DRUGS ON SKELETAL MUSCLE

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Summary

Drugs that affect neuromuscular transmission are therapeutically important. They are particularly used in anesthesia to produce muscle relaxation which is beneficial in surgery. These drugs include tubocurarine, the first neuromuscular blocking drug to be used in balanced anesthesia. Succinylcholine (suxamethonium), another neuromuscular blocking drug, acting as a depolarizing agent, has been in use at the same time as tubocurarine. Both drugs have marked limitations and there has been an ongoing search for alternatives, the most successful being pancuronium. But it too has drawbacks, particularly in terms of unacceptable times of onset and offset of action. Drugs such as vecuronium, rocuronium, atracurium and mivacurium have been developed and used. Strategies using anticholinesterase drugs are used to reverse unacceptably prolonged neuromuscular block but these too have limitations. Even now there is a quest for a shorter acting non-depolarizing drug and, while the search continues, a novel approach using suggamadex, a chelator of non-depolarizing drugs, is employed to hasten termination of action. Another clinically important drug which affects the contractility of muscle by impairing neuromuscular transmission is botulinum toxin. This drug is used both to treat serious muscle spasm and for cosmetic purposes. Other drugs exert their action directly on muscle fibers, and of particular clinical interest is dantrolene, which is an important antidote to the malignant hyperthermia which occurs after some volatile anesthetics and suxamethonium which are administered in general anesthesia. Dantrolene is also used to treat persistent muscle spasm. Knowledge of the pharmacology of skeletal muscle has developed at the same time as that of the autonomic nervous system and early observations in both systems have been critical for the general development of physiology and pharmacology of neurotransmission and muscle contractility.

1. Introduction

Skeletal muscle pharmacology embraces the effects of drugs on processes occurring during the time between the excitation of motor nerve terminals and the contraction, and subsequent relaxation, of skeletal muscle. Sequentially these processes are: synthesis of acetylcholine (ACh) in the axon terminal; storage of the neurotransmitter ACh in terminal vesicles; depolarization of the axon terminal; excitation-induced release of ACh from the vesicles; activation of post-junctional nicotinic ACh (nACh) receptors; depolarization of the muscle cell membrane; coupling of depolarization to contraction; and termination of contraction. Additional important aspects of neurotransmission are the termination of the action of ACh by the enzyme acetylcholinesterase (AChE), active

uptake of its metabolite, choline, into the axon terminal, and regulation of the transmitter-ACh release by nACh autoreceptors on the axon terminals. All of these processes are subject to modification by drugs.

Skeletal muscle pharmacology has been pivotal in the early development of pharmacology overall, in ways similar to studies on the autonomic nervous system. This was due largely to ready access to motor nerve-skeletal muscle preparations (such as in frogs and chickens), the ability to easily apply drugs such as curare and nicotine, and the ease with which muscle contractions could be observed. Thus Claude Bernard, in 1844, was able to demonstrate that curare prevented contractions of skeletal muscle when an electrical stimulus was applied to frog motor nerves, but not when the skeletal muscle itself was stimulated. His pupil Vulpian suggested that curare blocked contractions by an action at the motor endplate. Langley, in 1906, using nicotine, which (like motor nerve stimulation) caused skeletal muscle contraction, and curare, which blocked this action, was able to more accurately identify the site of action of curare by showing that nicotine and curare acted on the muscle rather than the nerve terminal. Langley subsequently proposed the existence of a 'receptive substance' in the muscle, and deduced that transmission from nerve to muscle was not electrical but caused by a nerve secretion. It was much later that Dale and colleagues, in 1936, identified ACh as the neurotransmitter. In their experiments they systematically examined the effects of tubocurarine (which was the active principle extracted from curare) on nerve stimulation and on the putative transmitter (ACh), and they were able to reinforce the theory that tubocurarine blocked the effects of ACh rather than prevent its release from nerves. As basic neuromuscular pharmacology was still being developed, tubocurarine was introduced into anesthesia (beginning around 1940) in the hope that the dangerously high levels of anesthetic drugs (diethyl ether and chloroform), that had to be used to paralyze reflex muscle movements, could be reduced. Thus the concept of 'balanced anesthesia' was born, and the search for better and better neuromuscular blocking drugs (and other anesthetic agents) began. The search continues.

