1.1. Introduction
Muscle relaxation is an important tool in the treatment of patients during anaesthesia for surgical and diagnostic procedures, and during treatment in the intensive care unit. Such muscle relaxation is obtained by pharmacological intervention with neuromuscular transmission with either depolarizing or non-depolarizing relaxants. Suxamethonium presently is the only depolarizer in clinical use, but has many adverse effects. A large number of non-depolarizing relaxants has been introduced into clinical practice; however, none of them matches perfectly with the 'ideal muscle relaxant'. This demands the development of new compounds. Some of the new development in neuromuscular transmission and its blockers are discussed.

1.2. Neuromuscular transmission
For normal muscular functions an impulse must be transferred from the nerve terminal to the muscle. Such a transfer takes place at the neuromuscular junction, which consist of the nerve terminal, a 50-100 nm wide junctional cleft, and the postjunctional muscle membrane [1].

In research on the neuromuscular junction it has been proven that the various morphological structures and their molecular interactions did not change significantly during evolution. Therefore, the results found in other species, and in different neurotransmitter involved transfer systems, can be extrapolated to the neuromuscular junction in humans.

1.2.1. The junctional cleft
The cleft forms a physical barrier to the continuity of the nerve impulse, that only can be bridged by a neurotransmitter, in this case acetylcholine (Fig. 1.1). The junctional cleft contains a basement membrane on which acetylcholinesterase is located. Acetylcholinesterase is produced in the nerve terminal and is secreted into the cleft. Its concentration is highly depending on nerve activity [2-3]. It is involved in the rapid specific hydrolytic breakdown of acetylcholine [4-6]. Each molecule of acetylcholinesterase can bind six molecules of acetylcholine [7]. Each binding site of the enzyme acetylcholinesterase possesses two active subsites: the esteratic site, which is involved in the hydrolysis of the ester binding, and the anionic site, in which an anionic subsite is involved in the binding of acetylcholine.

\[
\text{CH}_3\text{NCH}_2\text{CH}_2\text{OCCH}_3
\]

Acetylcholine

**Figure 1.1**
Structural formula for acetylcholine.
acetylcholine. The destruction of acetylcholine is very fast, most of a quantum being metabolized within 1 ms. It takes ca. 80-100 μs to split one acetylcholine molecule. Acetylcholinesterase can be blocked by anticholinesterases, which on the one hand can reverse a non-depolarizing neuromuscular blockade, but on the other hand can induce a desensitization block.

In the cleft a network of fibers, connecting the nerve terminal and the muscle membrane, is also present. This network ties the two elements together, but also forms a preferential pathway for acetylcholine diffusion across the cleft.

1.2.2. The nerve terminal
One of the major functions of the nerve terminal is the rapid secretion of acetylcholine in response to electrical signals. Acetylcholine is synthesized in the neuronal cytoplasm [8]. An acetyl group is transferred by choline-acetyltransferase from acetylcoenzyme A to a choline molecule. The enzyme is produced in the cell body of the neuron and transported down the axon. Acetyl-CoA is supplied by intracellular metabolic processes [9]. Choline is supplied by uptake from the extracellular fluid [10]. Fifty per cent of the choline comes from reuptake after acetylcholine hydrolysis [11]. The uptake is mediated by a sodium-dependent mechanism [12]. Choline transport is the rate-limiting step for the synthesis of acetylcholine [13]. The majority of acetylcholine is stored in vesicles (Fig. 1.2) [14]. The filling of the vesicle is mediated by an acetylcholine transporter [15]. A membrane-located ATPase-dependent proton pump first pumps protons into the vesicle. The protons are then exchanged for acetylcholine through the acetylcholine transporter [15]. Vesamicol is a potent selective inhibitor of acetylcholine uptake into prejunctional vesicles (inhibition of reuptake) [17]. Each vesicle contains approximately 10 000 molecules of acetylcholine, and is the basis for the quantal release of acetylcholine.

Vesicles containing acetylcholine that are available for immediate release, are located near the active zones in the nerve terminal (Fig. 1.2) [18]. Also there is a reserve store of vesicles that can be mobilized and they are located further away from the membrane (Fig. 1.2). The active zones are involved in the exocytosis of acetylcholine, and are located just opposite the openings of the junctional folds in the muscle membrane. The acetylcholine diffuses across the junctional cleft toward the acetylcholine receptors located on the crests of the muscle membrane with a density of 10 000-20 000 molecules/μm² [19-20]. Each acetylcholine receptor is firmly anchored in the endplate by fine cytoskeleton filaments [21]. Together with acetylcholine, a series of cotransmitters is released which are involved in the normal function of neuromuscular transmission. Most of the cotransmitters are peptides.

There is a delay of 0.5-0.6 ms between the arrival of the action potential at the nerve terminal and the discharge of acetylcholine in the synaptic cleft. This reflects the occurring prejunctional events [22]. The delay is temperature sensitive, increasing with hypothermia [23].

