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REVIEW ARTICLE

Structure, conformation, and action of neuromuscular blocking drugs†

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Since the introduction of (+)-tubocurarine (dTc),[45] many new neuromuscular blocking (NMB) agents have been brought into anaesthesia practice. The large molecular size and the quaternary ammonium structure of these compounds are unique among anaesthesia-related drugs. For practical purposes, understanding their structure–activity relationship (SAR) is a rational way to understand their actions and metabolism. A 1994 review by Hill and colleagues summarized contributions by previous reviewers and provided details on several series of compounds.[50] Besides molecular structure, this review will cover molecular conformation and attempt to offer explanations.

Historic and scientific background

History

The classic experiments of Claude Bernard led to the recognition of the anatomical gap between motor nerve and skeletal muscle.[7] Dale and colleagues identified acetylcholine (Ach) as the transmitter at the neuromuscular junction.[16 25] dTc was originally thought to be bisquaternary, but in 1970 its structure was revised to correctly indicate that it is monoquaternary.[38 65] The tertiary nitrogen (N) is only protonated, yet dTc nevertheless functions like a bisquaternary compound. Corresponding with the revision, metocurine (mTc) becomes *O*,*O*',*N*-trimethyl tubocurarine,[93] instead of *O*,*O*′-dimethyltubocurarine as it was originally called. It has two permanent quaternary onium heads (Fig. 1).

Hofmann described in 1841 the elimination of an α-β carbon radical from the quaternary N at high pH and temperature (100°C). This is generally called 'Hofmann elimination' (see Stenlake and colleagues[94] for details). In the conception of atracurium, the acyl group was designed to break away under physiological pH and temperature, leaving the tertiary benzylisoquinoline product laudanosine (Fig. 1).

Ach, skeletal muscle endplate AchR, and cholinergic agonists

Ach is a flexible molecule capable of adopting several conformations without significant energy penalty.[23] This allows it to be physiologically multifunctional. Its symmetrical conformers can flip easily. The structure of Ach, CH_3-CO-O-$(CH_2)_2$-$N^+(CH_3)_3$, although simple, has several important functional features, namely the methonium head centred on the positively charged quaternary N atom, the alcohol O atom that forms the ester (-O-), and the acetyl group with the carbonyl O atom (-CO-).

The skeletal muscle endplate Ach receptor (AchR) is generally modelled after the electric organ nicotinic AchR as a pentameric structure of $\alpha_2\beta\gamma$ (or ε) δ subunits arranged in a rosette around a sodium–potassium ionic channel (Fig. 2). Each receptor has two Ach receptive sites, one on each α subunit in a pocket near where the α subunit interfaces with its neighbour γ or δ subunit. Pedersen and Cohen proposed that it is unlikely for the β subunit to be between the α subunits, and accordingly, one likely arrangement of the rosette is αγαβδ, or αγαδβ viewed from the other direction.[58 82 84 92] It takes two Ach molecules acting

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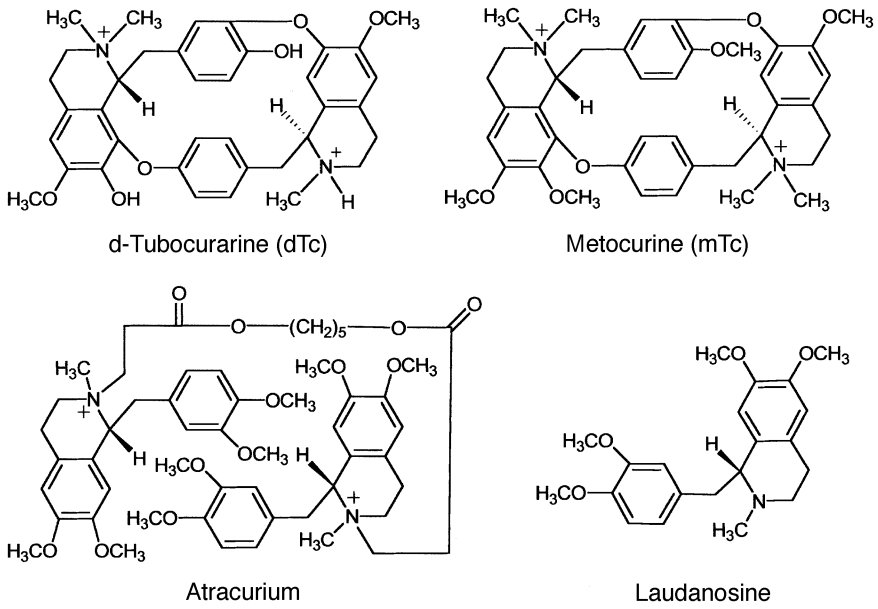


Fig 1 dTc, mTc, atracurium, and laudanosine. Atracurium is drawn in an unusual way of presentation (with the connecting chain around the benzylisoquinolinium heads) to orient its benzyl and tetrahydroisoquinoline moieties with the other compounds. Atracurium is also shown in Figure 6. Removal of the connecting chain of atracurium by Hofmann elimination yields laudanosine.

concomitantly, one on each receptive site, to open one AchR channel. The neighbouring subunits make the receptive sites different in affinity. The distance between the two receptive sites has been estimated to be about 50 Å between their outer limits and 30 Å between their inner limits[30 97] (Fig. 2A). The space available for NMB agents could be smaller, but the cross-section of the entire receptor is larger and could exceed 80 Å. The pentameric arrangement in a circle indicates that the two Ach receptive sites are not symmetrical or mirror image to each other (Fig. 2B).

Each receptive site, in turn, has two subsites, namely, an anionic subsite to attract the positively charged onium head and a hydrogen bond donor to attract the hydrogen bond acceptor of Ach (Fig. 2B). The asymmetrical arrangement makes it possible for subsites of like charges to completely avoid facing each other directly.

Various cholinergic receptors and cholinesterases have different conformational requirements or preferences of their agonists (or substrates in the case of cholinesterase) and antagonists. Of the cholinergic compounds, a 1970 report,[4] rarely quoted in literature on neuromuscular pharmacology, proposed that the distance from the centre of the cationic N to the van der Waals (*vdw*) extension of the respective O atom (or equivalent hydrogen bond acceptor) is important in determining whether a cholinergic agonist will be nicotinic or muscarinic (Fig. 3). A distance of 4.4 Å will impart muscarinic action, while a distance of 5.9 Å will impart nicotinic action.[4] For convenience, these two rules will be referred to in this review as the Beers and Reich's rule of 4.4 Å for muscarinic action and rule of 5.9 Å for nicotinic action, respectively. The ester O and the carbonyl O of Ach can fulfil these respective rules readily.