After early development of knowledge about neurotransmission, and the neuromuscular junction, more attention has been paid to mechanisms involved in the synthesis and release of transmitter ACh and drugs which control these events. On the muscle side of the junction, researchers and the pharmaceutical industry have also focused on events involved in the coupling of muscle membrane depolarization to contraction, and factors which determine the strength and duration of contraction of muscle fibers. On the basis of this knowledge, drugs which interfere with excitation-release coupling and skeletal muscle contractility are now used therapeutically. Drugs that affect these and all other aspects of the neuromuscular transmission and contraction are also used experimentally.

This Chapter explores drug actions on neuromuscular transmission and muscle contractility with an emphasis on beneficial and adverse actions of therapeutically useful drugs.

2. Neuromuscular transmission and contraction in skeletal muscle

Skeletal muscle consists of muscle fibers arranged in motor units. A motor neuron consists of a single axon (of which there are thousands in a motor nerve) that branches

into as many as 2000 terminal axons. Each terminal axon innervates a post-junctional motor endplate and there is usually one motor endplate per muscle fiber. It follows that when an axon in the motor nerve is stimulated, all the muscle fibers in the motor unit will contract synchronously. The axons in a motor nerve fire out of phase with one another so that the tetanic crest of voluntary gross muscle contractions is smooth (Figure 1).

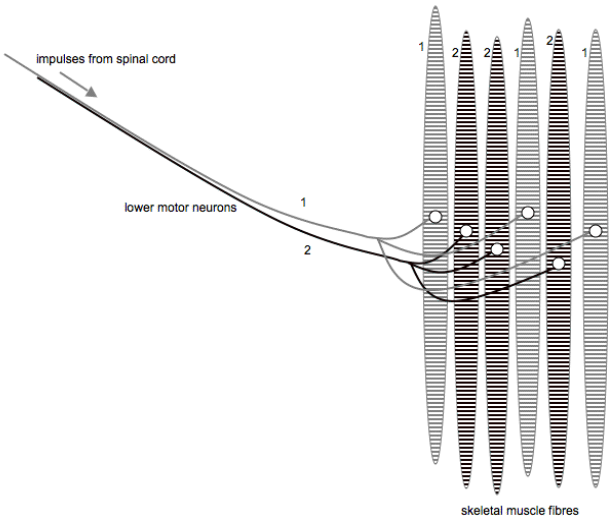

Figure 1. Representation of two motor units in skeletal muscle. Each motor unit comprises a lower motor neuron (LMN), its terminal axon branches and the skeletal muscle fibers that these branches innervate. The diagram represents two motor units, with LMN 1 and 2 each innervating three muscle fibers. During muscle contractions each LMN depolarizes slightly out of phase with other LMNs so that the tension crest developed by the muscle is smooth and sustained. In real life each somatic nerve contains thousands of LMNs and each motor unit consists of thousands of muscle fibers.

The ACh in the nerve terminal is contained in vesicles. Vesicles may spontaneously release their ACh contents by exocytosis but such individual events cause only slight depolarization of the motor endplate (miniature endplate potentials; mepps) which are much too small to initiate an action potential in the muscle fiber. However, when a nerve impulse passes to the axon terminal there is a synchronous release of up to several hundred vesicles that collectively deliver up to 10^6 to 10^7 molecules of ACh to the endplate nicotinic acetylcholine (nACh) receptors, which are ligand gated ion channels, thereby initiating an action potential in the muscle fiber. The firing of the action potential at a single motor endplate is an all-or-nothing event. However, particularly when the safety factor in transmission (see 2.1 below) is reduced, it is possible for some motor endplates to reach their action potential threshold whereas others may not. Thus for the aggregate muscle, consisting of thousands of muscle fibers, each with their own motor endplate, contractions may be graded in intensity.