When an action potential reaches the nerve terminal, a depolarization of the prejunctional membrane occurs resulting in the opening of voltage-operated calcium channels, and a small influx of calcium into the cytoplasm. Only 2% of the calcium channels need to be opened to finally cause acetylcholine release [24]. The initial calcium influx increases the membrane conductivity to Ca²⁺. The intracellular calcium concentration increases and the transmitter release machinery is activated by rendering it more sensitive to calcium (calcium voltage theory) [25]. Ca²⁺ activates a protein kinase C. This starts the phosphorylation of several proteins located on the cytosolic surface of the vesicle (the so-called v-SNAREs, i.e. synaptobrevin) docked near the active zones in the nerve terminal, and on the cytosolic site of the plasmalemma (the so-called t-SNAREs, i.e. syntaxin, Rab, SNAP-25) [26]. The two systems recognize each other and then interact after activation by synaptotagmin and facilitation by RabGTPases, to form the SNARE-complex [27]. It leads first to facilitation of the mobilization of vesicles [28]. Synaptotagmin is activated and docks vesicles at the active zones through binding to syntaxin or SNAP-25. Than a second docking system, involving synaptobrevin and syntaxin of SNAP, is activated, which also removes synaptotagmin from its site.

Thereafter a site is occupied by rabphilin, which binds to Rap (another protein). This causes double docking of the vesicle. Fusion of the double docked membrane starts and acetylcholine is released. The fused vesicle is rapidly replaced by a vesicle from the reserve pool of vesicles. It must be recognized that many neurotoxins act by binding to the SNAREs, and in this way disturb transmitter release and neuromuscular transmission.

The active zones are rich in calcium channels, and thus it is understandable that the process of docking,
fusión, and release is mainly concentrated at these active sites [29 30].

The membrane of the fused vesicle is recycled by endocytosis, and subsequently refilled with acetylcholine (Fig. 1.2). Endocytosis is also a complex process, again involving a series of membrane proteins [31]. Clathrin coats the membrane that originally constituted the vesicle, thereupon it invaginates, closes and separates from the plasmalemma. The clathrin coat is then removed [32]. The whole cycle takes less than a minute. Part of vesicle recycling is through immediate closure of the vesicle upon release of acetylcholine (‘kiss-and-run’) [33]. An important protein in endocytosis is the GTPase dynamin, which is involved in fission of the clathrin-coated vesicles [34]. Dynamin forms a ring around the stalk of the vesicle [35]. Vesicles are also produced by budding off from endosomes, after coating with clathrin [36]. Fission is obtained with dynamin. The recycled vesicles cluster.

In this process synapsin, a protein bound on the cytosolic vesicle surface, is involved [37]. Synapsin also binds to the vesicles to the cytoskeleton (vesicle clustering). Endocytosis is triggered by intracellular calcium.

One quantum of acetylcholine is released upon fusion of one single vesicle with the prejunctional membrane [38]. Upon a depolarizing nerve impulse, 400-500 quanta of acetylcholine (each 2000-10 000 molecules) are released. The discharge of acetylcholine molecules in the cleft must be fast in order to reach the necessary high concentration in the receptor area, and thus cannot depend on diffusion [39].

Apart from quantal release, non-quantal release of acetylcholine also occurs. Cytoplasmic acetylcholine leaks continuously into the junctional cleft. With nerve stimulation there is some increase in the release of cytoplasmatic acetylcholine [40]. Vesicles can also release acetylcholine spontaneously. The spontaneous release of acetylcholine causes miniature endplate potentials that are not able to depolarize the muscle cell membrane, but seems to play a tonic and trophic role for the muscle. Upon nerve stimulation acetylcholine is released in quanta by exocytosis of the vesicle. Sufficient miniature endplate potentials are then present, which summate and cause an endplate potential [41]. The number of acetylcholine quanta released is in excess of the amount required to evoke the actual degree of endplate depolarization for muscle contraction.

Only a limited amount of acetylcholine is available for immediate release, the remainder is stored in a depot or back-up store [42 43]. Apparently the normal level of transmitter mobilization is unable to supply sufficient acetylcholine for high frequency release. At high stimulation rates more acetylcholine must be mobilized. Mobilization is the process by which the nerve supply of acetylcholine is moved into the immediately available compartment as it is depleted by the process of vesicular exocytosis. The mobilization and release of acetylcholine is modulated by prejunctional acetylcholine autoreceptors. In this process both nicotinic and muscarinic autoreceptors are involved. The nicotinic prejunctional receptors serve a positive feedback mechanism: they enhance mobilization of acetylcholine [44]. Muscarinic autoreceptors serve as a negative feedback mechanism: they stop acetylcholine release when enough transmitter is present [45]. Similar effects have been found for the cholinergic transmission in brain and bronchial smooth muscles [46 47]. The prejunctional acetylcholine receptors contain only α and β subunits (see later) [48]. They, however, consist of several subtypes of α and β strains. Acetylcholine receptor antagonists (non-depolarizing muscle relaxants) decrease the acetylcholine mobilization and release by occupation of presynaptic receptors [49].

