NEUROMUSCULAR BLOCKING AGENTS: AN REVIEW

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Summary

Steroids have contributed a lot in the development of organic chemistry. The broad operations of biological activity and multiplicity of action make steroids one of the most intriguing classes of biologically active compounds. The area has been very fascinating for the study of reaction mechanisms and stereochemistry and a brief discussion on neuromuscular blocking agents is discussed below.

Keywords: Steroids, Neuromuscular blocking agents, Cholinergic receptors, Neuromuscular Junction, Nonsteroidal neuromuscular blockers

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Introduction

One of the most important advances in anaesthesia and surgery is the introduction of neuromuscular blocking agents. Research in the area of neuromuscular blocking agents rose dramatically on account of quest to find ideal muscle relaxants. There has been a considerable increase in the understanding of the mechanisms of action of the agents at the molecular level. As shown in **Figure-1** the receipt of the nerve impulse at the axon terminal promotes Ca^{2+} -activated fusion of acetylcholine storage vesicles with the terminal membrane, causes release of acetylcholine into the synaptic cleft[1].

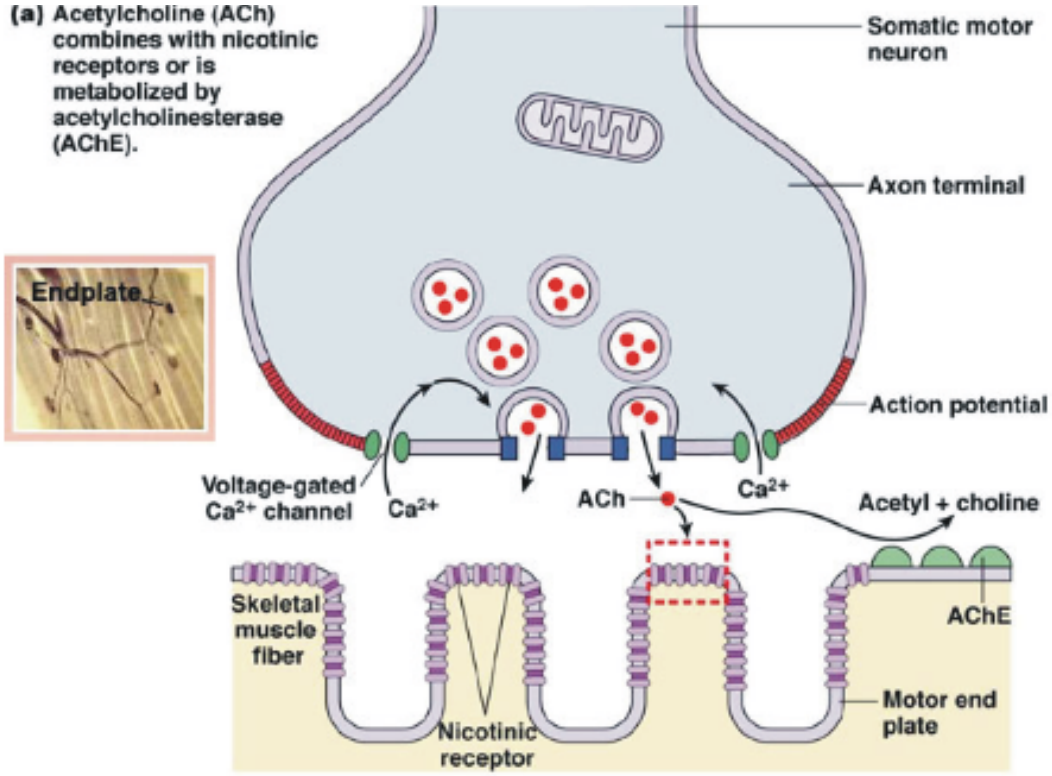


Figure-1

The neurotransmitter acetylcholine so released combines with specific lipoprotein receptors on the post-junctional membrane. This agonist-receptor combination leads to conformational changes in receptor, with the opening of ion channels, and as a result a massive flow of Na^+ and K^+ occurs across the muscle membrane. The inward flow of Na^+ ions is much more and faster than the outward flow of K^+, results into net inward flow of some 3000 univalent cations per acetylcholine molecule released. This surge in

membrane conductance and its rapid decay, all within 0.5 msec, causes the equally rapid fall (depolarization) and recovery of the membrane potential, which actuates muscle contraction. The course of events at the neuromuscular junction leading to transmission of nervous impulses has been described in a review on molecular interaction at the cholinergic receptor in neuromuscular blockade[2].