When the motor endplate receptors are occupied by a nACh agonist and the ligand-gated ion channels open, the muscle membrane begins to depolarize in response to a

rapid influx of Na^+. The opening of potassium ion channels with a resultant outflow of K^+ contributes to the repolarization of the endplate. This transmitter-mediated depolarization is called an endplate potential (epp). Ion pumps restore Na^+ and K^+ levels to their resting states.

The ACh released from the axon terminal is quickly inactivated (within 1 millisecond) by the enzyme acetylcholinesterase (AChE), which is concentrated on the axonal and motor endplate surfaces of the neuromuscular junction. This inactivation takes place before the endplate membrane repolarises in readiness for another action potential (i.e., hydrolysis of ACh by AChE occurs during the refractory period). Consequently, one depolarization of the axon terminal will produce only one propagated action potential along the muscle fiber that it innervates. The situation changes if AChE is inhibited, in which case ACh concentration remains high around the motor endplate nACh receptors so that one axon depolarization can cause sustained depolarization of the motor endplate and repetitive muscle action potentials.

Additional nACh receptors (autoreceptors) on the nerve terminal modulate (enhance) the exocytotic release of ACh caused by the nerve impulse. When the equivalent receptors at adrenergic nerve terminals (α_2-adrenoceptors) are activated by neuronally released norepinephrine (NE) subsequent release of NE is *inhibited* (negative feedback). But when ACh activates the nAChRs in cholinergic motor nerves, subsequent release of ACh is *enhanced*. This positive feedback helps maintain adequate transmitter release, so that the safety factor in transmission is not voided during the high frequency bursts of discharges associated with skeletal muscle contractions. It follows that nAChR competitive antagonists (non-depolarizing blocking drugs), will inhibit ACh release by blocking these prejunctional nACh autoreceptors, and this effect contributes to block of neuromuscular transmission caused by non-depolarizing blocking drugs. Recently another nACh autoreceptor has been identified and it is most easily demonstrated at low frequencies of stimulation. When activated, these nACh receptors *inhibit* subsequent stimulation-induced release of transmitter ACh, but their functional significance is uncertain.

Nicotinic ACh receptors (nAChRs) are grouped broadly into three main types: muscle, ganglionic (also called neuronal) and CNS types. With increasing characterization of receptors according to agonist and antagonist affinities, and molecular cloning and antibody studies, this grouping (which is based on location) is becoming less useful. Within each type there are many nAChR subtypes all of which function as ligand-gated ion channels. nAChRs are pentameric protein structures which span the muscle cell membrane. Each of the five (pentamer) subunits is designated as α, β, γ, δ or ε. Cloning has identified further types of each of these subunits. In humans there are 6 types of α subunit (α2 to α7 ; α1 is found in chickens) and 3 types of β subunit. A typical muscle nicotinic receptor is described as $(\alpha 1)_2\beta 1\delta\varepsilon$ (i.e., the pentamer consists of two α1 subunits, one β1 subunit, one δ subunit and one ε subunit).

nAChRs are found at the skeletal neuromuscular junction both at prejunctional and postjunctional sites. The prejunctional receptors are autoreceptors (see above) and those that mediate an increase in ACh release (which occurs at low frequencies of nerve

stimulation) are classed as neuronal receptors, whereas those that mediate a decrease in ACh release (seen at higher frequencies of stimulation) are the same as those found on the motor endplate and are therefore classed as muscle type receptors. Postjunctional receptors (on motor end plates) are archetypal muscle receptors. At autonomic and sensory ganglia the nicotinic receptors are found on pre- and postjunctional sites and are likewise heterogeneous, which suggests that different subtypes could play distinct roles, and that they might be activated according to particular stimulation conditions. CNS type nAChRs are particularly varied, with at least nine different sequences of α subunits and three different β subunits.

