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# Neuromuscular Blockade: Offset Anomalies. Are they Simply Potency-related Receptor Bonding Effects?

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## Abstract

Rapid making and breaking of bonds between quaternary ammonium compounds and cholinergic receptors is typical of ion-pair bonding, which is weak, and ion-pair reactions, which are extremely fast. These properties explain the observed rapid association and dissociation of turbocurarine at receptors.

The time course receptor offset is determined by two factors, buffered diffusion due to repetitive bonding, and a potency-related offset-retarding effect. The strength of the latter is a function of chemical structure, which determines the microscopic molecular rate of drug-receptor association and dissociation. Together, buffered diffusion and the potency-related offset-retarding effect provide a complete rational physico-chemical explanation for the marked, yet variable, differences between onset and offset times of non-depolarizing neuromuscular blocking agents.

The influence of a potency-related offset-retarding effect together with differing structural requirements for neuromuscular blocking potency and plasma carboxyesterase hydrolysis, suggests that a high potency ultra-short duration block is unlikely to be achieved in a non-depolarizing compound metabolized by plasma esterases alone.

Recovery from non-depolarizing neuromuscular blockade in both animals and man is always gradual and much slower than onset (Feldman & Tyrell 1970; Feldman et al 1990), even when compounds are rapidly metabolized. As set out below, there is now a considerable body of evidence to show that onset of neuromuscular block is essentially a mass-action effect. Recovery is clearly more complex, but it is evident that its delay reflects events at the neuromuscular junction itself, because the time-course for the onset of block by tubocurarine applied ionophoretically is very much shorter than that of offset (del Castillo & Katz 1957; Armstrong & Lester 1977, 1979) – an effect that is still evident when drug concentration in the biophase is reduced by washing the preparation and offset is encouraged by applying large ionophoretic pulses of acetylcholine.

Slow recovery was originally thought to represent the molecular rate of dissociation of the tubocurarine–receptor complex (del Castillo & Katz 1957), but it has since been shown that the dissociation of tubocurarine from receptors is sufficiently fast to be displaced significantly by brief ionophoretic pulses of acetylcholine (Blackman et al 1975). This and other evidence set out in detail below has led to the concept of buffered diffusion within the synaptic cleft (Armstrong & Lester 1979), which fits many of the facts. Some unexplained deficiencies still remain, however (Bowman 1992).

Studies of short-acting non-depolarizing neuromuscular blocking agents (Stenlake et al 1992, 1993; Dhar et al 1996) have meanwhile focussed our attention on correlations between potency and duration of action within groups of closely related compounds that have inherently similar pathways and rates of metabolism. This leads us to suggest that insufficient consideration has been given to the influence of receptor affinity on the time-course of receptor offset.

This factor has been considered by others but dismissed, we believe, because of a misunderstanding of the nature and speed of ionic bonding interactions.

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## Onset

The response to both tubocurarine (Matteo et al 1974) and pancuronium (Hull et al 1978; Shanks et al 1978) in man is directly correlated with plasma concentration. Evidence from a group of non-depolarizing steroidal agents (Bowman et al 1988; Bowman 1992) and from a group of atracurium isomers (Maehr & Wastila 1993) also suggests that onset is primarily mass-action driven, because faster onset is associated with lower neuromuscular blocking potency. High receptor occupancy is achieved more rapidly with the higher doses of less potent compounds necessary to achieve blockade. These create greater plasma/receptor-biophase concentration gradients, and hence faster onset, than the lower doses of more potent compounds. Likewise, the reduction of onset time obtainable with supramaximal neuromuscular blocking doses of a wide variety of agents in clinical use, including atracurium (Hughes & Payne 1983), vecuronium (Agoston et al 1980a) and mivacurium (Savarese 1990) also supports this conclusion.

## Offset of Neuromuscular Blockade

Isolated arm experiments (Feldman & Tyrell 1970; Agoston et al 1979; Feldman et al 1990) provide clear evidence there are factors in addition to mass action that retard receptor offset and extend recovery time. Thus blockade in the arm isolated by tourniquet then immediate release of the tourniquet causes massive and rapid dilution of the small paralysing dose used to

produce neuromuscular blockade in the isolated arm. Times for 25–75% recovery from neuromuscular blocking doses of non-depolarizing agents in such experiments are, nevertheless, several minutes longer than onset times, and full recovery times are several multiples of onset time.