**1.2.3. The postjunctional membrane**

Acetylcholine diffuses across the junctional cleft and binds to nicotinic acetylcholine receptors (Fig. 1.3). The nicotinic acetylcholine receptor is a transmembrane protein, forming a ligand-gated ion channel. Each motor endplate contains ca. 10 million acetylcholine receptors. The acetylcholine receptor is constituted of 5 homologous subunits (Fig. 1.3), α2 β ε δ in mature receptors and α2 β γ δ in immature receptors [51 52]. During maturation of the neuromuscular junction the γ-subunits are thus replaced by ε-subunits [53]. ARIA (acetylcholine receptor inducing activity), produced in the nerve terminal and secreted into the cleft, is involved in this replacement, and stimulates the production of acetylcholine receptors [54 55].

![Figure 1.3](image-url)

*Figure 1.3*

The 5 subunits of the acetylcholine receptor from an ion channel embedded in the membrane lipid layer.

However, ARIA has no effect on receptor accumulation. The maturation of the receptor results in acceleration of channel conductance, which is mediated by one single amino acid [56]. It was recently demonstrated that during development of muscles, most ARIA is produced by the muscles [57]. With nicotinic receptors some subtypes do exist, depending on the structure of the α-subunit [58].

Each subunit contains ca. 2330 amino acids. The order of the subunits is clockwise, α β α γ δ (immature) and α β ε δ (mature). The molecular weight of the α-subunit is 35 000 Dalton, of the β-subunit 37 000 Dalton, of the γ-subunit 45 000 Dalton, and the δ-subunit 44 000 Dalton [59]. Two receptors are always tied together with a disulfide bridge between their δ-subunits. When the bridges are disturbed, the functioning of the receptor is not interrupted.

**Immmature receptors are present in neonates and outside the neuromuscular junction (after denervation and other disease states)** [61]. Neuropeptides (sciatin,
Acetylcholine receptors are believed to keep the acetylcholine receptors in the endplate region [62 63]. An essential one in this regard is Agrin, a nerve-derived extracellular matrix protein that provides the signal for induction of acetylcholine clustering [64]. The neuronal nicotinic acetylcholine receptor is also a pentamer, but consists only in 1 or 2 different subunits [65]. Acetylcholine receptors are also fixed in the membrane with a number of proteins (laminin, rapsyn, utrophin, syntrophin, dystroglycan), some of which are extracellular, some intramembranous, and others intracellular [66].

The subunits form a large, relatively non-selective pore that allows passage of almost all cations with diameters of less than 6-7Å [67]. At its entrance the width is ca. 40 Å, and it narrows to ca. 7 Å in diameter at the level of the membrane surface [68]. Thereafter the diameter remains the same. At the extracellular side the receptor sticks ca. 60 Å into the cleft, the intramembranous part is ca. 40 Å long and the intracellular part ca. 20 Å. The open channel has an external width of 80 Å and a smallest width (in the membrane) of 6.5 Å [69]. The ion-channel opens upon binding of acetylcholine to both its α-subunits [70]. Apart from the high-affinity binding sites there are some low-affinity sites, which are involved in activation and desensitization of the receptor [71]. Other compounds than acetylcholine can also bind to such places, and thus interfere with the functioning of the ion channel.

The ion flux after ion channel opening results in a fall in muscle membrane potential, i.e. an endplate potential occurs. If sufficient quanta of acetylcholine are released the endplate potential will reach a threshold to activate voltage-dependent sodium ion-channels of the adjacent muscle membrane, thereby initiating an action potential and depolarization of the muscle membrane. This occurs when at least 5-20% of the ion-channels are opened. One quantum of acetylcholine is able to open ca. 1500 channels, causing an endplate potential of about 4mA. At the peak of an endplate potential ca. 340 000 channels are open, through each of which approximately 10 000 cations traverse during a mean channels open time. As a result the Donan equilibrium over the membrane is disturbed with a reduction in membrane potential. The residence time of each acetylcholine molecule on the receptor is only sufficient to activate one receptor molecule. Before it can affect another receptor, acetylcholine is hydrolyzed by acetylcholinesterase, or is diffused away.

The various acetylcholine receptor subunits are encoded in genes. The genetic information is transcribed into mRNA; they then direct the synthesis of subunits by ribosomes in the endoplasmic reticulum. The α-subunit can bind agonists already in the ribosomes, the subunits thus have undergone so-called conformational maturation. Partial assembly of subunits occurs in the endoplasmic reticulum, and so does the critical addition of carbohydrates [72]. Membrane lipoprotein is added, and the final product assembled into a vesicle, which is transported to the cell surface. Here the vesicle fuses with the membrane, incorporating the receptor in it.

