Neuromuscular function and transmission

Allan Fletcher

Abstract

The neuromuscular junction (NMJ) is a chemical synapse between a motor neurone and a skeletal muscle cell. It has been the most intensively studied synapse in the body owing to its comparatively large size, relative simplicity and accessibility. Commands from the central nervous system are transmitted along motor neurone axons, resulting in the release of the neurotransmitter acetylcholine from axon terminals. The transmitter activates nicotinic cholinergic receptors located on the muscle cell membrane. These receptors are ligand-gated cation channels. Upon binding of acetylcholine, the receptor channel opens to allow mainly Na^+ ions to enter the muscle cell, causing a partial membrane depolarization. This triggers action potentials in the muscle cell membrane, resulting in Ca^{2+} influx and muscle contraction. The structure and function of the NMJ are such that, even under the extremes of muscular exertion, the operation of the NMJ is highly reliable. This is because a large 'safety factor' is intrinsic to NMJ function in the sense that more neurotransmitter is released than is necessary to initiate muscle contraction. However, the normal function of the NMJ can be severely disrupted by drugs, naturally occurring toxins and disease. Myasthenia gravis, for example, is characterized by muscle weakness and easily fatigued muscles. It is an autoimmune disorder in which the body produces antibodies to the muscle nicotinic receptor protein.

Keywords Acetylcholine; chemical synapse; myasthenia gravis; neuromuscular junction (NMJ); nicotinic receptor; safety factor

Learning objectives

After reading this article you should be able to:

- draw and label a diagram of a neuromuscular junction, indicating the main ion fluxes that are responsible for its functioning in the pre-synaptic axon, the end-plate and the muscle
- give an account of the structure of the receptors and ligand-gated channels in the end-plate and explain how they respond in the presence of neurotransmitter
- explain how an end-plate potential is generated and how it triggers an action potential in the muscle

The chemical synapse is a specialized junction between a neurone and another neurone (or a muscle cell) which allows the transfer of information between cells that is mediated by the release of a neurotransmitter. Owing to its relatively large size, comparative simplicity and accessibility, most of the basic features of synaptic neurotransmission were discovered by studying the neuromuscular junction (NMJ), the synapse formed by a motor neurone with a skeletal muscle fibre.

The instruction 'telling' a skeletal muscle fibre to contract originates within the central nervous system and travels as action potentials along the axon of a motor neurone. The arrival of action potentials at the axon terminal triggers the release of acetylcholine, which activates post-synaptic receptors on the muscle cell membrane. These receptors are ion channels that open in response to the binding of the acetylcholine neurotransmitter, allowing the influx of Na^+ ions and thereby partially depolarizing the muscle cell membrane. This partial depolarization initiates action potentials (see page 258 of this issue) in the muscle cell plasma membrane, which induce a rapid increase in intracellular calcium concentration. It is the abrupt rise in intracellular $[Ca^{2+}]$ which activates the contractile mechanism of the muscle fibre. This short review examines this sequence of events in more detail in order to explain the main features of neuromuscular function and transmission.

The motor unit

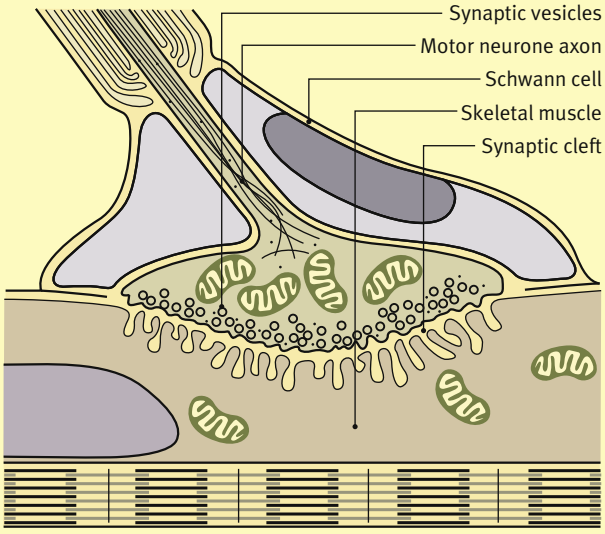
The motor neurones that induce contraction of the main force-producing fibres in skeletal muscle are called alpha motor neurones. A single alpha motor neurone may synapse on a number of individual muscle cells (fibres) because of the neuronal axon branching towards the terminal region (although each muscle fibre is innervated by only one motor neurone). A single motor neurone together with the different muscle fibres it innervates is referred to as a motor unit. One motor neurone may innervate more than a 1000 muscle fibres in large muscles (e.g. in the leg), whereas the motor unit is much smaller (as few as two or three muscle fibres per motor neurone) in muscles responsible for precisely controlled movements such as in the fingers or eyes. Skeletal muscle fibres are either of the rapidly contracting/quickly fatiguing type or of the slowly contracting type which is capable of more sustained contraction. The nature of the muscle fibre phenotype, together with other features such as clustering of the post-synaptic nicotinic receptors at the end-plates, is controlled by growth factors secreted from the nerve ending. Conversely, chemical signals (e.g. insulin-like growth factor) produced by muscle cells are thought to stimulate the development of the pre-synaptic nerve terminal during synaptogenesis.