From this it was postulated that non-depolarizing agents such as tubocurarine are bound strongly to the receptor site and, although binding is reversed as competing acetylcholine concentrations rise (either spontaneously or by application of an anti-cholinesterase, e.g. neostigmine), recovery only proceeds when there is a sufficient downhill concentration gradient between the synaptic cleft and perfusing blood to enable diffusion away from the synapse and so maintain the receptor in the unoccupied state. The anomalous reversal of block by decamethonium on release of the tourniquet (Feldman & Tyrrell 1970) and the prolonged blockade by α-bungarotoxin, although not adequately explained, might simply reflect potency-related affinity differences.

Further light on the slow release of non-depolarizing agents has come from interpretation of studies on *Electrophorus electroplaques* (Sheridan & Lester 1977). These indicate that compounds such as tubocurarine rapidly and repeatedly combine with and dissociate from receptors on a sub-millisecond time-scale. Similar ionophoretic studies on frog skeletal-muscle fibres (Armstrong & Lester 1977, 1979) have confirmed that both acetylcholine and tubocurarine have brief latencies. They have also shown that the rate constant for recovery from a pulse of tubocurarine varies only slightly with temperature, and that the kinetics of tubocurarine inhibition depend on the density of acetylcholine receptors in the synaptic cleft.

These findings led to the conclusion that the outward flow of tubocurarine from the synaptic cleft is slowed by repeated interaction with the large number of receptors present. In particular, the low temperature-dependence of recovery from inhibition is deemed to distinguish this buffered diffusion (Rang 1966; Thron & Waud 1968; Colquhoun et al 1972, 1977) from the alternative explanation—a low rate of drug-receptor dissociation for which the temperature coefficient would be expected to be much higher.

More recently, Glavinovic et al (1993) have confirmed the generality of buffered diffusion of quaternary ammonium compounds at the neuromuscular junction. Thus, six different agents (tubocurarine, pancuronium, vecuronium, rocuronium, atracurium and doxacurium) applied ionophoretically to the frog cutaneous pectoris preparation all showed small $Q_{10}$ temperature-dependence of recovery values between 15 and 25°C (ranging from 1·19 to 1·30) and even smaller $Q_{10}$ temperature-dependence of equilibrium dissociation constants (ranging from 1·07 to 1·28).

Although it is evident from these results that buffered diffusion in the synaptic cleft is a major factor determining the time-course of recovery, it does not necessarily follow that the effect of the microscopic molecular rate of drug–receptor dissociation can be completely ignored. Thus it has been pointed out that repeated receptor binding and unbinding of drug molecules would seem to effect the release of all blocking agents similarly, and would not readily explain different rates of recovery with different blocking drugs (Bowman 1992). It is, therefore, interesting to note that, irrespective of temperature, receptor offset times and equilibrium dissociation constants of the compounds in the temperature-dependence study (Glavinovic et al 1993) both increase in parallel with their increasing potencies.

It seems, therefore, that the time-course of offset is determined by two factors: repeated binding and a potency-related effect. Repeated binding, which delays release from the synaptic cleft, is primarily a consequence of the typical ionic bonding characteristic of quaternary ammonium compounds and high concentrations of receptors. The second factor, a differential receptor-offset retarding effect, is a function of chemical structure, and hence potency, and it is this that accounts for the different recovery rates of different blocking agents—a conclusion that is supported by our own studies described below.

## The Influence of Potency on Duration of Action

We have increasing evidence that structural features which determine high potency of non-depolarizing agents conflict with those that favour fast onset, short duration and rapid recovery—key objectives in the design of a clinically acceptable non-depolarizing alternative to suxamethonium (Stenlake et al 1992, 1993; Dhar et al 1996).

In particular, duration increases with increasing potency in a series of bis-thiazolium compounds (Stenlake et al 1993). The increases are small, because the potencies are relatively low and the effect is overshadowed by rapid metabolism (Fig. 1). In contrast, the increase is quite pronounced with the more potent pancuronium-related mono- and diacetoxy bis-quaternary steroids (Buckett et al 1973; Fig. 1), because metabolism is centred mainly in the liver and is comparatively slow (in the cat approximately 58% of pancuronium remains unmetabolized after 8 h; Agoston et al 1973). Likewise, the profile of the