The receptive sites are chiral-sensitive or chiral-selective, if not chiral-specific. For example, cisatracurium is more potent than its stereoisomers supposedly because it fits the receptor at the receptive site better. A conformation of Ach bound to a Torpedo nicotinic receptor has been published.[5] For medicinal chemistry and clinical anaesthesia, however, one must realize that species variation and status of desensitization may alter the conformation of Ach bound to the receptor and/or that of the receptor itself. Free Ach may prefer a bent configuration.[24]

Decamethonium and congeners, and succinylcholine

Pharmacologically, the classic work of Paton and Zaimis established that of the polymethylene bismethonium series of compounds, C5–C12 and C18, congener C10 (decamethonium) is optimal for neuromuscular block.[83] In a biphasic manner, other congeners longer and shorter alike lose NMB potency. Instead of being neuromuscular blockers, C5 and C6 are ganglion blockers. Congeners higher than C11 lose potency, but very long congener such as C18 shows a trend toward re-gaining potency while changing to a non-depolarizing mechanism of block.[50 83]

Chemically, each congener of C10 differs from its neighbour only by one methylene group in the polymethylene chain that connects the two terminal methonium heads. Besides molecular length and a possible slight increment in lipophilicity with each additional methylene

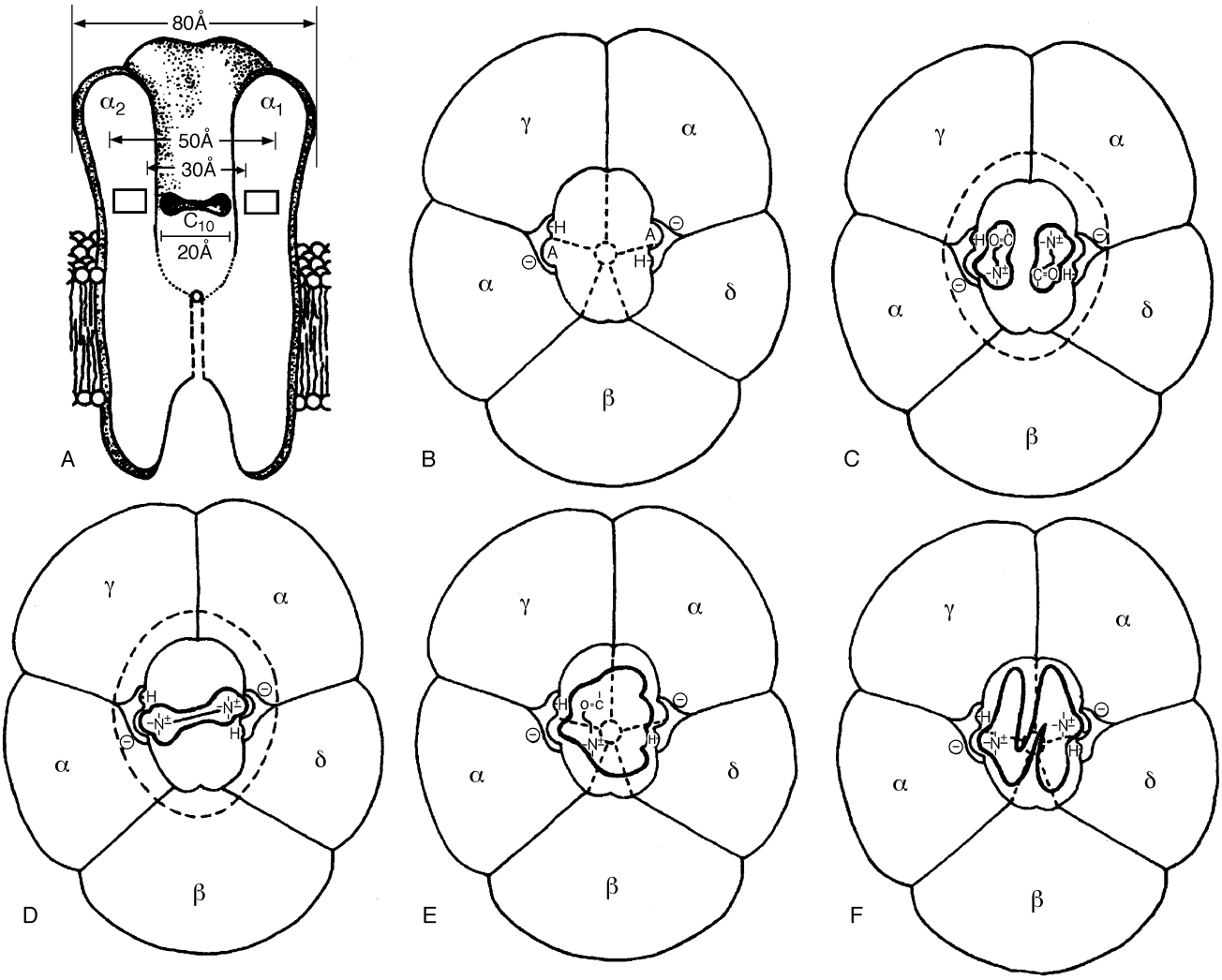


Fig 2 Diagram of a model of the muscle nicotinic AchR, vertical section (A), and cross-section at the level of the receptive sites (B–F). The pentameric rosette is arranged according to one version of Pedersen and Cohen.[84] (A) Vertical section showing receptor dimensions and hypothetically a molecule of C10 between the receptive sites. (B) Each receptive site has an anionic subsite (A) and a hydrogen bond donor (H) subsite. The circular arrangement maximizes the chances for subsites of unlike charges to face each other and promote closure of the resting channel, shown in dashed lines. (C) Ionic channel opened by two Ach molecules. (D) Ionic channel opened by decamethonium binding both anionic subsites. (E) Blockage by vecuronium (flipped α side up) binding the anionic and the H bond donor subsites of one receptive site. (F) Blockage by a long-chain bisquaternary bulky NMB molecule that bends to fit between the anionic subsites.

group, nothing else explains their different pharmacological profiles. Consequentially, one can assume that at some point of drug–receptor interaction the molecular length of C10 best fits the space available to NMB agents between the two receptive sites of the muscle nicotinic AchR (Fig. 2A and D). However, if the molecule of these congeners may bend to alter its N-N interonium distance with relative ease, such an assumption becomes insecure, and the superior NMB potency of C10 becomes unexplainable.

As is in the case of Ach, it takes two molecules of succinylcholine to open one AchR ionic channel.[30 73] (One wonders whether succinylcholine also exists in bent form and will, therefore, not span the two receptive sites across the channel (see below). Furthermore, if one Ach moiety of succinylcholine binds both the anionic and hydrogen bond donor subsites of one receptive site, its other Ach moiety would be prevented from pointing correctly to the other receptive site.)

Chemical classes and structures of NMB drugs

Ach, succinylcholine, and C10

Ach is the choline ester of acetic acid. Cholines are quaternary ammonium alcohols. Succinylcholine is two Ach molecules joined end-on-end, through the succinic acid connecting chain, HOOC-CH_2-CH_2-COOH, a diacid. In C10 congeners, a simple polymethylene chain of various

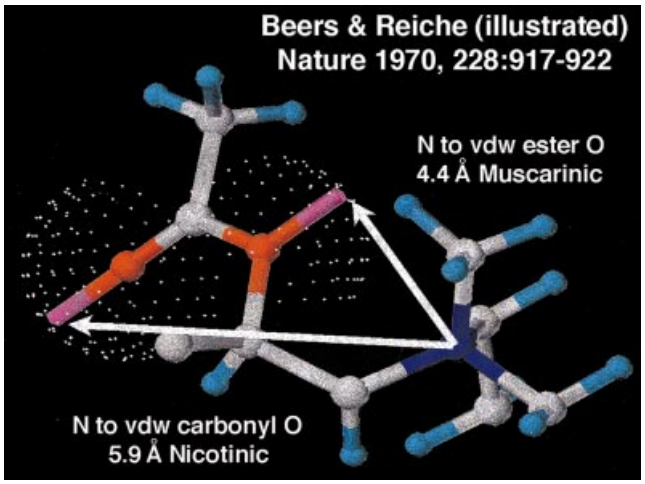


Fig 3 Beers and Reich's rule of 4.4 Å for muscarinic action and rule of 5.9 Å for nicotinic action illustrated together on a hypothetical Ach-like moiety. Red atoms are O, blue atom is N, white atoms are C and green atoms are H. Bars extended out of the O atoms show the direction and length of their *vdw* radii. Arrows point from the centre of N to the *vdw* extension of the O atoms.

