Review Article

The Role of Prejunctural Effects in Myoneural Transmission

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This article considers current views on the dynamics of transmitter release at the myoneural junction, with special emphasis on prejunctural drug actions and their significance to anesthetists. The reader interested in additional information about the physiology of the myoneural junction is referred to references 1-4. Details concerning research techniques are presented in references 5 and 6. The general subject of drug effects on prejunctural structures is covered in reference 7.

Junctional Anatomy and Physiology

Transmission of electrical signals from motor nerves to muscle fibers across a 200-A junctional gap is a complex phenomenon in which two structures play important roles. These structures are the motor nerve terminal (prejunctural) and the muscle endplate membrane (postjunctural). The present belief is that when the nerve action potential arrives at the motor nerve terminal it releases acetylcholine (ACh) into the junctional gap. ACh would then act as a carrier or "transmitter" of this action potential from pre- to postjunctural structures. This "transmission" is accomplished when ACh interacts with a lipoprotein (cholinergic receptor) in the muscle endplate to cause a transient increase in permeability to Na+, K+, and other ions, and thereby depolarization. This depolarization generates the endplate potential (EPP) which, upon propagation to the muscle fiber, triggers the muscle action potential (AP). Propagation may not take place if the amplitude of the EPP is below the threshold necessary to generate an AP. This occurs in nondepolarizing block. If the endplate is already depolarized, the EPP cannot propagate to an "accommodated" fiber, the situation in depolarizing block.1

Prejunctural anatomy involves description of motor nerve terminals. These structures (0.5 to 1 μ in diameter) lose their myelin sheaths for several microns before ending.10 It is believed that this myelin loss increases their vulnerability to muscle relaxants and ionized anesthetic agents.11 Nerve terminals have various patterns depending on type of muscle12,13; thus, the fast or "white" muscle has only single endings, while in the slow or "red" fibers these endings are multiple. At birth these two types of nerve terminals and muscle fibers are hard to differentiate, but upon maturation they become clearly distinct.14 Animal species is another factor determining the morphology of nerve terminals.15 In frog and reptilian muscles the endings run for more than 100 μ, making a long junctional contact with the muscle (synaptic gutter). In mammals they end in a smaller area (30 μ), forming a tight junction imbedded into the muscle fiber. The exact functional importance of these different morphologic patterns remains unknown, although in every species ACh is synthesized, stored, mobilized, and released at the motor nerve terminal.

The transmitter substance is partially or completely stored in small vesicles at the mo...

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† Throughout this review the terms "depolarizing" and "nondepolarizing" are used to describe muscle relaxants. This classification is helpful since it describes the effect of these drugs on the resting potential of the endplate membrane. However, it should not be used to indicate their mode of action, because the site or mechanism of neuromuscular depression is not necessarily related to changes in the resting membrane potential of the endplate.
tor nerve terminal. Some of these vesicles form a population, or pool, readily available for release. The others are not immediately available, but upon stimulation they can be "mobilized" and become part of the available transmitter pool. While there is little doubt that ACh depolarizes the endplate membrane, the manner in which ACh is activated to produce this effect is controversial. The actual process of transmitter "release" and the nature of the specialized cholinergic receptors have not been discovered.

One hypothesis states that ACh is bound to lipoproteins at both sides of the junctional gap. The nerve action potential liberates prejunctionally-bound ACh, which in turn creates an ionic current between nerve ending and endplate membrane. Transmission of this current to the postjunctional membrane initiates a secondary release of ACh, thereby generating the endplate potential (fig. 1). Unfortunately for this hypothesis, the "ionic current" has not been identified at the myoneural junction. It has been demonstrated at other synaptic junctions, however. In these cases, electrotonic transmission (the electrical signals "jump" the gap without transmitter assistance) is totally or partially involved in sending information across the synaptic gap.

A more widely accepted hypothesis states that ACh is stored in a large pool of units, or quanta. Arrival of the nerve impulse releases a fraction of this pool through a complex process in which Ca++, Na+, and an unspecified carrier (X) play important roles (fig. 1). According to this hypothesis, the release of transmitter is a quantal phenomenon.

THE QUANTAL HYPOTHESIS

The quantal hypothesis assumes that ACh is stored in a large number of preformed pockets, or quanta, which may correspond to the well-known synaptic vesicles. Each unit would have a small probability of being released by the nerve action potential. The average number of vesicles released to the synaptic gap determines the amplitude of the endplate potential.