Transmission of nerve impulse is interrupted by neuromuscular blocking agents at the skeletal neuromuscular junction. They are used clinically as adjuncts to general anaesthesia to produce paralysis, so that surgery, especially intra-abdominal and intra-thoracic surgeries can be conducted with fewer complications and are also used to facilitate intubation procedures in orthopaedics for manipulation of fractured or dislocated bones[2,3], management of spasticity[4,5], control convulsions in tetanus and electroconvulsive therapy of psychiatric disorders[3]. The use of neuromuscular blockers in patients requiring mechanical ventilation as a part of intensive care[6] and management of muscle cramps has been reviewed[7]. There are electrophysiological differences in their mode of action.

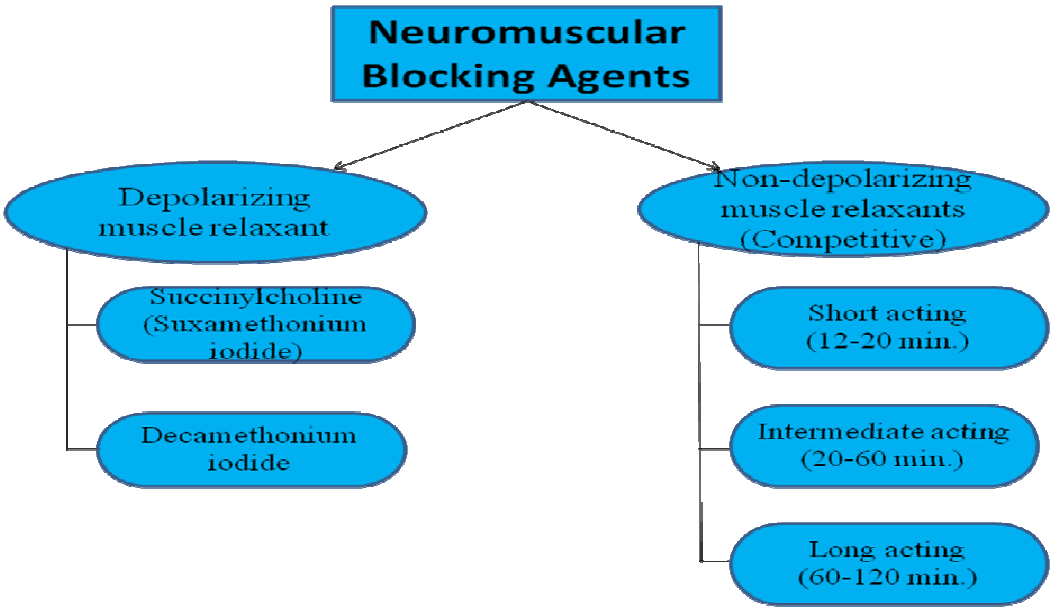


Figure-2

As depicted in above **Figure-2**, they are classified on the basis of duration of action as nondepolarizing (competitive, stabilizing, curariform, antidepolarizing) or as depolarizing agent.[8] The nondepolarizing agents block neuromuscular transmission by competing with acetylcholine for receptor sites on the motor end-plate, thus reducing the response of the end-plate to acetylcholine; their action is usually reversed by anticholinesterases. The depolarizing agents interrupt neuromuscular transmission by

producing a sustained partial depolarization of the motor end-plate, which renders the tissues incapable of responding to the transmitter. The only depolarising agent in clinical use, suxamethonium chloride, binds to and provides prolonged activation of postjunctional acetylcholine receptors; this prevents repolarisation of the postjunctional membrane.[9] Anticholinesterase agents such as neostigmine and edrophonium can reverse the action of nondepolarising neuromuscular blocking agents by increasing the concentrations of acetylcholine at the neuromuscular junction, but do not reverse the primary neuromuscular block caused by depolarising neuromuscular blocking agents[10]. Generally, the nondepolarizing agents, with a prolonged action, are used in major operations, while the depolarizing agents, with a much shorter effect, are used for minor operations[11].