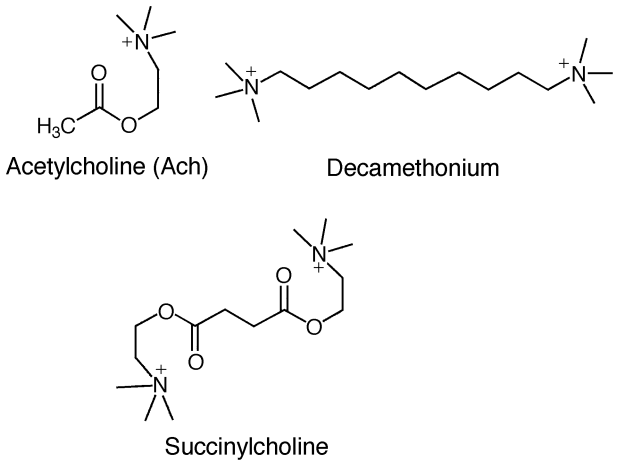


Fig 4 Ach, decamethonium, and succinylcholine. While decamethonium prefers a straight conformation, Ach and succinylcholine prefer bent geometry because of electrostatic interactions between the functional groups (methonium head, carbonyl group and ester O atom).

lengths, -$(CH_2)_n$-, connects the two methonium heads, without any hydrogen bond acceptor (Fig. 4). Although succinylcholine and C10 have the same number of heavy atoms (C, O) between their methonium heads, succinylcholine has complex functional groups and is a shorter molecule. Both succinylcholine and C10 have small trimethyl onium heads and flexible links.

Aminosteroids

Pancuronium, vecuronium, rocuronium, rapacuronium, dacuronium, malouètine, duador, dipyrandium, pipecuronium (Arduan), chandonium (HS-310), HS-342 and other HS- compounds[1 8 28 29 32 37 41–43 62 75 76 80 81 98] are among the aminosteroid NMB agents. The steroid nucleus provides a rigid bulky structural base (Fig. 5). Of the two Ach moieties of pancuronium, the A-ring Ach has *trans* geometry, with the charged N group pointing up (β) while the acetoxy group points down (α). The D-ring Ach has *cis* geometry, with both functional groups pointing up (β). In the case of vecuronium, the A-ring Ach moiety is tertiary, without a methyl quaternizing group. Vecuronium is, therefore, D-ring Ach monoquaternary. Its A-ring Ach is a 'NOR' ('Nitrogen ohne Radical' in German, or nitrogen without radical), as in the trade name (Norcuron®). As pancuronium and vecuronium are roughly equipotent, their NMB action can be attributable to their common *cis* D-ring Ach moiety. Details of conformation–activity relationships (CAR) of the A-ring and D-ring Ach moieties, especially in connection with their different NMB *vs* cardiovagal blocking profiles, are discussed in a separate section below. Strangely, some conformationally rigid small *cis* analogues of Ach are inactive.[21]

Tetrahydroisoquinoline derivatives

Atracurium, mivacurium, and doxacurium are benzylisoquinolinium compounds based on the tetrahydroisoquinoline moiety (Fig. 6).[2 3 6 71] On their benzylisoquinolinium heads are 4, 5, or 6 methoxy groups, in that order. The connecting chain between the two onium heads is long, and flexible except for the double bond of mivacurium. Other benzylisoquinolinium NMB compounds may have rigid segments in their connecting chain.[50] The connecting chain for doxacurium is succinate, although doxacurium is not readily metabolized by plasma cholinesterase. In the case of atracurium, the ester linkage is reversed, meaning that the ester O and the carbonyl groups (CO) are transposed, -CO-O-$(CH_2)_n$-O-OC-, instead of -O-OC-$(CH_2)_n$-CO-O-. By definition, the benzylisoquinolinium structure of atracurium is an acid, not alcohol or choline, and the esters are not cholinester.

On first sight, dTc and mTc do not look like atracurium, mivacurium, or doxacurium. However, they are benzylisoquinolinium compounds. Their onium heads are interconnected by ether linkages between the tetrahydroisoquinolinium group of one benzylisoquinolinium and the benzyl group of the other, instead of by a long diester chain between the quaternary N atoms (Figs 1 and 6).

Gallamine and other chemical classes

Gallamine is a trisquaternary ether, with one ethonium head each attached to the 1, 2, 3 positions of a phenyl ring through an ether linkage -O-$(CH_2)_2$-$N^+(CH_2CH_3)_3$ (Fig. 7).

Many other chemical structures, such as fazadinium (AH8165), alcuronium (alloferin), diadonium, anatruxonium, and tropeinium,[14 15 17 26 27 48 52 53 57 59 88] have been exploited for NMB properties. Some of these illustrate SAR

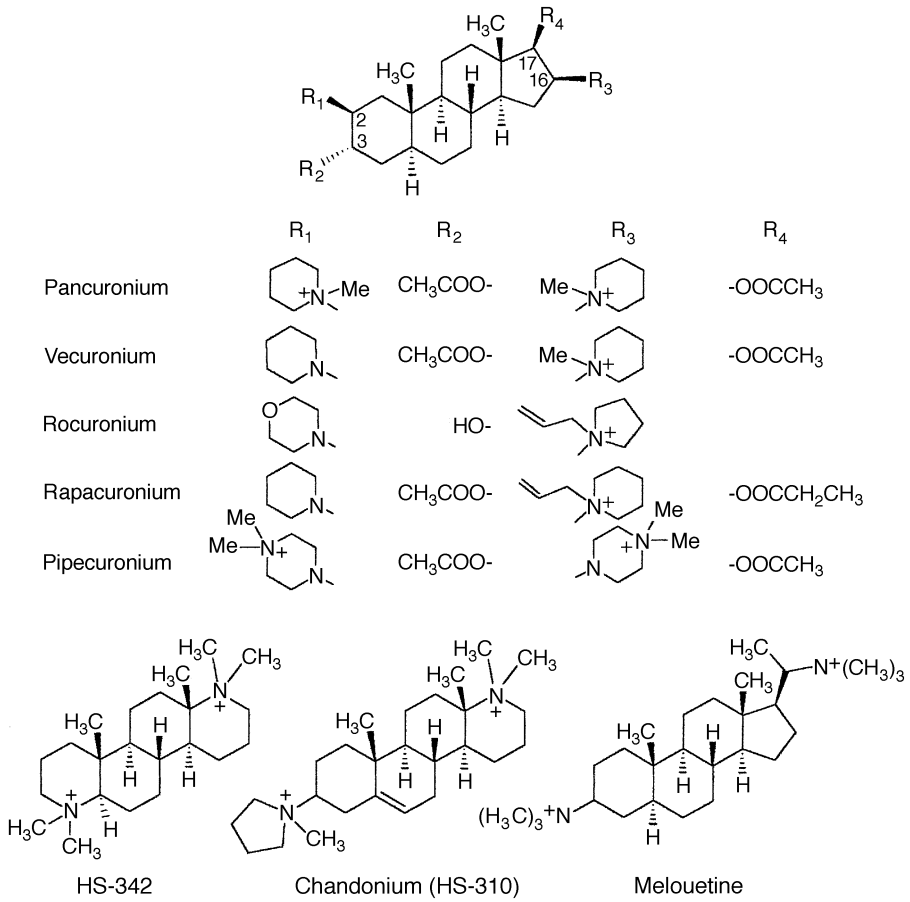


Fig 5 Molecular structures of some aminosteroid NMB agents. The beta (up) surface of the molecule is crowded with, *clockwise*, R1, two methyl groups, R4 and R3. The R1, R3–4 groups are tilted outward sidewise. Longer groups on R3 or R4 replacing the methyl group decrease potency, see text. The quaternary N atoms on pipecuronium are farthest out. HS-342, chandonium and malouètine differ, among others, in interonium distance.