The unit packet, or quantum, contains about 10^3 molecules of ACh. Under ordinary circumstances quanta are released spontaneously from motor terminals, generating miniature endplate potentials (MEPP's) of variable amplitude (100 μV to 3 mV). The endplate potential is then formed by summation of MEPP's. Consequently, the quantal content (m) of the EPP can be determined directly by dividing the amplitude of the EPP by that of the MEPP, m = EPP/Δ MEPP. Mean values from a few hundred measurements are normally used. Indirectly, m can be calculated through statistical analysis of the endplate potential amplitude distribution.

† According to the proponents of the quantal hypothesis, the probability of release is small and the transmitter pool large. Thus, the amplitudes of the EPP's should follow a Poisson distribution. In a Poisson distribution a given event occurs according to a low probability; if the probability is large the distribution becomes binomial. Most investigators support the quantal hypothesis, but some have doubts about the applicability of the Poisson distribution to transmitter release and advocate use of the binomial distribution for this calculation.
To support the quantal hypothesis the m obtained by direct measurements (EPP/MEPP) and that obtained through statistical analysis should coincide. This is indeed the case provided transmitter release is depressed to less than 1 per cent of its normal level. However, if transmitter output is normal, m is very large and its calculation, using statistical methods, is unreliable owing to nonlinear summation of quantal units, and to the geometry of EPP propagation. The output of ACh may also be approximated by collecting and bioassaying the perfusate during indirect stimulation. This technique is difficult to use in muscle at the present time, but it is of great value in the study of transmitter output in other systems.

The foregoing explanation of transmitter release remains hypothetical. The existence of prejunctural vesicles containing ACh is not proof that they constitute the unit of release, or quantum, although some electromicrographs from the electric organ of Torpedo are suggestive of direct release of ACh from the vesicles. However, not all recent work confirms the idea that ACh released by nerve stimulation is the same ACh stored in vesicles.

In summary, release of ACh from motor nerve terminals is not well understood. It has been compared to a quantal phenomenon in which the nerve action potential would momentarily liberate a number of quanta into the synaptic gap. The actual number depends on the store of ACh available before the arrival of the nerve impulse, and on the probability of each quantum's being liberated by this impulse.
TRANSMITTER MOBILIZATION

According to the quantal hypothesis, transmitter is depleted by each nerve impulse in proportion to the probability of release. It becomes clear that a mechanism is necessary to recharge the nerve terminals in order to maintain efficient transmitter output (transmitter mobilization), and thus permit myoneural transmission during normal muscular contractions requiring nerve discharges at frequencies of 20 to 60 cycles per second.

The turnover of ACh, or transmitter mobilization, takes place at the nerve terminals. Synthesis of ACh depends on the availability of external choline. Uptake of the latter is mediated by a specific sodium-dependent transport process, normally activated by nerve impulses. The release of ACh is calcium-dependent and is affected by the resting membrane potential of the nerve terminals. Newly formed ACh is released to the synaptic gap, hydrolyzed, and the choline recaptured to form new ACh to start the cycle over again.

The study of changes in amplitude in a group of 40 or more endplate potentials evoked by tetanic stimulation of motor nerves gives an idea of transmitter mobilization. The first 5 to 20 EPP’s suffer a progressive reduction in amplitude until a point at which successive EPP’s have uniform size is reached. The rundown of EPP amplitude corresponds to the latent period during which mobilization is being fully activated and reflects the initial size of the pool. This period is followed by the plateau or steady-state amplitude. During the latter period transmitter output is equal to its mobilization (fig. 2). Studies of mobilization, based on this observation, indicate that this process is linearly related to the frequency of motor nerve stimulation, and that greater fractions of transmitter pool are mobilized at higher frequencies.

Evidence for Presynaptic Actions of Drugs

The pharmacologic importance of the myoneural junction was unveiled by the classic work of Claude Bernard with curare in 1850. Initially, it was enough to localize drug effects “somewhere between the nerve and the muscle,” an area known today as the “junction” or “synapse.” With new knowledge the complexity of transmission of impulses from nerve to muscle became evident. Discovery of the role of ACh in synaptic transmission focused physiologic and pharmacologic research on the cholinergic receptors. Little attention was given to the role of nerve terminals in the dynamics of transmitter release, although studies by Langley in 1915 had indicated a principal role for nerve terminals in the pharmacology of the myoneural junction. Masland and Wigtan in 1940 again suggested the pharmacologic importance of these structures.

More recently, Riker and his group considered the motor nerve terminals a likely site for drug action. Later, Standaert developed an in-vitro preparation to study neuromuscular transmission and nerve activity simultaneously. His interpretation of results supported a predominant role of motor nerve terminals in the pharmacology of muscle relaxants and anesthetic agents. Subsequently, several authors using this preparation have reported “prejunctional effects” of all muscle relaxants and anesthetic agents investigated.