2.1. Safety factor in transmission

Normal neuromuscular transmission has a feature which has an important bearing on skeletal muscle pathology and the actions of neuromuscular blocking drugs: that the amount of ACh released by a single depolarization of the axon terminal greatly exceeds that required to bring the endplate up to the threshold for initiation of a muscle fiber action potential. Thus there is a *safety factor in transmission* of about 400%, which means that it is necessary to block about 80% of the receptor sites before neuromuscular block begins to occur. But this safety factor in transmission can be reduced in disease (such as myasthenia gravis), and by antibacterial drugs and other drugs that interfere with the release of transmitter ACh, and, of course by non-depolarizing neuromuscular blocking drugs (see section 4). In these situations the amount of ACh released by the nerve impulse is reduced to a point where some motor endplates no longer reach the threshold for initiation of an action potential. In other situations, where the safety factor in transmission is still present, but to a reduced extent, subjects become much more sensitive to interventions that impair neuromuscular transmission. Drugs that reduce the safety factor in transmission (and at higher doses cause transmission failure) include the non-depolarizing neuromuscular blocking drugs and botulinum toxin (both are used clinically), and drugs such as antibacterial agents and antimalarial quinolines that are noted for their side effects on neuromuscular transmission. Other drugs, such as hemicholinium and vesamicol, act by impairing transmission but are only used experimentally. Finally, pathology of the neuromuscular junction, particularly in myasthenia gravis, also reduces the safety factor in transmission, and, as disease progresses, causes failure of transmission. These drugs and their effects on transmission are further discussed below and summarized in Table 1.

Class of Drug	Subclass	Example(s)	M of A	Clinical Use/Significance
Neuromuscular blocking drugs	non-depolarizing	tubocurarine, pancuronium, vecuronium	competitive antagonist ACh receptor	surgery requiring muscle relaxation
	depolarizing	decamethonium, succinylcholine	mimics ACh	short surgical procedure; tracheal intubation
Drugs affecting axonal ACh	affects synthesis	hemicholinium	competes with choline for uptake	none; experimental only

	affects storage	vesamicol	inhibits storage in vesicles	none; experimental only
Neuromuscular block reversing agents	anticholinesterases	neostigmine	enhances junctional concentration of transmitter ACh	reversal of non-depolarizing block; myasthenia gravis
	chelating agents	suggamadex		reversal of non-depolarizing block
Antispasmodics	neuromuscular action	botulinum toxin	inhibit release of ACh	wrinkles; muscle spasm
		dantrolene	prevent Ca^{2+} release from SR	malignant hyperthermia
		azumolene	same as dantrolene (30 times more water soluble)	
Centrally acting antispasmodics	GABAergic transmission	baclofen	reduced motor nerve activity	spasm
		benzodiazepines	reduced motor nerve activity	spasm
	glycinergic transmission	mephenesin	reduced motor nerve activity	spasm
Drugs which enhance contractility	methylxanthines	caffeine	enhanced release of Ca by SR	none; experimental only
Drugs which reduce contractility	β_2-adrenoceptor agonists	salbutamol	enhanced reuptake of Ca by SR	adverse effect of tremor

Table 1. Actions Of Drugs On Neuromuscular Transmission And Muscle Contractility.

2.2. Train of four and tetanic stimulation

In order to assess depth and nature of block when neuromuscular blocking drugs are used in anesthesia, a technique called *train of four stimulation* is used. The anesthetist places a stimulating electrode on the skin of the wrist above the ulnar nerve. Depth of block is assessed by observing adduction of the thumb, or EMG activity of the adductor pollicis, each time the nerve is stimulated. A train of four stimulation is applied at 2Hz for 2 seconds every 10 or 20 seconds. Before onset of block, each contraction (or EMG activity) in the train of four is of the same intensity. However, with onset of block with a non-depolarizing drug there is a successive diminution of the response between the first and last contraction in the train of four. This is because each stimulation of the nerve releases less transmitter ACh than the previous one and, because the postjunctional nACh are occupied by the antagonist (non-depolarizing) blocker, fewer endplates reach threshold for propagation of an action potential with each successive stimulation. The reason why there is no diminution of response before onset of block is because of the large safety factor in transmission (see Section 2.1) so that, even though there is a successive reduction in ACh release from the first to the last stimulation in the train, there is still enough ACh released to successfully stimulate all motor endplates to

an action potential threshold.

