimat PA, Sonderegger P, Schaffner AE, Nirenberg M: Three cholinergic neuroblastoma hybrid cell lines that form few synapses on myotubes are deficient in acetylcholine receptor aggregation molecules and large dense core vesicles. Brain Res 324:201–210, 1984
69. Fischbach GD, Schuetze SM: A postnatal decrease in acetylcholine channel open time at rat endplates. J Physiol (Lond) 305:125–137, 1980
88. Gronert GA, Matteo RS, Perkins S: Canine gastrocnemius disuse


106. Feldman JM: Cardiac arrest after succinylcholine administration in a patient who was exposed to guanin-barre syndrome. Anesthesiology 72:942–944, 1990


Brown JC, Charlton JE: Study of sensitivity to curare in certain neurological disorders using a regional technique. J Neurol Neurosurg Psychiatry 38:34–45, 1975


Dwersteg JF, Pavlin EG, Heimbach DM: Patients with burns are resistant to atracurium. ANESTHESIOLOGY 65:517–520, 1986


Martyn JA: Clinical pharmacology and drug therapy in the burned patient. ANESTHESIOLOGY 65:67–75, 1986


220. Simpson LL: The effects of acute and chronic butylamine toxin treatment on receptor number, receptor distribution and tissue

221. Tonge DA: Chronic effects of botulinum toxin on neuromuscular transmission and sensitivity to acetylcholine in slow and fast skeletal muscle of the mouse. J Physiol (Lond) 241:127–139, 1974


248. Chang CC, Chen TF, Chuang ST: Influence of chronic neostigmine treatment on the number of acetylcholine receptors and the release of acetylcholine from the rat diaphragm. J Physiol (Lond) 230:613–618, 1973