Incorporation of the receptor in the membrane takes ca. 3 h [73]. With denervation the synthesis of acetylcholine receptors is enormously increased [74]. Also the encoding of the α-subunit is increased after denervation [75].

Acetylcholine receptors are broken down. This involves an ATP-dependent internalization of the receptors and proteolysis in the lysosomes. The turnover time of acetylcholine receptors is usually 2 weeks for junctional receptors, and less than 1 day for extra-junctional receptors.

The metabolic stability of the receptors is controlled exclusively by evoked muscle activity.

1.2.4. The muscle
Muscle fibres are divided in fast and slow contracting fibres. Each muscle fibre contains myofibrils constituted of filaments of interdigitating actin and myosin, mitochondria, and the sarcoplasmic reticulum. The reticulum has a function in storing the calcium ions necessary for contraction. At rest these proteins are inhibited by troponin and tropomyosin. When a high calcium concentration is present after depolarization of the membrane, an interaction between actin and myosin occurs and bridges are formed. The filaments then slide along each other and muscle contraction is seen. When formation of calcium bridges ceases, the muscle relaxes.

The capacity to contract and develop tension is modulated by the recruitment of more or less motor units. Relaxation of the muscle requires the distraction of calcium from the sarcoplasm [76 77]. It is taken up in the sarcoplasmic reticulum and bound to the protein calsequestrin (43 molecules of calcium per molecule calsequestrin).

1.3. The margin of safety of neuromuscular transmission
Under normal conditions the amount of acetylcholine released is abundant, and the number of acetylcholine-receptors activated is much larger than the number needed to initiate a muscle action potential [78].

There is thus a large margin of safety both in the presynaptic (acetylcholine release) and postsynaptic (acetylcholine receptor activation) components of neuromuscular transmission [79]. This is reflected in the effect of neuromuscular blocking agents. Approximately 75% of the acetylcholine receptors must be blocked by antagonists before a reduction in contraction force can be observed, while at least 95% must be blocked before complete absence of contraction occurs.

1.4. Up- and down-regulation of the acetylcholine receptor
In general chronic exposure to agonist does result in a down-regulation (decrease in number of receptors) of the receptor. Chronic exposure to antagonists leads to up-regulation (increase in number of receptors) of the receptor. This self-regulation constitutes a homeostatic regulatory mechanism of membrane receptors. In muscle, sympathetic neurons, and in autonomic ganglion nicotinic receptors, chronic nicotine exposure causes down-regulation [80 81]. However, in brain nicotinic acetylcholine receptors it has been demonstrated that chronic exposure to nicotine (the agonist) results in up-regulation [82 83]. It is caused by
an increase in receptor density, without a change in receptor affinity. The muscarinic brain receptors are not affected by nicotine [84]. Acetylcholine itself causes down-regulation both in muscarinic and nicotinic brain receptors [85]. Down-regulation is the result of internalization of acetylcholine receptors.

Receptor up-regulation is typically associated with increased sensitivity for agonists and resistance to competitive antagonists, down-regulations is associated with hyposensitivity for agonists and extreme sensitivity to antagonists. Up-regulation is seen in denervation, disuse atrophy, thermal muscle trauma, infection, and chronic non-depolarizing relaxant or anti-epileptic drug administration [86]. In up-regulation the ε-subunit is replaced by a γ-subunit. Increased sensitivity to agonist leads to lethal potassium release after suxamethonium administration.

Chronic neuromuscular blockade by competitive antagonists leads, even in subparalytic dosages, to proliferation of acetylcholine receptors [87]. A cascade of reactions involving multiple proteins occurs in docking and fusion of prejunctional vesicles. This effect is independent from the up-regulation seen from immobilization. Furthermore, in the same study it was demonstrated that chronic exposure to an antagonist leads to tolerance. It is not certain whether the up-regulation is the result of a prejunctional effect (decrease in acetylcholine release), or of a postjunctional effect (receptor occupation). The up-regulation is accompanied by an increase in extrajunctional acetylcholine receptors. These are responsible for the increased sensitivity and potassium release seen with suxamethonium administration. Such potassium release may cause death [88,89].

Down-regulation of muscle acetylcholine receptors has been demonstrated in situations where increased acetylcholine concentrations were present [90]. It is also seen in myasthenia gravis.

1.5. Blockade of prejunctional acetylcholine receptors
Prejunctional acetylcholine receptors are, as described above, involved in the regulation of acetylcholine release. Acetylcholine mobilization and release is augmented in conditions where under normal levels of release insufficient acetylcholine is supplied for release during high frequency nerve depolarization. Pharmacological interference with the presynaptic receptors thus has an effect on neuromuscular transmission [91].