The structure of the neuromuscular junction

Figure 1 shows a detailed diagrammatic representation of the structure of an individual neuromuscular synapse. The pre-synaptic terminal of each axonal branch is expanded to form a pre-synaptic bouton, from which the acetylcholine transmitter is released into the synaptic cleft (the gap between pre-synaptic and post-synaptic membranes is approximately 15–30 nm). The post-synaptic receptors for acetylcholine are ligand-gated cation channels located in the post-synaptic muscle cell membrane. The post-synaptic area of the muscle cell membrane is called the motor end-plate and is approximately 3000 μm^2 in area. Activation of the post-synaptic receptors results in Na^+

Allan Fletcher *BSc is currently an e-tutor for the Department of Biomedical Sciences, University of Ulster, UK, following several years as a Teaching Fellow at the University of Durham, UK, lecturing in Biomedical Sciences (mainly pharmacology and neuroscience).*
Conflicts of interest: none.

Sagittal section through a neuromuscular junction



The synapse formed by a motor neurone terminal at a skeletal muscle fibre. A glial Schwann cell is shown in close contact with the pre-synaptic terminal. The post-synaptic membrane is highly folded to optimize nicotinic receptor activation, acetylcholine hydrolysis and voltage-gated sodium channel activation

Figure 1

influx which partially depolarizes the muscle cell membrane to trigger action potentials, resulting in muscle contraction. One or more glial cells (peri-synaptic Schwann cells) form close contacts with the pre-synaptic nerve ending and are now thought to participate actively in NMJ function.

Physiology of neuromuscular transmission

Pre-synaptic events

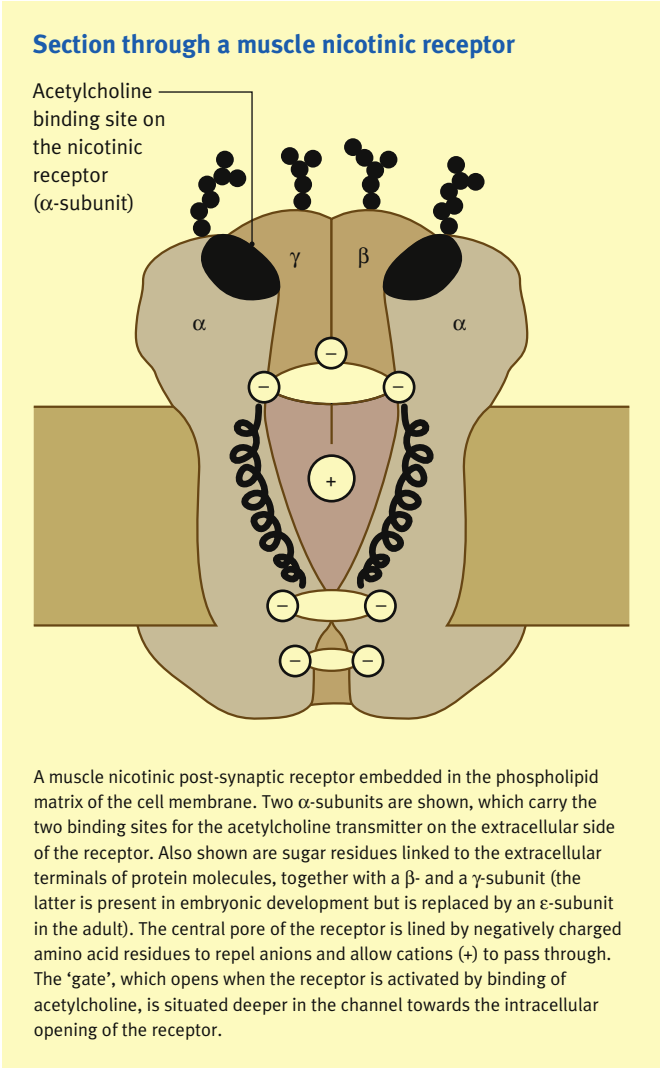
When the bouton membrane is depolarized by the arrival of an action potential, voltage-gated calcium channels in the membrane open to allow the rapid influx of calcium ions down their concentration gradient. The rise in intracellular Ca^{2+} concentration triggers the release of acetylcholine by a complex mechanism which mediates the fusion of intracellular transmitter-containing membrane vesicles with the pre-synaptic membrane. The release mechanism is not yet completely understood but involves the interlinking of specialized proteins (SNARE proteins) embedded in the vesicle membrane and in the pre-synaptic membrane. This process is initiated and controlled by other nearby membrane-bound proteins and by soluble cytoplasmic proteins. The 'calcium sensor' which responds to increased cytoplasmic calcium concentration to initiate the release mechanism is a membrane-associated protein called synaptotagmin. Once a vesicle has fused with the pre-synaptic membrane and emptied its contents into the synapse (exocytosis), it 'buds off' intracellularly (endocytosis) to be recycled and refilled with transmitter for subsequent release (the so-called 'vesicle cycle'). Therefore, neurotransmitter release is quantal (i.e. acetycholine is released in discrete 'packets' or quanta — a single quantum being the contents of a single pre-synaptic vesicle (approximately 5000 to 10,000 molecules of acetylcholine)). This phenomenon was initially discovered by detailed analysis of the post-synaptic effects of spontaneous neurotransmitter release which occurs at a steady low level in the absence of motor neurone stimulation (see below).