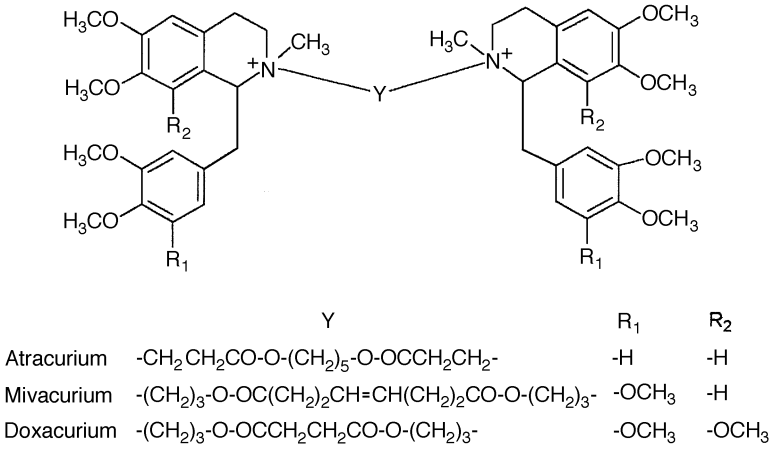


Fig 6 Atracurium, mivacurium and doxacurium have 4, 5, and 6 methoxy groups, respectively, on each of their onium heads and their NMB potency increases in the same order. The connecting chain of atracurium has two reversed esters. The tertiary product of Hofmann elimination from atracurium will be laudanosine.

well (Fig. 8). For more information, refer to individual publications and the reviews by Hill and colleagues, and Kharkevich.[50 62] The review by Kharkevich is a useful liaison to significant works published in Russian.[60 61 63]

Novel NMB agents

Of timely interest are a new series of asymmetrical diester isoquinolinium compounds[10] and a series of new bis-

benzyltropinium compounds (Fig. 9).[46 47] Both were recently introduced as candidates for the earlier defined 'ideal' ultra-fast and ultra-short-acting non-depolarizing NMB agent.[89] The former series of compounds differ from their benzylisoquinolinium predecessors by having asymmetrical structures, such as with one phenyl and one benzyl isoquinolinium head. The connecting diacid chain can also be asymmetrical, such as chlorofumarate (-CO-CH=CCl-CO-). Initial human trials have produced promising results. The latter group, that includes G-1-64, is bistropinium salts of various diacids. The onium head may have a benzyl group that is acyloxylated.[46] They are undergoing extensive pre-human testing (personal information).

SAR

Pachycurares and leptocurares

A rule of SAR that was discovered early on and still seems valid is that bulky and rigid ('pachy' meaning thick) molecules or onium heads usually cause non-depolarizing block, while slender, small and flexible ('lepto' meaning thin) compounds usually result in depolarizing block.[11] Rigidity often co-exists with bulk, as large numbers of atoms form specific ring structures. Methonium is a small onium head and succinic and polymethylene groups are small and flexible links (Fig. 4). They make succinylcholine and C10 depolarizing. The steroid nucleus is bulky and rigid, so are benzylisoquinolinium and tropinium heads, and they are the structural basis of potent non-depolarizing NMB agents (Figs 1 and 5–9).

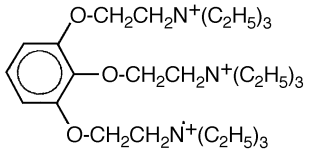
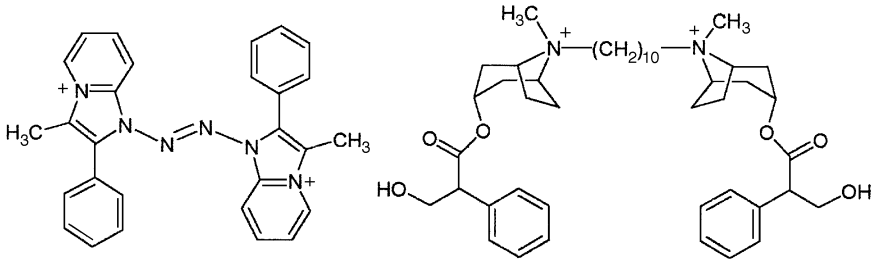


Fig 7 Gallamine is tris-quaternary, with three ethonium heads attached to the phenyl ring through ether links. See Figure 10 regarding its CAR.

Tertiary, monoquaternary, bisquaternary, and multiquaternary compounds

Succinylcholine and C10 are bisquaternary. dTc is monoquaternary, but the tertiary N is protonated. mTc has both its N atoms permanently quaternized and is more potent than dTc.[87 93] Pancuronium was the most potent NMB agent known at the time of its introduction. Of the HS series, bisquaternary compounds are much more potent than their monoquaternary analogues.[43] Before the discovery of vecuronium, these observations were convincing evidence that potent NMB agents, depolarizing or non-depolarizing, must be bisquaternary.

The discovery of vecuronium established the value of some monoquaternary aminosteroids as superior NMB agents (Fig. 5). It has been said that between pancuronium and vecuronium, in the early days of development of the aminosteroid series of NMB agents, pancuronium was



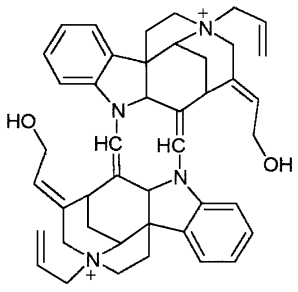


Fig 8 Fazadinium has a short interonium distance. Alcuronium has a rigidly fixed interonium distance, but the number of interposing atoms is debatable. Decamethylene bis-atropine consists of two atropine molecules connected by a quaternizing C10 linkage, which rotates only within a narrow range of interonium distance.

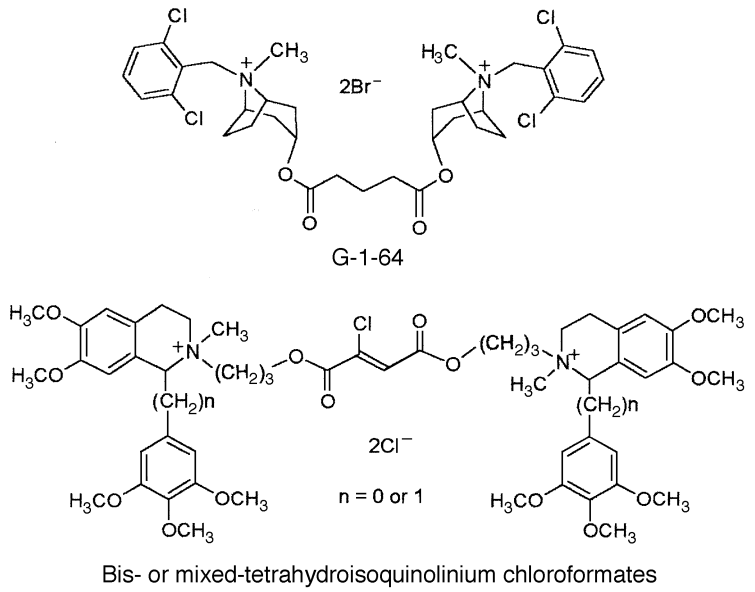


Fig 9 Novel compounds as candidates for ultra-short and ultra-fast-acting NMB agents. G-1-64 is prototype of a large series of benzyl-quaternized bistropine diesters, many of which have an acyloxylated benzyl group.[46 47] The chlorofumarates can have dissimilar onium heads. Chlorofumaric acid is itself an asymmetrical diacid.

developed ahead of vecuronium because of its bisquaternary structure.[33 85] It was also said that Nicholas Durant, then a PhD student, while learning the 'bisquaternary rule', 'accidentally' discovered the superior NMB profile of vecuronium (personal communication).