Suxamethonium has an agonistic effect on the receptor, and thus stimulates the presynaptic receptor, leading to increased acetylcholine release and initial overshoot in response to peripheral nerve stimulation (until paralysis occurs). Clinically used non-depolarizing muscle relaxants block prejunctional acetylcholine receptors [92]. This decreases prejunctional acetylcholine release via a feedback mechanism, and may contribute to muscle relaxation [93-95]. Decrease in prejunctional acetylcholine release results in fade in the responses to tetanic or train-of-four stimulation. The amount of fade produced by different non-depolarizing drugs varies, and so, presumably, does the effect on the nerve terminal [96].

With tubocurarine it was demonstrated that at low frequency stimulation an increase in acetylcholine occurs, and at high frequency stimulation a decrease. With vecuronium only an increase was seen [97]. Hexamethonium has the same results as tubocurarine [98]. It is suggested that the increase is mediated through neuronal-type nicotinic receptors, normally reacting in negative feedback on spontaneous acetylcholine release, and the decrease by a muscular-type receptor, normally reacting in positive feedback on evoked release.

Magnesium is an antagonist of the action of calcium in the nerve terminal, and thus will decrease acetylcholine release. Some other metals have the same effect [99]. The black spider venom, α-latrotoxin, increases prejunctional vesicle fusion and inhibits vesicle recycling. This depletes the nerve terminal from releasable acetylcholine [100].

1.6. Blockade of postjunctional acetylcholine receptors
Occupation of acetylcholine receptors with substances other than acetylcholine prevents the binding of the neurotransmitter with its receptor, and thus may lead to inactivation of neuromuscular transmission. When an agonist is administered, approximately 25% of the receptors need to be occupied (as with acetylcholine) to see an effect. With administration of an antagonist it must be prevented that 25% of the receptors can be occupied by acetylcholine, and thus the antagonist must occupy 75% or more of the receptors to exert effect [101]. The receptor binding is characterized by a constant association and dissociation of the relaxant or acetylcholine [102]. The rate of receptor association (association constant) and dissociation (dissociation constant) determine receptor affinity, and thus they are important factors in the 'onset' and 'offset' of the neuromuscular blockade. As long as there is relaxant in the vicinity of the receptor, it can be occupied again, and thus neuromuscular blockade will be maintained.

The duration of neuromuscular blockade thus depends on the concentration of receptor in the receptor compartment (biophase). The concentration in the biophase is determined by the plasma concentration and the physico-chemical characteristics of the relaxant. There is, therefore, a relationship between the plasma concentration of the relaxant and receptor occupation [103-106].

When receptors are in the open state, particular molecules may enter the channel. Because the channel does not have the same width along its whole length, the molecule will plug the channel. None of the channel blocking compounds is clinically used to induce neuromuscular relaxation; however, many drugs co-administered perioperatively do have this effect (for example, antibiotics, steroids, some muscle relaxants, anticholinesterases, local anaesthetics, inhalational and intravenous anaesthetics), and thus they may interfere with neuromuscular transmission [107]. Such a channel block is non-stereospecific, whereas receptor block is stereospecific.

1.6.1. Depolarizing neuromuscular blockade
Muscle relaxants with a depolarizing mechanism of action have an intrinsic effect on the acetylcholine receptor. They thus stimulate the opening of the ion-channel, resulting in depolarization of the muscle
membrane, causing muscle fasciculations. Since the compounds are not metabolized by acetylcholinesterase in the synaptic cleft, the receptor remains occupied, and repolarization is impossible, thus leading to muscle relaxation. A depolarizing block is characterized by decreased contraction force in the response to single twitch stimulation (T1). There is no fade in the response to tetanic (Tet) or train-of-four (TOF) stimulation. The only clinically used depolarizing muscle relaxants is suxamethonium.

1.6.2. Anticholinesterase-induced neuromuscular blockade
With sustained presence of acetylcholine at the receptor, receptor desensitization occurs [108]. In the situation one of the subunits bends and distorts the receptor, so that it no longer responds to an agonist by opening the channel [109]. Such a situation can occur if acetylcholine is not metabolized due to acetylcholinesterase inhibition. Overdose of anticholinesterases have been shown to cause such problems [110 111].