Desirable characteristics of an ideal nondepolarising neuromuscular blocking agent has been enlisted by Savarese and Kitz.[12] An ideal NMBA should show a rapid onset of action, rapid dissipation of neuromuscular blockade, lack of cumulative effects, antagonism of the block by a suitable antidote, absence of pharmacological action or toxicity of metabolites, high potency, lack of histamine release, and acceptable cardiovascular effects. A total absence of cardiovascular effect is desired. Yet, compounds with mild vagal blocking effect are acceptable, as most advanced anaesthetic techniques lead to a relative bradycardia and hypotension. Even drugs with mild ganglion blocking effect may have a use, as in hypertensive patients and operations under induced hypotension. This is declared that a mild degree of vagal blocking and / or ganglion blocking action may be beneficial during intubation of the trachea, to prevent bradycardia secondary to vagal refluxes, and to prevent hypertension due to the stimulus of intubation.

Anatomy of Neuromuscular Junction

Claude Bernand[13] introduced the concept of the existence of neuromuscular junction, who showed that although curare could cause paralysis, it did not effect nerve conduction or prevent the muscle from contracting when directly stimulated. The fundamental anatomy of the frog neuromuscular junction was described by Birks *et al*. in 1960, and serves as a model for other species.[14] The three parts neuromuscular junction are: prejunctional nerve ending, the junctional cleft and postjunctional membrane. As shown in **Figure 3** below nerve terminal contains not only the mitochondria and other common subcellular structures, but also numerous vesicles about 70 mm in diameter.[15]

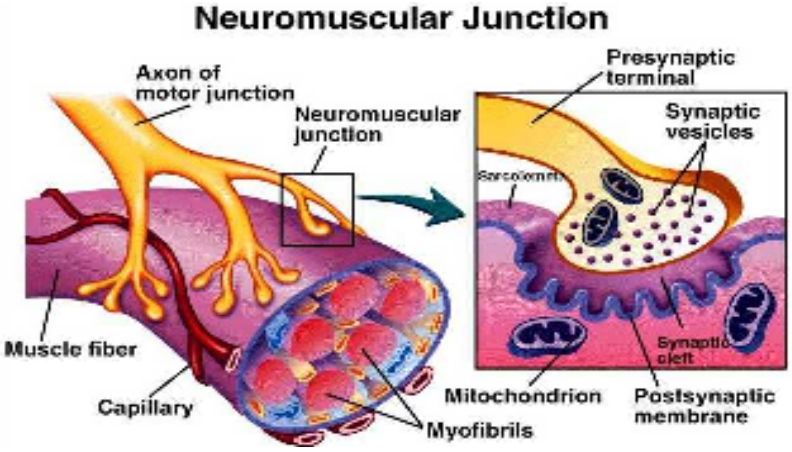


Figure 3

These synaptic vesicles are already filled with acetylcholine on the prejunctional membrane, transverse band can be seen, and these have been called 'active zone' because they are believed to be sites of acetylcholine release. There are such one thousand active zones at each nerve ending.[16] The synaptic cleft is about 60 mm across and having a basement membrane material that is composed of mucopolysaccharide. Acetylcholinesterase exists within this basement membrane, although it is particularly concentrated in the folds of the postjunctional membrane.[17] The presynaptically released acetylcholine has to travel the cleft membrane before it reaches the receptor on the postsynaptic membrane. The postjunctional membrane is thrown into folds (secondary clefts), with the acetylcholine receptors organized in discrete clusters located on the shoulders of those folds. This means that they are in direct opposition of the active zones of the nerve terminals.[18] There are more than 10,000 receptors/μm[19], each of which is inserted through the phospholipid bilayer of the postsynaptic membrane. The receptors exist as dimmers, and it is likely that there is cooperation between the two components of each receptor pair.