Gallamine is trisquaternary. There are also tertiary amine NMB agents, and compounds with more than three onium heads.[50 62 74] They are not superior NMB agents, some also producing block pre-junctionally.

Pipecuronium and other aminosteroid NMB agents

The aminosteroids illustrate several important SAR and CAR and these will be discussed under separate headings. Meanwhile, it is noted that pipecuronium is chemically bisquaternary, with dimethylpiperazinium heads on the A and D rings of the steroid nucleus (Fig. 5). Its functional groups are far from being Ach-like in geometry, indicating that pipecuronium probably blocks neuromuscular transmission as a bisquaternary compound (with both onium heads), in contrast with vecuronium and analogues that block with their D-ring Ach moiety as monoquaternary. Pipecuronium matches or exceeds pancuronium in potency.

Separation of the charged quaternary onium heads

Ten heavy (C or O) atoms separate the quaternary N atoms of C10 and succinylcholine (Fig. 4). When two molecules of quaternized atropine (Fig. 8) or other suitable onium heads are interconnected, a polymethylene chain of 10 C atoms imparts greatest NMB potency.[50 62 64] For dTc, mTc, and pancuronium, one can also count 10 atoms along one path or another from one N to the other (Figs 1, 5 and 8). This is the so-called '10-atoms rule' for NMB potency.

Separation of the two quaternary N atoms (by 5–6, 10–12 or 15–18 C atoms) determines the cholinergic (ganglionic *vs* neuromuscular) profile of the congeners of C10.[83] In days past, some concepts were passed down to anaesthesia trainees that cholinergic 'receptors' might be spaced 5-carbon lengths apart, so that potent nicotinic antagonists span 1, 2, or 3 such lengths. This is obsolete because, obviously, receptors are, in fact, much larger (Fig. 2).

The '10-atom rule' of NMB potency is flawed by the fact that the interonium distance maintained by the 10 atoms differs markedly among potent NMB agents. Succinylcholine tends to assume a bent conformation, and is, therefore, shorter than C10 (Fig. 4). The two N atoms in mTc, pancuronium, and alcuronium are not fixed along a straight line. An interonium distance of 14 Å for them is a frequent misquote (Figs 1, 5 and 8). Equally ambiguous is the 15–18-atom interonium distance for the return of NMB potency. The connecting chains of modern long-chain muscle relaxants are too flexible and variable to support the 15–18-atom distance hypothesis (Figs 6 and 9). They may bend to fit the 10-atom distance instead.

It is the linear interonium distance, not the number of interposing atoms, which generally differentiates between NMB and ganglion blocking potencies. C5 and C6, dTc, the bisquaternary HS compounds and AH8165 show that bisquaternary NMB agents with shorter interonium distance are associated with increased ganglionic block.[15 75 93] Conformational details of this are discussed below.

Importance of one methyl substitute on the functional groups for potency

Most potent NMB agents have at least one methyl group on the active quaternary N atom. In place of the methyl group, rocuronium and rapacuronium have a propenyl (-CH_2-$CH{=}CH_2$) and gallamine has all ethyl quaternizing groups (Figs 5 and 7). As a result, rocuronium and rapacuronium are much weaker than vecuronium.[50 77 90 99] Likewise on the functional hydrogen bond acceptor, acyl groups larger than acetyl also reduce potency. Rapacuronium, the newest aminosteroid NMB agent, has 16-N-propenyl and 17-propionyloxy (CH_3CH_2COO-) groups. For losing both methyl groups, it is more than 10 times weaker than vecuronium.[8 44]

Lipophilicity and the potency of aminosteroid NMB agents

Wierda and colleagues reported that among several aminosteroid compounds, the NMB potency is inversely related to the lipophilicity.[99] One wonders whether this inverse correlation with lipophilicity is an independent determinant of potency or coincidental with other factors, such as steric hindrance.

In general, a 16-N quaternizing group larger than methyl that makes the aminosteroid a weaker NMB agent is also more lipophilic. So is a 17-acyloxy group larger than acetoxy. Conformationally, any group bulkier than the methyl might distance the functional groups from the action centres of the receptor and impose charge dilution and steric hindrance, which in turn reduce NMB potency. Pipecuronium has least hindered and highly hydrophilic onium heads (Fig. 5), and it excels in potency.

Methoxy groups increase potency of benzylisoquinolinium NMB agents and reduce side effects

Both hydroxy groups of dTc are methylated in mTc (Fig. 1). As a result, mTc has four methoxy groups, while dTc has two, and mTc has 2–3-fold greater potency and specificity than dTc.[86 87] Atracurium, mivacurium, and doxacurium have on each of their benzylisoquinolinium heads 4, 5, and 6 methoxy groups, in that order (Fig. 6). Their potency increases in that same order.[2 3] The reduction of histamine release could also be attributed to the increased number of methoxy groups, by improved specificity or indirectly by reduction of dose requirement.

Hydrogen bond acceptor requirement

To be a potent monoquaternary NMB agent, a properly positioned hydrogen bond acceptor must accompany the active onium head. This requirement is observed in the weak non-acyloxylated monoquaternary aminosteroid compounds (including the HS series), and in the 17-desacylated products of vecuronium and analogues.[43 62 75 85 99]

Metabolizable bonds, degradation, and excretion relationships

Molecular structure of most NMB agents relates to their metabolic pathways, although exceptions exist. Even without enzymatic catalysis, short-acting NMB agents, especially the ultra-short ones, may break down spontaneously. The new asymmetrical isoquinolinium[10] and bistropinium diester compounds (personal information) most likely break down spontaneously *in vivo*. These compounds have unusual destabilizing molecular structures, such as molecular asymmetry (Fig. 9) or acyloxy groups around their onium heads (personal information).

As non-cholines, and, therefore, not substrate of any cholinesterase, the reversed ester linkages of atracurium and cisatracurium are made to break down by Hofmann elimination, and/or non-specific (i.e. non-choline) esteratic hydrolysis, both in plasma.[22 39 94] Plasma cholinesterases hydrolyse succinylcholine and mivacurium, but not doxacurium.[40 71] The exception of doxacurium illustrates our incomplete understanding of the structural requirements and quantitative SAR for hydrolysis by plasma cholinesterases.

Esters hydrolyzed by hepatic esterases are usually non-cholines, as the cholinesters are already broken down in the plasma or tissue. In general, very water-soluble NMB agents undergo renal excretion, while the liver takes up less water-soluble ones for metabolism and/or biliary excretion. Among the aminosteroids, the hydroxy products are generally less water-soluble than their parent esters,[32] and pipecuronium is more dependent on the kidneys for excretion than its 16-onium,17-aceloxy analogues.