Analysis of the endplate potentials (epps) and miniature epps (mepps) during train of four stimulation show that both the quantal size (the number of neurotransmitter molecules released by a single synaptic vesicle during exocytosis) and the quantal content (number of vesicles released per nerve stimulation) decline from stimulus to stimulus. The latter effect is due to a presynaptic inhibitory action of the antagonist (bearing in mind that at the neuromuscular junction ACh normally acts on presynaptic receptors to *enhance* ACh excitation-release coupling).

During block by non-depolarizing neuromuscular blocking agents, anesthetists use the train of four procedure to establish whether block is deep enough for surgery to begin and, at the end of the operation, to determine if it safe to administer an anticholinesterase drug to reverse the block. A major consideration is that an anticholinesterase will *not* reverse block by a competitive antagonist until the amount of ACh at the neuroeffector junction (which builds up because ACh is not being metabolised by cholinesterase), is great enough to successfully displace the antagonist at the nACh receptors. Experience shows that there must be significant recovery from block (due to a fall in the concentration of the blocking drug at the neuromuscular junction) before this happens, and that, by otherwise increasing the dose of anticholinesterase serious parasympathomimetic actions due to cholinergic overstimulation (e.g., copious respiratory secretion, bronchospasm, gut spasm) may result.

Anesthetists also use train of four stimuli to determine the depth and nature of block by depolarizing blockers, such as succinylcholine, which act by stimulating nACh receptors. Train of four contractions are different in subjects who have received succinylcholine compared to those given non-depolarizing blockers, and changes as the subject proceeds from so-called Phase I to Phase II block. In Phase I block (block by depolarization due to persistent activation of receptors) successive stimuli in the train of four do not produce a fall in tension of contractions (or associated EMG activity) because the depolarizing blocker is not acting as an antagonist at nACh receptors. If Phase I block proceeds to phase II block then the train of four responses take on the characteristics of competitive type non-depolarizing block. Phase I and Phase II block are described in more detail in Section 5.

Tetanic stimuli are also applied by anesthetists as a way of confirming depth and nature of block. During block by non-depolarizing (competitive antagonist) drugs there is a predictable fade of tension in 50 Hz tetanic contractions (Wedensky inhibition) as the neurally released acetylcholine quantal size and content diminishes with each successive impulse. Additionally there is a post-tetanic enhancement of tension of train of four contractions due to the build up of ACh at prejunctional facilitatory nACh receptors during the tetanus. In contrast, during Phase I of block by a depolarizing drug (nACh receptor agonist; noncompetitive), a tetanic stimulus produces a tetanus of small but sustained intensity, similar in shape to that seen before the drug (i.e., no Wedensky inhibition), and, after the tetanic stimulation, the train-of four stimuli produce muscle contractions equal in intensity and character to contractions before the tetanus (i.e., no post-tetanic facilitation).

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Biographical Sketch

Michael Nott is an Australian who graduated with honours in pharmacology at the University of Melbourne and undertook PhD studies in pharmacology at the Department of Pharmaceutical Science at Strathclyde University with WC Bowman as supervisor. His thesis and early published papers (with Bowman and colleagues) dealt with the effects of adrenoceptor agonists on skeletal muscle with a particular emphasis on tremorigenic actions of β_2-adrenoceptor agonists used to treat asthma. The intention of the research was to determine mechanisms causing tremor and to see if it was possible to distinguish between lung and muscle adrenoceptors which mediated the actions. Since then Nott has continued work on skeletal muscle and published and supervised student work in cardiovascular pharmacology.

Nott taught pharmacology at Strathclyde University and then returned to Australia to the University of Melbourne where he has spent most of his academic career. His commitment to teaching and learning saw him take the position of Director of the Science Multimedia Teaching Unit from the mid 1990s to 2000. He has spent one year at the University of Washington working on intelligent databases for the drug treatment of cancer, and another sabbatical in computer based learning at the Open University (UK). From 2000 until the present he has taught pharmacology at LaTrobe University in Bendigo (Victoria, Australia) and at the Fiji School of Medicine in Suva (Fiji) and is currently Senior Lecturer in Pharmacology at RMIT University in Melbourne.