Nicotinic acetylcholine receptor is a pentamer of five glycoprotein subunits, **Figure-4**, which together form a central cation channel[20-22]. Two α subunits of the receptor are identical, others are slightly larger and known as β, δ and ε-subunits in the mammalian adult. The ε-subunits dose not exists in the foetus and various other species, being replaced by the γ-subunits. In the initial few weeks of life, the γ-subunits disappear to be replaced by the ε-subunits[23]. The two α-subunits each carry a single recognition site that binds acetylcholine, other agonists, toxins[24] and reversible antagonists, for example tubocurarine[25-27].

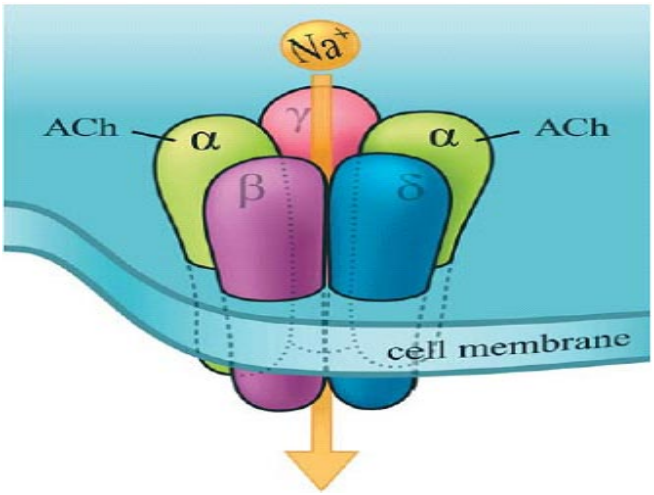


Figure-4

Two α –subunits have the same amino acid sequence, but both reside in different environments. One α-subunit is having the β and ε adjacent to it, while the other subunit is surrounded by the δ and ε-subunits. So properties of the two sites being different, and they have been shown not to behave in an identical fashion in their interaction with tubocurarine[28]. Recent immunological studies have shown that the majority of the antibodies raised against the acetylcholine receptor in myasthenia gravis are directed against a single region, the "main immunogenic region". This region is located on the extracellular part of α –subunits[29].

The nicotinic acetylcholine receptor has depicted as an example of ligand-gated, it consists of several subunits that are inserted into a membrane and provide ligand-gated conductance[30]. In contrast, a functional voltage- gated ion channel (e.g. the sodium channel) consists of a single large protein unit[31].

Physiology of Neuromuscular Junction

Acetylcholine is the transmitter at the neuromuscular junction. Acetylcholine synthesis, storage, mobilization, releases and recycling[32] is performed in presynaptic region. Acetylcholine is formed from the acetylation of choline under the influence of enzyme choline acetyltransferase, a soluble enzyme prepared within the cell body. Choline is supplied both from plasma and from the breakdown products of acetylcholine. It enters the nerve terminals under the influence of an active transport system. Acetate is supplied bound to coenzyme A, and synthesis of acetylcholine is energy dependent process.

Subsequently acetylcholine is actively loaded and densely packed into the synaptic vesicles. The mechanism of this process has been studied using vesamicol[33].

Acetylcholine is present both in the vesicles and free in the axoplasm. Most of the vesicles are situated at a short distance from the inside of the cell membrane, with the remainder lying very close to the cell membrane behind the region of the active zones. The former clusters of vesicles are regarded as being the reserve, while the latter are immediately available source. Amount of quantal acetylcholine release is determine by the size of immediately available pool following stimulation of the motor nerve. The movement of the vesicle from reserve to immediately available stores is the process of transmitter mobilization and probably takes place in an energy dependent process involving calcium ions. It has been proposed that phosphorylation of synapsin I in the presence of calcium is involved in the release of synaptic vesicle from its location on an internal cytoskeleton, thus allowing it to move down towards the release sites[34]. The course of events at the neuromuscular junction leading to transmission of nervous impulses has been described in the review on molecular interactions at the cholinergic receptor in the neuromuscular blockade [35].