The ester structures on positions 3 and 17 of the aminosteroid NMB agents are susceptible to hepatic esterases for de-acylation to 3-OH, 17-OH and/or 3,17-dihydroxy metabolites.[9 32] With a steroid link, these pharmacologically Ach-like moieties are chemically distant from Ach. They are, therefore, not substrates for plasma or tissue cholinesterases. Rapacuronium deserves special mention. It is rapidly hydrolyzed to 3-OH rapacuronium, spontaneously or catalysed 'by esterases of unknown identity and site' (quoted from official package insert of Raplon®), not in the liver. As a result, its NMB action is not significantly altered in hepatic failure,[35 96] although its metabolite, (see below), is active and theoretically may be cumulative.

C10 has no metabolizable bonds. Gallamine has three and mTc has two ether (besides methoxy) links. Although ether inhalational anaesthetics are metabolized to some extent, ether NMB agents are hardly metabolized. These agents, as well as doxacurium, are eliminated intact through the kidneys. Methoxy group breakdown or removal is not recognized as a metabolic pathway for NMB agents.

While plasma cholinesterase may be atypical, inhibited or deficient, and hepatic esterases may be deficient in hepatic failure, non-specific esterases have a greater margin of safety and Hofmann elimination or spontaneous breakdown rarely fails under clinical pH or temperature. The above provides the basis for choice of NMB agents in managing patients with organ failures or atypical or inhibited plasma cholinesterases.[20 34 35 54–56]

Structure–onset relationship

Unfortunately, little is known about the independent relationship between structure and onset of NMB action, which so far has only been observed to relate inversely to potency.[12 66 78] Bowman and colleagues made this observation on the aminosteroid compounds,[12] as did Wierda and Proost,[99] and then Nigrovic and colleagues.[79] Kopman and colleagues expanded this observation to include NMB agents of all ranges of duration of action.[66] Being weaker and faster than their predecessors, the new aminosteroids, rocuronium and rapacuronium, are no exceptions.[99] The rapid onset of rocuronium appears to qualify it as an outlier,[37] but not really an exception to the rule considering its relatively low potency.[77] The new asymmetric isoquinolinium compounds appear unusually fast for the potency.[10]

The inversed potency–onset relationship is a disappointment. Potency of action is often determined by specificity of receptor binding. In other words, it is a means towards freedom from side effects. Unfortunately, sacrificing some specificity appears so far to be the only known method to speed up onset of action, as was done in the development of rocuronium and rapacuronium. Rapacuronium incurs more side effects than its predecessors.[8 44] Donati and colleagues proposed a plausible model to explain the inversed potency–onset relationship.[31] Weak compounds have more molecules to diffuse from the central compartment into the effect compartment. Once in the effect compartment, all molecules act promptly.

Chemical groups associated with specific side effects

Benzylisoquinolinium structure tends to cause histamine release, although SAR exercises have largely removed this side effect from mTc, doxacurium, cisatracurium, and the novel asymmetric isoquinolinium compounds.[10] Tropine derivatives tend to cause tachycardia.[46] It is hoped that the tachycardia in humans that may follow administration of the novel bistropinium NMB agents, if any, will be mild, equally short-acting, or even protective against the reflex bradycardia that may complicate tracheal intubation. Laudanosine may cause convulsions. The improved potency of cisatracurium reduces laudanosine production.[13 19 36]

Pro-drug or cumulation of active metabolites

A pro-drug is not a cherished idea for NMB agents. Conversion of the pro-drug to the active drug takes time and fast onset is desirable. In other words, active metabolites are not desirable. On prolonged use, cumulation of NMB effect may follow accumulation of metabolites, especially in renal failure, but, so far, metabolites of NMB agents have been weaker than their parent compounds.[8 18 22 44 90 91 96] Rapacuronium is an exception. Its metabolite, 3-desacetyl rapacuronium, is more potent, slower and longer acting than rapacuronium itself.[90]

New structural features of the novel NMB agents

In the new bisquaternary isoquinolinium diester series of candidates for the 'ideal' NMB agent, the asymmetry of the chlorofumaric connecting link and the onium heads[10] is intriguing because the two receptive sites of the muscle nicotinic AchR are now known to differ.

While previous improvements on the benzylisoquinolinium compounds have succeeded in increasing the NMB potency by addition of methoxy groups, improvements in the tropinium diester series, which includes prototype G-1-64,[47] have resulted from incorporation of acyloxy groups onto the benzyl ring that quaternizes the tropinium head.[46] Acyloxylation of existing NMB relaxants, such as dTc or other isoquinolinium compounds, could make interesting future SAR exercises.

From the above, one recognizes that exceptions and outliers to the SAR rules deserve attention. For example, vecuronium qualified as an exception to the 'bisquaternary rule'. Doxacurium is an exceptional benzylisoquinolinium for not releasing histamine. Its resistance to plasma cholinesterase is an exception in the wrong direction. For its potency, rocuronium has outstanding speed of onset. Desirable outliers are often those with a wider autonomic margin of safety.[86]

Besides the classical SAR, computer-aided 'quantitative structure–activity relationship' (QSAR) has enhanced application of SAR in the development of new therapeutic agents. The traditional SAR is mainly qualitative. Its application cycle consists typically of *conception >> chemical synthesis >> pharmacological testing >> conception of modified compounds*. QSAR incorporates *computer-based quantitative analyses* in the cycle, often before chemical synthesis. It facilitates creation, screening, and prioritization of competing ideas 'on paper', or on the monitor. QSAR has been extensively utilized in the pharmaceutical industry, but its beneficial application in the research of NMB agents still awaits exploration and proof of viability.

CAR

While SAR relates structure to action, it is imprecise and there are numerous exceptions to the rules. Even where rules of SAR hold, and many have held and facilitated the conception of new NMB drugs, they often do not explain the mechanism of action.

As more is learned about the muscle nicotinic AchR, further progress in understanding the effect of structure on the action of NMB agents may focus on molecular conformation. Obvious conformational questions of interest include, for example, how the D-ring Ach moiety of pancuronium imparts NMB action while the A-ring Ach moiety imparts cardiovagolytic action, how methoxylation improves the NMB potency of the benzylisoquinolinium compounds, and how stereoisomerism affects potency.

Molecular shape is a fundamental concept of organic chemistry.[95] In the past, various stereomodels made of plastic or other materials have provided useful estimation of the size, shape, interatomic distance, and flexibility of molecules. Although computer chemistry is a fast growing science, the computer-aided conformational study of NMB agents is primitive at best.

Molecules prefer to exist in low energy conformations, and flexible molecules incur a smaller energy penalty to change conformation. In general, conformational flexibility means low selectivity. For practical application by non-computer chemists, user-friendly computer programs are becoming available for reliable search of low-energy molecular conformations. Besides energy minimization and conformational searches, computer modelling allows vivid visualization and accurate measurement of molecular dimensions.

Like SAR, CAR is commonly studied without considering the environment the molecule is actually in. One obvious concern is that the NMB agents may change conformation in the milieu of the body fluid and on interaction with the receptor.[5] Advanced modelling may take this into consideration in the future. Meanwhile, such caveats do not necessarily invalidate simple CAR concepts, as they have not discredited SAR rules, if cautiously interpreted. Many useful rules of SAR and CAR have been recognized in the past using plastic models.