These findings gave rise to a controversy between the proponents of cholinergic receptors as the principal site of drug action and those supporting the motor nerve terminal site. Disagreement between these groups is not surprising, as their investigative methods differ considerably. The former group uses “direct” techniques of investigation in paralyzed muscles in vitro. Intracellular electrodes located at or very near the endplate are used, and transmitter output is estimated from statistical analysis of endplate potential amplitude. Changes in the responses of the endplate membrane to directly applied ACh (iontophoresis) indicate the extent of postjunctional drug action.

The latter group usually works with “indirect” techniques in-vitro. Depression of the force of muscular contractions in response to stimulation of motor nerves is observed. In the absence of reduced force with direct stimulation of muscle it is assumed that the drug is acting at the myoneural junction. Depression of nerve action potentials traveling “backward” or antidromically through motor fibers, as part of the indirect techniques, has been used as a proof of prejunctional drug action. This antidromic activity can be seen after tetanic stimulation of ventral roots in cats anesthetized with
chloralose. It is a phenomenon originating at the nerve terminals and depressed by subparalytic doses of muscle relaxants. Depression of antidromic potentials is alleged to indicate reduced transmitter output. The logic is not convincing, since repetitive antidromic firing is not directly involved in transmitter output. Depression of this phenomenon indicates the existence of a prejunctional effect, but does not specify its nature or functional importance. Although there is a relationship between depression of antidromic repetitive firing and depression of neuromuscular transmission, it may be coincidental and not causal.

Further problems of interpreting experimental results arise with the intra-arterial administration of ACh to localize postjunctional sites of action in the indirect techniques. A reduction in the amplitude of muscle contracture produced by intra-arterial ACh is taken as an indication of blockade of postjunctional cholinergic receptors. However, this test ignores the fact that these receptors also occur at motor nerve terminals and that their activation may stimulate the muscle indirectly. This objection is also applicable to the iontophoretic administration of ACh used as part of the direct techniques. Such uncertainties present a formidable obstacle to the study of pure drug-receptor interactions.

Despite the above technical limitations in describing "presynaptic effects," it is important to specify their nature insofar as possible. Pharmacologic agents may depress synaptic transmission by acting on motor nerve terminals through several mechanisms: a) on the propagation or the amplitude of the nerve action potential; b) on the size of the transmitter pool; c) on its rate of mobilization; or d) on the quantal probability of release.

**Propagation and Amplitude of the Nerve Action Potential**

Blockade of neuromuscular transmission may occur because the nerve action potential cannot fully invade the unmyelinated nerve endings. Depolarization, hyperpolarization, or stabilization of these structures may produce this effect. Hypoxia, hyperkalemia, toxic increases in Na⁺ permeability, and the initial administration of succinylcholine and decamethonium depress or block neuromuscular transmission to a large extent by depolarizing motor nerve terminals even though there may be an initial phase of greater transmitter release. As a cause of neuromuscular blockade, hyperpolarization does not have clinical importance. Membrane "stabilization," the effect commonly associated with local anesthetics, has little importance as the sole cause of blockade in clinical situations, but it may play some role when a combination of drugs is used. Small doses of local anesthetics and barbiturates, which do not affect nerve conduction, have been shown to prevent full invasion of nerve endings by the action potential, perhaps by "stabilizing" the membrane of the nerve terminals. Other compounds, tetrodotoxin (TTX) and batrachotoxin, prevent transmitter liberation by selective actions on membrane permeability to Na⁺ and other ions.

**Size of the Transmitter Pool**

Reversible depression of transmitter synthesis by lecithinolium-3 is an example of neuromuscular blockade caused by a reduction of the transmitter pool. According to recent findings in stretched muscles, d-tubocurarine appears to reduce the size of the transmitter pool, an effect that could explain the neuromuscular fatigue during tetanic stimulation characteristic of this drug, as well as the "decurarizing" effect of choline administration. However, some investigators deny that d-tubocurarine depresses the output of transmitter at the myoneural junction, based on statistical analysis of EPP amplitudes.