The process of neuromuscular transmission is extremely fast. The time from the stimulus to the first detection of a postjunctional event (synaptic delay) is as little as 0.2 msec., within this period, the arrival of the nerve action potential has to trigger the release of acetylcholine, which must than diffuse across the synaptic cleft, combine with and activate the receptors. Transmitter released is affected by the arrival of the action potential, which cause voltage dependent calcium channels in the nerve terminal to open with a resultant rise in the calcium concentration close to the synaptic vesicles. The calcium binds to a receptor that is calcium-binding protein, probably synaptotagnin,[36] and this is related to the activation of a second messenger system[37]. The vesicles fuse with the prejunctional membrane, expelling acetylcholine, and subsequently are directly recycled[38].

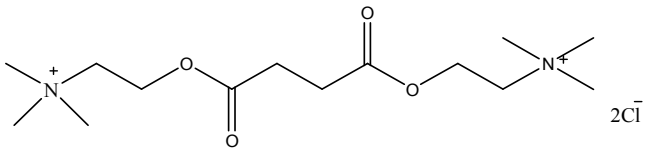
The released acetylcholine diffuses across the synaptic cleft to interact with the postjunctional receptor. The acetylcholine receptor is not a receptor in the classical sense, but rather as integral signal transducer. It contains in it a protein agonist moiety, the binding sites for acetylcholine and its agonists and antagonists (receptor function), the ligand gated cation channel (response function), and several types of modulation sites (modulation function). The postjunctional response starts simultaneously as acetylcholine interacts with receptor. The binding of the acetylcholine facilitates the binding of the second. This sequential receptor saturation will induce a chain of conformation transitions, one of which

is the active state (a state of high probability of channel opening). This state is long lasting when two molecules of transmitter are bound, but also exists for partially occupied or even unoccupied receptors[39]. Other multiple low binding sites have been proposed, in addition to the two high affinity agonist-binding sites per receptor. Opening of a channel permits small cations (sodium and calcium particularly) to travel down their concentration gradients. These concentration gradients, together with the electrical potential across the membrane, result in the main movement being an influx of sodium ions. The mean open time of the channel varies with the activating against. The time constant acetylcholine activation approximately 3 msec at resting membrane potential of -80 mV and a temperature of 15 ^{o}C[40].

Each molecule of acetylcholine probably exists long enough to activate a single receptor before it is destroyed by acetylcholinesterase. An increase in the acetylcholine concentration will increase the frequency with which the ion channel opens. Prolonged exposure to the transmitter (receptor saturation), however, may lead to subsequent conformational changes that decreases receptor channel conductivity "desensitization". The current of sodium ions that flows inwards through the whole motor endplate membrane (endplate current) will change the potential across that membrane sufficiently to depolarize it, producing the end plate potential. When the endplate potential reaches a critical threshold, it will trigger a muscle action potential that subsequently activates the contractile mechanism. There is a large safety factor in the transmission process, both in the amount of acetylcholine released and in the number of receptors available upon which for it to act. Both are in very much large number than required to produce the critical level of endplate potential necessary to initiate a muscle contraction.[40]

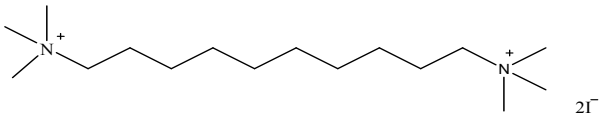
DEPOLARIZING BLOCKING AGENTS

Depolarizing neuromuscular blocking agent is a form of neuromuscular blocker which depolarizes the motor end plate e.g Suxamethonium chloride **(1)**. Depolarizing blocking agents work by depolarizing the plasma membrane of the muscle fiber, similar to acetylcholine. However, these agents are more resistant to degradation by acetylcholinesterase, the enzyme responsible for degrading acetylcholine, and can thus more persistently depolarize the muscle fibers. This differs from acetylcholine, which is rapidly degraded and depolarizes the muscle for the short period of time.