1.6.3. Non-depolarizing neuromuscular blockade
The non-depolarizing relaxants bind to the acetylcholine receptor, but have no intrinsic effect. Therefore the ion-channel remains closed, and flaccid paralysis is seen. A non-depolarizing block is characterized by decreased contraction force on single twitch stimulation (T1), fade in the response to tetanic (TET) and train-of-four (TOF) stimulation, and in post-tetanic facilitation. Non-depolarizing muscle relaxants are charged quaternary nitrogen compounds with a fixed interonium distance. They compete with acetylcholine for the acetylcholine binding places at the two α-subunits of the receptor. More than 75% of the postjunctional receptors must be occupied before an effect can be seen [112]. The non-depolarizers jump on and off the receptor, exerting effects within ms of binding. These compounds also occupy presynaptic receptors (results in fading) and ion channels. Non-depolarizing muscle relaxants are now used routinely in clinical anaesthesia and in intensive care. A wide variety of such compounds have been introduced, e.g. tubocurarine (1942), gallamine (1947 [113]), alcuronium (1961 [114]), pancuronium (1967 [115]), metocurine (1948 and reintroduced in 1972 [116]), dioxonium (1972 [117]), fadazolinium (1974 [118]), vecuronium (1980 [119 120]), pipecuronium (1980 [121]), atracurium (1981 [122 123]), doxacurium (1988 [124]), mivacurium (1988 [125]), and rocuronium (1991 [126]). Although the newer relaxers are nearer to the ‘ideal’ requirements (see Table 1.1.) of a neuromuscular blocker, and provide more flexibility than the older ones, is there still no single compound that can be used in all circumstances.

A large number of experimental relaxants have been studied in animals and in a small number of patients, but have not come into routine clinical use. Examples are tropanyl esters [127], bulky esters (dioxonium, diadonium, anatroxonium, cyclobutonium, and truxilonium [128], steroidal relaxants (chandonium [129], Org 6368 [130], Org 7617 [131 132], Org 8764 [133], Org 9991 [134], and OR 9616 [32], and benzylisoquinolines (BW 252C64 [135] and BW 403C65).

1.6.4. Drugs with a mixed depolarizing and non-depolarizing mechanism of action
Coryneine, a compound with a quaternary ammonium derivative of dopamine structure, and derived from the aconite root, has a relaxant effect which is mixed competitive and non-competitive [136]. Dioxonium is a relaxant with mixed effect, having an initial depolarizing mechanism, but with higher dosages converts into a non-depolarizing mechanism [137 138]. When the non-depolarizing stage is reached, the blockade is reversible by anticholinesterases. In studies, cardiovascular or other adverse effects were not observed. A disadvantage is that these seems to be tachyphylaxis for dioxonium upon repeated administration. The clinical value is thus not well established.

1.7. The ideal muscle relaxant
Based on clinical experience with the existing muscle relaxants, the requirements for the ideal muscle relaxant can be defined (see Table 1.1). Some additional ‘desires’ can be defined as well. It would be ideal if blockade of particular muscles could be selectively obtained. Then, for example, selective blockade of the hypopharyngeal and laryngeal muscles would make intubation in spontaneously breathing patients possible. Selective paralysis of abdominal muscles would allow intra-abdominal surgery in spontaneously breathing patients under general anaesthesia. Another desirable point is a smaller variability in effect. If it were possible, a gaseous relaxant which would depend on ventilation for its elimination, and thus being independent from hepatic or renal function, would seem to be ideal. Organ independency could also be reached if metabolism was through hydrolysis in plasma, but independent from plasma cholinesterase activity. This might be possible if specific esterases (i.e. carboxylesterase) are involved in the metabolism.

**Table 1.1 Requirements for the ideal muscle relaxant**

<table>
<thead>
<tr>
<th>Requirement</th>
<th>Details</th>
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<tbody>
<tr>
<td>1. Non-depolarizing mechanism of action</td>
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<td>2. Rapid onset</td>
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<td>3. Appropriate duration</td>
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<td>4. Rapid recovery</td>
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<td>5. Non-cumulative</td>
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<td>6. No serious cardiovascular side-effects</td>
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<td>7. No histamine release</td>
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<td>8. Reversible effect</td>
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<td>9. Lack of drug interaction</td>
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<td>10. Pharmacological inactive metabolites</td>
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<td>11. Independent from organ excretion (liver, kidney)</td>
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<td>12. Absence of nervous systems effects (ICU use)</td>
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<td>13. Absence of muscular effects (ICU use)</td>
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<tr>
<td>14. Stable in solution/ready for use formulation</td>
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1.8. Pharmacodynamics of muscle relaxants

1.8.1. Potency, latency time, and onset
With non-depolarizing neuromuscular blockers, a correlation between potency and rate of onset appears to exist; the less potent the compound, the faster the onset [139 140]. With low potency non-depolarizing muscle relaxants, recovery is fast, whereas, with high potency drugs, it is slow [141]. This may be related to a high receptor association rate, making dissociation from the receptor more 'difficult' (low dissociation constant). This effect may, however, be masked if the relaxant remains in the vicinity of the receptor, i.e. if the plasma concentration is not decreasing rapidly [142]. For short duration of action, low affinity of the molecules to the receptor is necessary. This automatically implies low potency. This has recently been confirmed to be applicable for the offset of effect of all non-depolarizing relaxants, giving in humans the sequence of gallamine < rocuronium < tubocurarine < atracurium < pancuronium < vecuronium < doxacurium [143]. Some studies have suggested that the rate of diffusion from the plasma to the receptor was more important than the rate and affinity of drug-receptor association [144]. This agrees with clinical data obtained for fazadinium, pancuronium, tubocurarine and suxamethonium [145].