**Transmitter Mobilization and Quantal Probability of Release**

Each nerve impulse mobilizes a small fraction (0.022 per cent) of the transmitter pool, as shown by direct collection of ACh from perfused rat diaphragms; but greater mobilization is calculated from measurement of end-plate potential amplitude during rapid rates of stimulation. In the latter experiments quantal probabilities of release ranged from 10 to 30 per cent, depending on temperature, muscle, and animal species. Except for the depression of this probability supposedly induced by low Ca²⁺ and/or high Mg²⁺, there is little pharmacologic information bearing on transmitter mobilization and release. Low doses of
d-tubocurarine reduce absolute mobilization rate and increase quantal probability.\textsuperscript{43}

\textbf{Relative Importance of Presynaptic Actions of Drugs}

From the foregoing it is apparent that pharmacologic depression of neuromuscular transmission may be the result of both pre- and postjunctional drug effects. A controversy arises when one asks the relative importance of these two sites of action. Owing to the use of many different experimental preparations, animal species, and ranges of temperature at which these observations are made, different studies yield different answers. At slow rates of motor nerve stimulation it is easier to detect the "static" postjunctional effects. However, if one studies the "dynamics" of synaptic transmission using near-physiologic rates of stimulation (20-50/sec), failure of the various prejunctional functions is uncovered. Therefore, the relative importance of prejunctional impairment is evident only when the functions of motor nerve terminals are fully activated.

Still another complication is introduced by the "margin of safety" concept. The amount of transmitter released by each nerve impulse is approximately five times greater than the minimum required for efficient functional transmission.\textsuperscript{46} The EPP amplitude can be depressed to 20 per cent of its original size by \textit{d}-tubocurarine or high Ca\textsuperscript{2+} before neuromuscular transmission begins to fail.\textsuperscript{28} In fact, the factor of five might be an underestimate. Recent direct determinations indicate that the output of ACh can be depressed 90 per cent before the beginning of transmission failure.\textsuperscript{49} This finding is consistent with electrophysiological findings in muscle under the effect of pancuronium.\textsuperscript{59} Due to the large margin of safety, functional neuromuscular blockade may sometimes be uncovered only by tetanic stimulation of a partially depressed junction.\textsuperscript{56} This junction may still be capable of transmitting single pulses despite depression of pre- and/or postjunctional structures. Another way to look at this functional effect is by correlating the effect of a given dose of muscle relaxant with frequency of stimulation. The faster the muscle is indirectly stimulated, the more effective is the muscle relaxant in blocking transmission.\textsuperscript{71}

\textbf{Roles of Prejunctional Events in Certain Clinical Phenomena}

\textbf{Alterations in Environment of Myoneural Junction}

The function of motor nerve terminals can be affected by environmental changes. For example, lowering the temperature of mammalian myoneural junctions increases the refractory period of prejunctional terminals, reducing not only the speed at which they propagate nerve impulses but also their ability to mobilize transmitter.\textsuperscript{12} Both of these factors contribute to failure of transmission during tetanic stimulation at low temperature. Depletion of transmitter stores by prolonged periods or high rates of stimulation may also cause neuromuscular blockade in the presence of various pharmacologic agents.\textsuperscript{31, 32, 65} In addition, motor nerve terminals, through processes yet unknown, control certain properties of the muscle fiber and, in particular, properties of the endplate membrane. The loss of these functions is seen following denervation. There is then atrophy with partial depolarization of muscle fibers, while their sensitivity to ACh extends beyond the endplate area.\textsuperscript{72} The latter effect may account for the greater K\textsuperscript{+} loss from denervated muscles in response to administration of succinylcholine.\textsuperscript{73}

\textbf{Response to Tetanic Stimulation}

The effectiveness of neuromuscular transmission is usually evaluated in man by measuring strength of muscular contractions in response to single and tetanic stimulation of motor nerves.\textsuperscript{74} In the awake unanesthetized person the strength of contractions in response to single stimulations is uniform and well maintained for long periods. Response to tetanic stimulation is also well maintained at frequencies up to 300 Hz provided the tetanus lasts for only a few seconds (fig. 3). The appearance of posttetanic facilitation or depression depends on the duration and frequency of stimulation during the tetanus. At low frequencies (20-30 Hz) there is potentiation; at high frequencies (100-300 Hz) there is depression (fig. 3). The response to tetanic stimulation is a sensitive test of the function of the myoneural junction, but it is necessary to keep in mind that in addition to neuromuscular transmission, the tetanus is also testing
Fig. 3. Effect of tetanic stimulation—3-second duration—on strength of muscular contractions recorded from adduction of the left thumb of a healthy, awake 32-year-old man during an axillary block. Bipolar stimulation of the ulnar nerve was accomplished with suprathreshold electrical pulses of 0.05-msec duration at a rate of 0.2 Hz before the tetani. There is posttetanic potentiation after tetani at frequencies below 50 Hz. Posttetanic depression occurs at higher frequencies. Note the two speeds of recording. The 2-minute time calibration applies to the slowest part (thick traces). In the fast portion of the record this calibration corresponds to 5 seconds (Galindo, A., and Wyte, S. R., unpublished observations).