Neurotransmitter-containing vesicles are clustered at 'active zones' of pre-synaptic release such that acetylcholine is released directly onto post-synaptic clusters of nicotinic receptors. In addition to stimulating post-synaptic nicotinic receptors, released acetylcholine can also activate muscarinic and nicotinic receptors located in the pre-synaptic membrane to modulate transmitter release under certain circumstances (e.g. very high frequencies of motor neurone stimulation). The action of released acetylcholine is rapidly terminated by enzymatic hydrolysis of the neurotransmitter. This is mediated by the enzyme acetylcholinesterase, which splits the acetylcholine molecule into choline and acetate. Choline is transported from the synaptic gap into the pre-synaptic terminal by carrier proteins located in the membrane. It is then recycled for more acetylcholine synthesis by the enzyme choline acetyl-transferase.

In addition to acetylcholine, the pre-synaptic vesicles also contain adenosine triphosphate (ATP) in an approximate ratio of 1 ATP molecule to 5 acetylcholine molecules. ATP stabilizes acetylcholine within the vesicle and is co-released upon pre-synaptic membrane depolarization. Synaptic ATP (and adenosine formed by ATP hydrolysis) has both pre-synaptic and post-synaptic actions to modulate acetylcholine release and to facilitate nicotinic receptor activation respectively.

Post-synaptic events

The post-synaptic membrane is folded into a series of 'clefts' or 'valleys' (Figure 1) with the post-synaptic cholinergic receptors being clustered on the crests between, and at the entrances to, each cleft. Acetylcholine activates different types of receptor located in different tissues — the post-synaptic receptor at the neuromuscular junction is the muscle-type nicotinic receptor (which differs slightly from neuronal nicotinic receptors located on brain and autonomic neurones or on adrenal medullary cells). The nicotinic cholinergic receptor (Figure 2) is a ligand-gated ion channel (i.e. it is 'gated' (opened) by the binding of a neurotransmitter ligand, as opposed to voltage-gated channels which are opened by changes in membrane electrical potential gradients — voltage). Two acetylcholine molecules must bind to the receptor simultaneously before it undergoes a conformational change, resulting in opening of the central ion channel. The nicotinic cholinergic receptor is composed of five individual protein subunits which are closely associated in the membrane to form a central pore or channel. There are five distinct subtypes of subunit (designated α,β,γ,δ and ε), with nine different isoforms of the α subunit (α_1 to α_9) and four different isoforms of the β subunit (β_1 to β_4). The muscle nicotinic receptor present during human embryonic development comprises two α_1 subunits, and one each of β_1, δ and γ; in the adult, the γ subunit is replaced by ε. Other proteins associated with the nicotinic receptor control the turnover of the receptor in the membrane (rate of removal of receptors and replacement by new receptors) and the clustering



A muscle nicotinic post-synaptic receptor embedded in the phospholipid matrix of the cell membrane. Two α-subunits are shown, which carry the two binding sites for the acetylcholine transmitter on the extracellular side of the receptor. Also shown are sugar residues linked to the extracellular terminals of protein molecules, together with a β- and a γ-subunit (the latter is present in embryonic development but is replaced by an ε-subunit in the adult). The central pore of the receptor is lined by negatively charged amino acid residues to repel anions and allow cations (+) to pass through. The 'gate', which opens when the receptor is activated by binding of acetylcholine, is situated deeper in the channel towards the intracellular opening of the receptor.