Molecular orientation

When receptor-bound, a molecule presumably has its functional side facing the receptor and the other side exposed and facing away. Ach bound to the nicotinic receptor exposes a continuous lipophilic surface of insulating methyl groups.[5] The molecule of mTc has a convex hydrophilic side that is supposed to interact with the receptor.[93] The steroid structure of HS-342 shows crowding on the β (up) side, and Gandiha and colleagues speculated that it interacts with the receptor on the less hindered, α side.[43] Vecuronium and analogues have the D-ring Ach on the crowded β side, but their functional groups protrude out of the steroid nucleus sideward (Fig. 5). Side-specific orientation of functional groups attached to rigid NMB molecules may be important to potency.

Stereospecificity of benzylisoquinolinium NMB agents

dTc (*d*-tubocurarine) is 20 times as potent as its *l*-isomer. The benzylisoquinolinium head of cisatracurium has an R-R *cis* conformation that increases potency and reduces its side effects.[19 36] Doxacurium has three, all *trans* stereoisomers. Mivacurium has *trans–trans* (52–60%), *trans–cis* (34–40%) and *cis–cis* (4–8%) stereoisomers. The *trans–trans* and *trans–cis* isomers are favourable.[49 71] The *cis–cis* has slow elimination, but usually poses no significant risk of cumulation in clinical use because it is a minor low-potency component. The conformational commonality of the advantageous benzylisoquinolinium heads has not been well studied.

Molecular length of C10, mTc, pancuronium, gallamine and pipecuronium, and the hypothetical distance between the receptive sites of the muscle endplate AchR

If C10 can be presumed to be a straight molecule, its molecular length may hypothetically be a credible measure of the space between the receptive sites of the muscle nicotinic AchR that is available to NMB agents. Conformational studies have shown that C10 congeners indeed strongly prefer a straight conformation (personal data). Including the *vdw* radii, the lowest energy conformer of C10 measures approximately 20 Å (Fig. 10). It is interesting that low energy conformers of gallamine also prefer this molecular length (19–20 Å) (Fig. 10).[68] The middle ethonium head of gallamine is said to stabilize the two other ethonium heads.[50] This reviewer supposed that it keeps them pointed outward to span 20 Å.[68]

mTC is among the most rigid NMB agents. It measures about 18 Å in total length. Pancuronium measures 19 Å. Pipecuronium measures 21 Å, with its quaternary N atoms farthest out and least hindered among its analogues (Fig. 5). The dimension of these potent rigid NMB agents follows the ranking order of their potency, indicating that their molecules size the 'inter-site working space' of the receptor. These NMB agents are the most potent among their respective chemical classes. Together, they suggest 20–21 Å as an optimal dimension to fit the space actually available to NMB agents between the receptive sites of the muscle nicotinic AchR (Fig. 2).

The different interonium distances for neuromuscular and ganglion blocking potencies

The interonium distances of AH8165 (7.5 Å), pentamethonium (C5, 7.7 Å), HS-342 (8.0 Å), hexamethonium (C6, 9.0 Å), HS-310 (chandonium, 10.2 Å), protonated dTc and mTc (10.8 Å), pancuronium (11.4 Å), malouètine (12.2 Å), decamethonium (C10, 14.0 Å), and pipecuronium (16.0 Å) illustrate a trend that a short interonium distance imparts

ganglion block, while a long interonium distance favours neuromuscular block.[15 75 93] Depending on the conformation of the onium head, molecular length and the interonium distance may coincide.

Among aminosteroids, pipecuronium has an interonium distance farthest away from that which is optimum for ganglion block. Correspondingly, it is among the least likely NMB agent to cause such side effects.[42 51]

Succinylcholine and C10 differ in conformation, neuromuscular block and side effects

As mentioned above, both Ach moieties of succinylcholine likely exist in a bent conformation (Fig. 4),[70] making it unlikely for one molecule of succinylcholine to span 20 Å. Furthermore, each Ach moiety of succinylcholine retains the conformation and flexibility of Ach, complete with hydrogen bond acceptor, to enable it to conform to multiple cholinergic receptors. If succinylcholine binds like Ach, with both the methonium head and the carbonyl O of one Ach moiety, its other methonium head may be prevented from pointing correctly to the other receptive site.[70] This may explain why it takes two succinylcholine molecules, as it takes two Ach molecules, to open one nicotinic Ach channel.[30 72] It also explains why succinylcholine differs from C10 in its side effects.[67]

Conformation and activity of A-ring vs *D-ring Ach moieties of aminosteroids*

The A-ring Ach moiety of pancuronium has *trans* conformation. From its centre, the charged N atom can assume a distance of 4.4 Å to the *vdw* extension of the ester O, but not a distance of 5.9 Å to the *vdw* extension of the carbonyl O. According to the rules of Beers and Reich, this A-ring Ach can be muscarinic, but not nicotinic. By contrast, the D-ring Ach moiety of pancuronium and vecuronium is *cis*. It obeys the rules of Beers and Reich to be nicotinic but not muscarinic.[69] Dacuronium (17-OH pancuronium) has an incomplete D-ring Ach moiety. Like pancuronium, however, it has an intact A-ring Ach moiety, and therefore high cardiovagal blocking potency.[1 85]

These and other concurring and contrasting examples[85] strongly suggest that while D-ring Ach is a pharmacophore for skeletal muscle nicotinic AchR, the A-ring Ach is a pharmacophore of the cardiac m_2 muscarinic receptor.[51] It explains why pancuronium and vecuronium have similar NMB potency while pancuronium alone causes a tachycardia.

Conformation of potent aminosteroid metabolites

The 3-OH metabolites of potent 3,17-diacyl aminosteroid NMB agents retain significant potency,[9 18 85 90] and 3-desacetylvecuronium is thought to cause prolonged paralysis in critically ill patients.[32 91] As discussed above, 3-OH rapacuronium is even more potent than rapacuronium itself. By contrast, the 17-OH counterparts in general are weak. This discrepancy is understandable, because the 17-acyloxy group complements the 16-onium head to complete the D-ring Ach moiety that affects neuromuscular block. It suggests that potent monoquaternary NMB agents must bind both the anionic and the hydrogen bond donor subsites of the same receptive site on the muscle nicotinic AchR.

Pipecuronium differs from other 3,17-diacyloxy aminosteroids

Pipecuronium has four functional groups, namely, 2- and 16-piperazinium heads and 3- and 17-acetoxy groups (Fig. 5). Its functional groups, however, resemble neither free Ach nor the A-ring or D-ring Ach moiety of pancuronium, and the quaternary N atoms are 3-atom distance farther away from the steroid nucleus. Without the like of the A-ring Ach moiety, pipecuronium, unlike pancuronium, does not cause tachycardia.[32 42 98] Without the like of a D-ring Ach moiety, pipecuronium probably blocks neuromuscular transmission as a bisquaternary, with two exposed and well-positioned onium heads, instead of as a monoquaternary, with a poor 16-onium,17-acyloxy pharmacophore.

Potent long-chain NMB agents

Most of these are bisquaternary, with flexible connecting chains that are 10 C-atoms long, or longer but bend without difficulty.[62 64] It is plausible to assume that they can conform to an optimal interonium distance (Fig. 2). Their stretched-out two-dimensional presentations (Fig. 6) do not meaningfully relate molecular dimensions to potency.

Conformational mechanism of the anti-muscarinic action of gallamine

Gallamine is highly cardiovagolytic.[51] The distance from the centre of N to the *vdw* extension of its ether O can readily be 4.4 Å without any energy penalty, obeying the rule of Beers and Reich for muscarinic action (Fig. 10).[68]

Conformational mechanism of neuromuscular block

The conformational features recapitulated below must be taken into consideration in explaining how NMB agents work.