Tetanic contractions may not be well maintained if drugs interfere with propagation of EPP's, electromechanical coupling, Ca** mobilization, or metabolic processes of muscle.11, 25, 26

Rapid decay in strength of muscular contractions in response to tetanic stimulation above 20 Hz is known as "fade," "fatigue" or "Wendsky inhibition."17 Its presence has been associated with d-tubocurarine, and is said to characterize the partial neuromuscular blockade induced by "nondepolarizing" drugs. On the other hand, a partial blockade where tetanus is well maintained is said to be caused by "depolarizing" neuromuscular blockers (succinylcholine, decamethonium). These associations may be true in most patients, but are not sufficiently reliable in themselves to identify mechanisms of drug action.76 Nevertheless, the presence or absence of fade in response to tetanic stimulation is a useful clinical tool. It is, therefore, important to understand its causes.

Studies in an isolated rat diaphragm preparation allow simultaneous recording of endplate potentials and strength of muscle contractions during rapid rates of stimulation. These experiments suggest that fade during tetanus may occur through prejunctional mechanisms, including those activated by hypoxia, hypothermia, hypercarbia, procaine, pentobarbital, d-tubocurarine, or phase II block of "depolarizing" muscle relaxants.9, 11, 45, 70 In addition, fade may occur in this preparation due in part to depression of postjunctional mechanisms by d-tubocurarine, pancuronium or neostigmine.68, 61

Recent clinical reports of fade following
various types of tetanic stimulation clearly indicate the usefulness of this simple test. Neuromuscular depression caused by anesthetic agents or by long-lasting effects of d-tubocurarine has been detected by fade in response to tetanus. The strength of contractions during single-pulse stimulation was little depressed. However, there could be some confusion in interpreting results from tetanic stimulations performed at different frequencies, lasting various periods, and repeated randomly, as reported in different papers. There is, then, practical importance in establishing clinical standards of testing to facilitate communication as well as understanding of the pharmacology of muscle relaxants and anesthetic agents. These standards should include the frequency and duration of the tetanus, the rest period between tetani, and the interval between the tetanus and the first single stimulation.

**Myoneural Events in Disease States**

Investigation of the strength of muscular contractions in response to tetanic stimulation of motor nerves is a helpful tool in a variety of pathological conditions. The myasthenic and pseudomyasthenic syndromes are characterized by poor responses to tetanus. In these two clinical syndromes, impairment of transmitter release has been found. Patients affected by them are more susceptible to muscle relaxants of either type. Interestingly, succinylcholine (SCh) and decamethonium (C10) in these patients may induce phase II block without an appreciable degree of phase I block initially. Some antibiotics are known to induce neuromuscular block in man. The site of this blockade has also been localized at the motor nerve terminals, where these drugs impair the release of transmitter.

Experiments on initially unparalyzed rat diaphragms obtained from starved rats indicate that phase II block with SCh or C10 is produced by impairment of the transmitter release mechanism and possibly not postjunctional desensitization as originally believed. This finding strongly suggests prejunctional failure as the reason for the special effect of "depolarizing" drugs on carcinomatous, undernourished and myasthenic patients. The parenteral administration of ionized calcium has been reported to reverse not only the muscular weakness of myasthenic and pseudomyasthenic patients, but also the prolonged apnea induced by succinylcholine. The important effect of calcium on output of transmitter is well known, and its effectiveness in the above clinical conditions may be explained mainly through reversal of prejunctional failure.

**Use of Anticholinesterase Drugs**

Anticholinesterases often reverse phase II neuromuscular block. Unfortunately, this is not a predictable effect, and in some instances more profound depression follows their use. Anticholinesterase (anti-AChE) drugs (neostigmine, edrophonium) are used with the simple pharmacologic purpose of lengthening the life span of ACh, thereby allowing displacement of drugs occupying cholinergic receptors (nondepolarizing muscle relaxants). In view of experimental evidence that anti-AChE drugs may increase transmitter output, there is the possibility that the presynaptic effect of these d-tubocurarine-reversing drugs is also significant. Large doses of neostigmine can also block neuromuscular transmission. Consistent with multiple sites of drug action, anti-AChE drugs appear to interact with cholinesterase at both the endplate and nerve terminal membranes.

In summary, present ignorance of the role of prejunctional cholinergic and cholinesterase sites is an obstacle to complete understanding of the mode of action of anesthetic agents, muscle relaxants, ACh, and anti-AChE. There is little doubt that these compounds act on both sides of the myoneural junction, and that effects on nerve terminals, because of the complex and multiple functions of these structures, may be more significant than presently accepted in producing neuromuscular blockade by most depressant drugs.

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