Theoretical considerations indicate that onset time must have a limit, because it cannot be shorter than the circulation time. The circulation time is the shortest latency time possible. The latency time is furthermore determined by the receptor association rate and the amount of relaxant present in the biophase. Because ca. 90% of receptors must be blocked for full relaxation, and thus an equal number of relaxant molecules must be administered, this means that potency also is limited. The likely non-depolarizing equivalent to suxamethonium would thus have, compared to other compounds in the same chemical group of substances, low potency.

A variety of methods to decrease the onset of relaxants, and thus to decrease the delay between administration of the relaxant and endotracheal intubation, have been used with the relaxants presently available. The onset time of an individual compound decreases with an increase in the dose, as has been demonstrated for atracurium [146] and vecuronium [147]. In a clinical study, the administration of respectively 100, 200, 300, and 400 μg/kg vecuronium in 10 patients each demonstrated a decrease in onset time with an increase in duration and recovery rate. Side effects, however, were not seen [148]. These results were confirmed in another study in which the onset time of vecuronium halved when 0.3 mg/kg was administered instead of 0.1 mg/kg [149]. The duration of action, however, was increased markedly with the higher doses. This has also been seen with other relaxants, but it leads to a marked increase in duration of action and side-effects. With the longer acting relaxants, the duration of action can become extremely long and unpredictable. When larger doses of relaxants are administered, there is a frequent need to antagonize the block produced. With benzylisoquinolines the increase in dose may lead to histamine release.

With the 'priming principle' technique, one third of the calculated dose of relaxant is administered 3-5 min before induction of anaesthesia, the remainder is then administered immediately after induction. Although it has been demonstrated that the 'priming principle' decreases the onset time, there are a number of unwanted side-effects. In one study, the onset times after priming with vecuronium, atracurium and pancuronium were compared with that after suxamethonium. They approached each other. The optimal dose for priming with vecuronium was 0.012 mg/kg, pancuronium 0.015 mg/kg, and atracurium 0.09 mg/kg. The optimal time interval between the doses of relaxant was 3-5 min [150]. However, with all these relaxants, double vision, difficulty with breathing and swallowing occurred frequently. This has been confirmed in other studies [151 152]. The side-effects of priming vary with the interindividual variability in sensitivity for relaxants.

The administration of combinations of particular relaxants, e.g. pancuronium and metocurine, gallamine and metocurine, and tubocurarine and pancuronium, can lead to a faster onset of action, but it can also lead to a potentiation or prolongation of effect [153]. This can be difficult to predict because of the large interindividual variability in response to relaxants. The interaction is probably due to the differences in the mechanism of action of the various relaxants. Because some relaxants have a more pronounced presynaptic effect than others, and because some cause more ion-channel plugging than others, and also because some depend more on redistribution and others more on metabolism to terminate their effects (different pharmacokinetic behaviour), marked interactions between relaxants are possible. Although the technique of administration of relaxant combinations has been advocated in the past, further studies are necessary before it can be considered safe for routine use. In one study, it was demonstrated that rocuronium and mivacurium potentiate each other. Not only was the duration of the combination longer, but also the onset was shorter. Interestingly, a combination of 150 μg/kg rocuronium + 37.5 μg/kg mivacurium produced a blockade with a rapid onset (114 s) and short duration (14.7 min); a combination of 300 μg/kg rocuronium + 75 μg/kg mivacurium produced a shorter onset (69 s) and a longer duration (34 min); a combination of 600 μg/kg rocuronium + 150 μg/kg mivacurium did not produce a faster onset, but did lead to a significantly prolonged duration (55.2 min) [154]. An explanation for this was not given, but may lie in the breakdown of mivacurium by plasma cholinesterase. This esterase is usually inhibited by aminosteroidal relaxants.

The onset time is not necessarily the same as the intubation time. With most relaxants, it is possible to intubate the trachea under excellent conditions when the neuromuscular blockade, measured at the adductor pollicis muscle is 70-80%. This is due to the fact that the pharmacodynamic profile of the relaxants varies with the different muscles. Intubation with rocuronium, for example, is possible with excellent conditions, comparable to those after suxamethonium, within 60-90 s after administration of twice the ED95 dose.

1.8.2. Duration of action and recovery rate
The duration of action of a relaxant depends on its
dissociation rate from the receptor and on the amount of relaxant in the vicinity of the receptor. This amount depends on the dose administered, the rate of metabolism, and the rate of (re)distribution and elimination. Most relaxants depend on redistribution for termination of their effect. Some are rapidly inactivated in the biophase, or have a built-in self-antagonistic action, while others are metabolized in the plasma or by enzymatic processes in the liver, or they are excreted unchanged via the kidneys. The recovery rate also depends on the speed with which the acetylcholine receptors are freed from the relaxant. If there is a high receptor affinity (i.e. high potency), then the relaxant binds again to the receptor. With a lower affinity, it may diffuse away, or it may be metabolized. The recovery rate is, however, independent from the degree of neuromuscular blockade. Depending on the pharmacokinetic characteristics of each relaxant, organ failure can lead to a change in the pharmacokinetic behaviour of the relaxant. This can alter the pharmacodynamic profile of the relaxant.