(1)

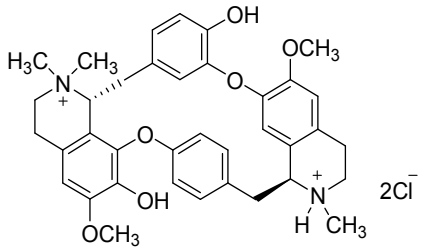
Depolarizing blocking involves two phases: During phase I (*depolarizing phase*), they cause muscular fasciculation's (muscle twitches) while they are depolarizing the muscle fibres. Subsequently, after sufficient depolarization has occurred, phase II (*desensitizing phase*) sets in and the muscle is no longer responsive to acetylcholine released by the motor neurons. At this point, full neuromuscular block has been achieved. The prototypical depolarizing blocking drug is succinylcholine (suxamethonium). It is the only such drug used clinically. It has a rapid onset (30 sec) but very short duration of action (5–10 min) because of hydrolysis by various cholinesterases (such as butyrylcholinesterase in the blood). Succinylcholine was originally known as diacetylcholine because structurally it is composed of two acetylcholine molecules joined with a methyl group. Decamethonium iodide **(2)** is sometimes, used in clinical practice.[11]



(2)

NONSTEROIDAL NEUROMUSCULAR BLOCKERS

The well known examples of nonsteroidal neuromuscular blocking agents are: tubocurarine chloride **(3),** gallamine triethiodide **(4),** which are competitive in action, and decamethonium iodide and suxamethonium iodide having depolarising mechanism of action[11]. Tubocurarine **(3)** was earlier considered to be a bis-quarternary compound[42] but laters it was found that one nitrogen is tertiary[43-45].



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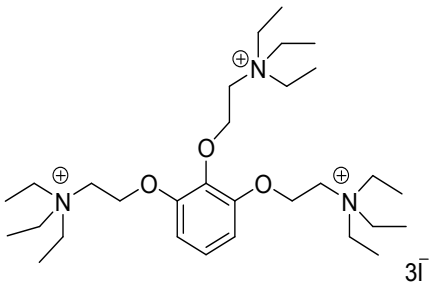
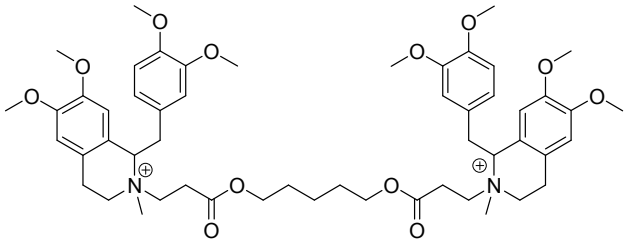


(4)

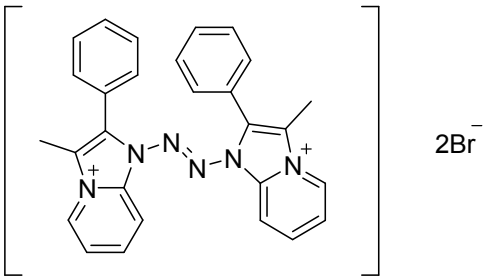
Comparatively non-depolarizing agents are bulky and rigid molecules, whereas depolarizing agents generally have a more flexible structure which enables free bond rotation[46-48]. While the interonium distance in the flexible depolarizing agent can vary up to the limit of the maximal bond distance (1.45nm for decamethonium), the distance for rigid competitive blocker is usually 1.0±0.1 nm. A study of crystal of (+)-tubocurarine revealed that interonium distance is 0.897nm in dichloride[44] and 1.066nm in dibromide salt[46]. Atracurium besylate **(5)** is a nondepolarizing neuromuscular blocking agent[49, 50]. The drug is the result of collaborative programme of research at the Universities of Strathclyde and the Wellcome Research Laboratories (UK). The rationale underlying the



(5)