1. The model nicotinic AchR arranges its pentameric subunits in a rosette. Both receptive sites must be activated for the ionic channel to open.
2. The rule of 5.9 Å of Beers and Reich for nicotinic action applies to NMB agents, as validated by the D-ring Ach moiety of pancuronium and vecuronium.

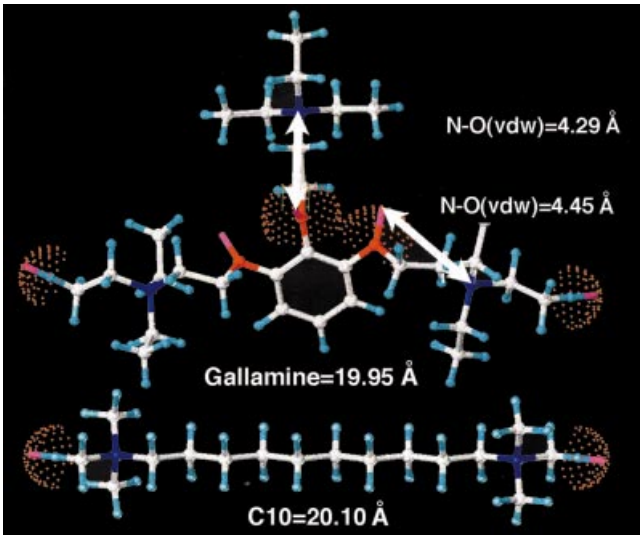


Fig 10 Molecular conformational mechanism of actions of gallamine and C10 illustrated in their low energy conformers.[68] Red atoms are O, blue atoms are N, white atoms are C and green atoms are H. Red dots around the ether O atoms and the terminal H atoms show their *vdw* radii extended in the direction marked. Total length including the *vdw* extensions is close to 20 Å for both molecules. The N-N interonium distances differ (11.30 Å, gallamine and 14.03 Å, C10). The double-arrows point to the centre of the N and the *vdw* extension of the ether O in two parts of gallamine, both of which conform to the Beers and Reich's rule of 4.4 Å for muscarinic activity.

3. C10, dTc and mTc, pancuronium and vecuronium, gallamine, and pipecuronium are among the most potent NMB agents of their respective chemical classes. Chemical diversity and variation in interonium distance notwithstanding, they all measure 18–21 Å in total length. The most potent of them, pancuronium, vecuronium, and pipecuronium measure 19–21 Å. Conformationally and pharmacologically, succinylcholine differs from other potent NMB agents. Gallamine has a third ethonium head to stabilize its molecular length.
4. Monoquaternary analogues of vecuronium depend on a properly positioned hydrogen bond acceptor that complements the active onium head for potency.
5. Bisquaternary NMB agents do not depend on hydrogen bond acceptors that complement their onium heads. Potent long-chain benzylisoquinolinium NMB agents have flexible connecting links that allow them to adopt shortened molecular lengths.
6. NMB agents lose potency if they lack a methyl quaternizing group or have the methyl group replaced by a longer alkyl or alkenyl group. Similar replacements on the 17-acyl group also reduce the potency of vecuronium analogues.
7. Pipecuronium lacks the Ach-like moiety that other aminosteroids depend on to act as potent monoquaternary NMB agents. Instead, its highly hydrophilic dimethyl onium heads protrude unhindered to permit pipecuronium to block as a potent bisquaternary.

The following conformational mechanisms of NMB action accommodate the above conditions.

- Potent NMB agents have at least two functional groups and bind two points. Potent bisquaternary agents bind the anionic subsite of both receptive sites with their onium heads, 'inter-site' (Fig. 2D and F). Potent monoquaternary agents bind the anionic subsite of one preferred receptive site and also form a hydrogen bond with the hydrogen bond donor, 'intra-site' (Fig. 2E). The D-ring Ach moiety of pancuronium and vecuronium is a NMB pharmacophore of precision and specificity.
- Alternative binding of two subsites other than in the above-specified combinations is less effective in cancelling the inter-site electrostatic attractions. Additional binding of the remaining subsites, as succinylcholine, pancuronium, and pipecuronium may do with their multiple functional groups, may slightly increase potency. However, onium heads greater in number than two (the number of anionic sub-sites) will not increase potency, except possibly by optimizing the conformation of the two binding heads.
- Depolarizing NMB agents initially bind both anionic centres while allowing depolarizing ions to flow through the channel around them (Fig. 2D). Non-depolarizing NMB agents block not only by receptive site occupation with functional groups, but also by bulk hindrance across the channel (Fig. 2E and F). Their molecules optimally measure 20–21 Å or adapt to that dimension.
- Replacing the functional methyl group with anything larger reduces NMB potency by distancing the functional groups from the receptive subsites. It also may reduce electrostatic interactions by increased lipophilicity.

This theory represents an up-dated snapshot based on the SAR and CAR rules reviewed, and is not intended to imply that receptor-relaxant interactions are static. It is open to speculations such as that long-chain NMB agents may be weakened by a rigid interonium link of non-optimal length. Like all SAR rules, species variations and exceptions must exist. How it relates to 'channel block' is beyond the scope of this review.

Conclusions

Imperfect and antiquated as they are, classical rules of SAR and their exceptions have helped the conception and development of numerous successful NMB agents. However, even with extensive SAR, refinement of any series of useful compounds still requires exhaustive trials and failure of many in multiple hopeful candidates. Like the discovery of vecuronium, any major breakthrough in NMB compound development is likely to continue to be somewhat 'accidental', because exceptional compounds are often exceptions to the rule.

The nicotinic AchR model for the skeletal muscle endplate is a pentameric macromolecule with two receptive sites, one on each α subunit where it joins its neighbouring δ or γ subunit. Two Ach molecules, one binding each receptive site, are required to open the ionic channel. Consequentially, blockage of one receptive site suffices to block neuromuscular transmission. This is the theoretical basis of action of potent monoquaternary NMB agents, which usually have two functional groups for the one receptive site.

The 'pachycurare' rule of the non-depolarizing mechanism of NMB action stands. The 'bisquaternary rule' stands, but in the case of monoquaternary aminosteroids, it is substituted by the 'two-point rule'. Potent NMB agents can block by either an 'inter-site' or 'intra-site' mechanism. The '10-carbon distance rule' applies only to the decamethylene connecting chain. Otherwise, it is the linear interonium distance and length of the molecule that determine NMB and ganglion blocking potencies, along with other factors. Onset of action, however, appears to follow no apparent rules of molecular structure or conformation, except that it is generally inversely related to potency.

Decamethonium appears to have an optimal length, 20 Å *vdw* radii included, to span the receptive sites. Potent bisquaternary non-depolarizing NMB agents of fixed molecular length measure 18–21 Å, with 20–21 Å appearing optimal. Long-chain NMB agents may have an accommodating connecting chain to adapt to the 20 Å model. Succinylcholine likely exists in short bent conformation, and takes two molecules to open one receptor ionic channel.

The 5.9 Å rule of Beers and Reich for nicotinic action can be validated for NMB agents by the D-ring Ach moiety of vecuronium and pancuronium. This Ach moiety is to date the most credible pharmacophore for the receptive site of the muscle nicotinic AchR. The 4.4 Å rule of Beers and Reich for muscarinic action can be validated by gallamine and by one O atom of the A-ring Ach moiety of pancuronium. The local steric geometry of an onium head is important to how it fits the anionic subsite of the receptor. CAR may play an increasing role in the future study of neuromuscular pharmacology, including drug design.

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