1.9. Pharmacokinetics of muscle relaxants

The pharmacokinetics of the currently used relaxants can be described in a two- or three-compartment model, with a rapid distribution phase (distributional clearance) in which they are transferred from the central compartment ($V_{DC}$) into one or two peripheral compartments (Fig. 1.4). This is followed by one or two slower elimination phases, consisting of biotransformation and excretion (metabolic clearance). For most relaxants, a two-compartment model is suitable and thus two half-life times be determined: the half-life of distribution ($t_{1/2D}$) and the half-life of elimination ($t_{1/2E}$).

Due to the high water solubility the volume of distribution of relaxants is small. After intravenous administration, the initial volume of distribution can vary from 80 to 150 ml/kg, and the subsequent volume of distribution (central plus peripheral compartment, $V_{D\text{area}}$ or $V_{DS}$) from 200 to 450 ml/kg. This is somewhere between the volume of the extracellular water compartment and the total body water in humans. $V_{DC}$ governs the peak plasma concentration after a rapid bolus injection, and $V_{D\text{area}}$ the tissue penetration of the compound. These distribution volumes and the clearances of relaxants can be markedly affected by disease states such as hepatic and renal failure and cardiovascular disturbances. In hepatic and renal diseases, but also in other diseases, the metabolism and excretion of drugs may be affected leading to changes in plasma clearance ($C_{LP}$) and $t_{1/2p}$. They can also change the volumes of distribution. Disease states are thus frequently connected with changes in the pharmacokinetic behaviour and the pharmacodynamic profile of relaxants. Because drug distribution in the tissues depends on tissue perfusion, the cardiac output is an important factor in the pharmacokinetics of relaxants. Reduction in cardiac output usually leads to a redistribution with lengthening of the $t_{1/2E}$, a slower onset of action, and to a greater effect. With increased cardiac output, tissue perfusion is elevated. This means more rapid and widespread distribution. A higher dose is, therefore, needed for the same effect. In hypovolaemic shock, the $V_{DC}$ is smaller, and thus a higher peak concentration occurs. This leads to a greater than normal clinical effect.

Protein binding of muscle relaxants varies between 30 and 85%, and is another important factor in their pharmacokinetic behaviour [155]. Both changes in the protein concentration in disease status and binding of concurrently administered drugs to proteins influence the protein binding of relaxants. This binding can alter the volume of distribution, metabolism, and the excretion of the relaxants. Protein binding also plays an important role in maternal-foetal drug equilibration during pregnancy.

1.10. The effect of animal toxins on neuromuscular transmission

Many animal toxins have a neuromuscular blocking effect, the study of which may be helpful in the future development of new neuromuscular transmission models.
blocking agents. In an excellent review the effect of a variety of venoms has been described [156]. Some of them block neuronal sodium channels (tetrodotoxin, saxitoxin, batrachotoxin, cicuatoxin, 13- and 12-scorpionotoxins, sea anemone toxins), potassium channels (dendrotoxin, scorpio toxins, apamin, 13- and 12-scorpionotoxins, sea anemone toxins), block postjunctional acetylcholine receptors (13-bungarotoxin, cobratoxin, sea snake venom, 13-conotoxin). The ones inhibiting postjunctional acetylcholine release frequently interfere with the SNARES involved in vesicle docking and fusion [157]. Yet other toxins interfere with muscle contractility (viper venom, honey bee, notexin, scorpio toxin). Many of the toxins were used to study the development, maturation, and functioning of the neuromuscular junction, and to elucidate the effect of chemical compounds on neuromuscular transmission. 13-latrotoxin, the active part of the black widow spider venom, causes a reduction in quantal acetylcholine release by blockade of exocytosis [158]. A number of toxins do irreversibly inhibit acetylcholine receptors. Lophotoxins are very specific selective irreversible inhibitors of nicotinic acetylcholine receptors [159].

1.11. Stereoisomerism

Many drugs, including some relaxants, are available as a racemic mixture of enantiomers. Each enantiomer can have different physicochemical properties, and hence can have a different pharmacological profile. Some isomers are each other’s mirror, but cannot be superimposed upon each other. Geometric-isomers show cis-trans isomerism. Examples of muscle relaxants with isomers are atracurium, containing 10 stereoisomers, and mivacurium, containing 3 stereoisomers. The 1R- cis-1R’- cis isomer of atracurium (cisatracurium, 51W89) is currently under development as a non-depolarizing relaxant.

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