Figure 2

of nicotinic receptors at the end-plate. Rapsyn associates with the receptors causing them to cluster, and agrin (released from the motor neurone terminal) promotes clustering via activation of a tyrosine kinase receptor (MuSk). Muscle denervation results in the spread of nicotinic receptors over the entire muscle cell membrane, primarily because of the absence of neuronally released agrin.

The opening of nicotinic receptor channels at the end-plate allows Na^+ ions to flow down their concentration gradient to the interior of the muscle cell. This induces a partial depolarization (called the end-plate potential; EPP) of the membrane, which is sufficient to initiate an action potential by triggering the opening of adjacent voltage-gated sodium channels (clustered deep within the clefts of the folded post-synaptic membrane). The translation of action potentials in the muscle membrane into muscle contraction is known as excitation–contraction coupling. It must be emphasized that the end-plate potential (which corresponds to the excitatory post-synaptic potential (EPSP) in neurone-to-neurone synapses) is fundamentally different from the action potential; for example, it is localized to the end-plate region (non-propagating) and its magnitude is graded (proportional to the number of activated nicotinic receptors) rather than being an 'all-or-none' response. Even in the absence of motor neurone stimulation, there is a steady low level of spontaneous acetylcholine release which evokes small post-synaptic depolarizations called miniature end-plate potentials (MEPPs). Detailed analysis of MEPPs revealed that their magnitude had a minimum value, and that bigger MEPPs always had a magnitude which was a multiple of the minimum value. This could be explained only by assuming that acetylcholine was released quantally (as 'packets', rather than 'dribbling' out of the pre-synaptic membrane) and that the smallest possible MEPP was evoked by the release of a single quantum. With the subsequent development of electron microscopy the pre-synaptic vesicles could be visualized, leading to the realization that the 'quanta' were actually individual vesicles containing neurotransmitter.

Safety factor

The structure and function of the NMJ are designed such that successful neurotransmission is virtually guaranteed; in the healthy human body, even under the most extreme limits of muscular exertion, the NMJ functions satisfactorily. One of the main reasons for this reliability is that more transmitter is released from the motor neurone terminal than is required to trigger action potentials in the post-synaptic membrane. This ensures that, at prolonged high-frequency stimulation of muscle contraction, neurotransmission is still reliable. This reserve capacity of neurotransmitter release gave rise to the concept of a large 'safety factor' in NMJ transmission. Structural features of the NMJ also contribute to the failsafe nature of transmission: pre-synaptic Ca^{2+} entry occurs mainly at the active zones where the voltage-gated Ca^{2+} channels are clustered near to the vesicles; the active zones are positioned opposite the clusters of post-synaptic receptors to minimize the distance the acetylcholine molecules must diffuse in order to bind to the receptors; voltage-gated sodium channels are clustered in the depths of the post-synaptic folds where the membrane depolarization is greatest following nicotinic receptor activation; there is a high density of nicotinic receptors at the end-plate (10,000–20,000 receptors per μm^2); and the magnitude of the end-plate potential is greater than the threshold required for triggering action potentials.

Pathophysiology of neuromuscular junction function

Although normal NMJ function is a highly reliable process, neurotransmission can be severely disrupted by drugs, naturally occurring toxins and several disease states. Muscular paralysis induced by curare results from the nicotinic receptor antagonist action of the main active constituent, D-tubocurarine. Another receptor antagonist, α-bungarotoxin, is a potent constituent of some snake venoms. Other toxins block transmission by disrupting the pre-synaptic acetylcholine release mechanism; for example, the bacterium *Clostridium botulinum*, responsible for many serious outbreaks of food poisoning, produces a toxin which is a mixture of proteolytic enzymes targeting the different proteins mediating the release process; black widow spider venom contains α-latrotoxin which also disrupts release. Myasthenia gravis, a disease characterized by progressive voluntary

muscle weakness, is an autoimmune disorder in which the postsynaptic nicotinic receptors are attacked by antibodies. Another autoimmune disorder, Lambert–Eaton myasthenic syndrome (also characterized by muscle weakness and rapid onset of muscle fatigue), is a consequence of antibody attack on the presynaptic voltage-gated calcium channels. ◆

FURTHER READING

Bear MF, Connors BW, Paradiso MA. Neuroscience: exploring the brain. 3rd edn. New York: Lippincott, Williams and Wilkins, 2007.

Ganong WF. Review of medical physiology. 21st edn. New York: McGraw-Hill, 2003.