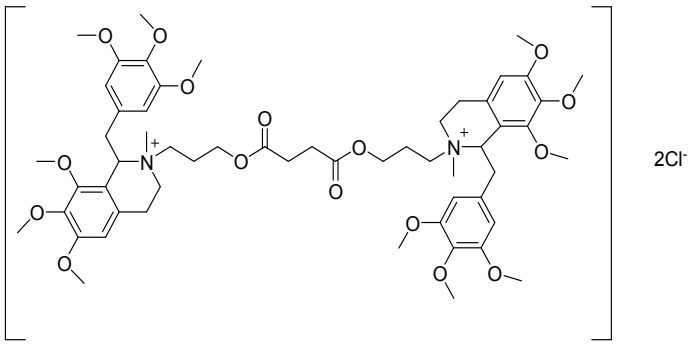
design of atracurium was described by Stenlake *et al*.[51] The bisquaternary ammonium neuromuscular blocking agent incorporates Hofmann elimination and ester hydrolysis biodegradation pathways. The breakdown products are relatively innocuous and are of no pharmacological importance.[52] The chemical breakdown by Hofmann elimination is rapid at physiological *p*H and temperature, whereas ester hydrolysis is enzyme catalyzed.[53] Atracurium and its metabolites are readily excreted in bile and urine. In toxicity studies in animals, atracurium was found not to produce any specific adverse effects[54]. Its solution did not cause local irritation. It was not mutagenic in the Ames test. Clinically, the drug is the potent nondepolarizing agent with no cardiovascular effects at dose required for neuromuscular paralysis[55]. Intravenous doses of 0.3-0.9 mg kg^{-1} produce a complete neuromuscular block.[56] The

present consensus[57] is that atracurium is a drug of intermediate duration and has a relatively slow onset of action. Allen and Hanburys Limited (UK) [58,59] , firstly discovered Fazadinium bromide (AH 8165) **(6)**. It produces nondepolarizing type of action in animals, with rapid onset and short duration of action[60]. However, the action was found to be slower and longer in man.[61-63]



(6)

Pharmacokinetic studies have shown that there as a wide tissue distribution of the drug, which is partially metabolized into inactive metabolites[64]. The urine and bile of man appear to be principal excretion routes[65], however, in the dog, the kidney acts only as a secondary elimination route[66]. In most cases, serum level follows a two compartment open kinetic model[65-67]. Doxacurium chloride (7) (Nuromax) introduced by Wellcome is an injectable, noncumulative, nondepolarising neuromuscular blocking agent which exhibits no significant cardiovascular effects[70-72]. It is a mixture of isomers of a bis-benzylisoquinolium diester. It provides satisfactory intubation with duration of action similar to (+) –tubocurarine **(3).**



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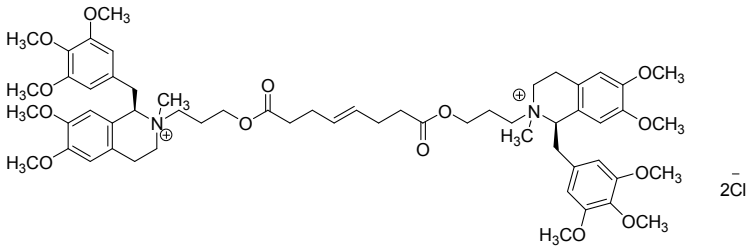
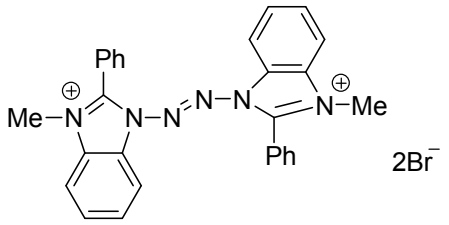


(8)

Mivacurium chloride (mivacron) **(8)** introduced by Wellcome mixture of three stereoisomers[73], intravenously administrated, short-acting[74] skeletal muscle relaxant introduced as an adjunct to general anaesthesia. Structurally it is closely related to doxacurium chloride **(7)**[75]. In comparison to other nondepolarising agents doxacurium chloride was found to have shorter duration of action and more rapid rate of spontaneous recovery. In extensive clinical trials, mivacurium chloride was well-tolerated with few side effects chloride was well-tolerated with few side effects.



(9)

AH 10407 **(9)** is another drug of Allen and Hanburys, which has rapid onset and short duration of action in animals and man[68,69]. It is degraded rapidly to inactive products in the presence of basic ions, such as the bicarbonate of plasma. The drug has not been pursued further, as its inherent instability poses problems in its bulky synthesis and formulation.

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