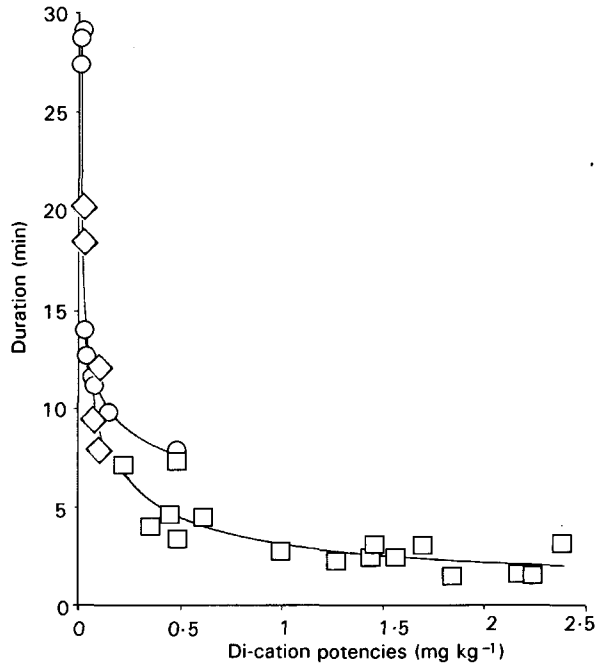


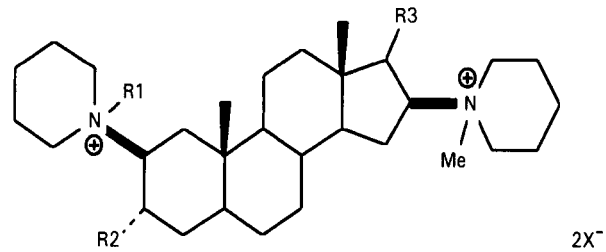

FIG. 1. Relationship between potency (calculated as mg $kg^{-1}$ di-cation) and duration of action of neuromuscular blocking agents: □ bis-thiazolium di-esters (measured on cat tibialis muscle), ◇ mono- and diacetoxy bis-quaternary steroids (measured on cat gastrocnemius muscle), ○ a group of six atracurium stereoisomers (measured on cat tibialis muscle).

six atracurium isomers (Maehr & Wastila 1993) shown in Fig. 1 is similar though less steep, reflecting their slightly lower potencies and shorter duration of action. It is also relevant that Waser (1953) clearly showed, though without comment on the relationship, that the duration of action of the calabash curare alkaloids increased with increasing potency.

Unfortunately, none of the profiles in Fig. 1 can be ascribed unequivocally to the effect of potency, because differences between the rates of metabolism of compounds in each series cannot be entirely excluded. Although not directly relevant to neuromuscular blockade, the effects of the alkyltrimethylammonium compounds $Me_3N^+(CH_2)_nMeX^-$ at muscarinic cholinoreceptor sites in the guinea-pig ileum (Paton 1961) provide an interesting parallel in a group of quaternary ammonium compounds that are stable and free from ester or other metabolizable functions. Thus the rate constant for their dissociation from receptor sites decreases with each additional methylene group in the alkyl chain between n = 7 and n = 11, i.e. there is a direct correlation between chemical structure and increasing receptor-complex stability, apparently attributable to increasing potential for hydrophobic bonding.

It appears, therefore, that in addition to the conventional expression of structure-activity relationships in terms of an effect response (i.e. potency or intrinsic activity), such relationships might be equally capable of expression as structure-duration effects. If that is indeed so, then chemical structure not only determines the potency of neuromuscular blockade but also accounts, at least in part, for the duration of the response. It follows therefore, that chemical structure provides a rational physicochemical basis for the different offset times of neuromuscular blocking agents.

Table 1. Potency, duration and acetoxy substitution in pancuronium and vecuronium derivatives.




| Compound* | R1 | R2 | R3 | Potency† | Duration‡ (min) |
|---|---|---|---|---|---|
| **1a** | Me | $CH_3COO$ | $CH_3COO$ | 21·5 | 15·0 |
| **1b** | Me | H | $CH_3COO$ | 63·0 | 7·5 |
| **1c** | Me | H | H | 113·0 | 9·8 |
| **2a** | H | $CH_3COO$ | $CH_3COO$ | 37·5 | 11·1 |
| **2b** | H | H | $CH_3COO$ | 110·0 | 8·0 |
| **2c** | H | H | H | 443·0 | 4·6 |

*Structures represented as ionized in-vivo at pH 7·4 (ca 99% dication). †Mean potencies; ED95 ($\mu g\ kg^{-1}$); cat tibialis anterior muscle. ‡Time from injection to 90% recovery.

## Chemical Structure and Offset Effects

*Ionic bonding and buffered diffusion*

Rapid association and dissociation is consistent with ion-pair complexation of quaternary ammonium antagonists and anionic (aspartate or glutamate) sites on acetylcholine receptors in an aqueous environment. This is so because ion-pair bond lengths are long (ca 7 Å) and the dielectric constant of water is high (80). Consequently, ion-pair bonds are weak (4–8 kJ $mol^{-1}$). Ionic reactions are, in addition, extremely fast. It follows, therefore, that associated ion pairs will dissociate into their constituent ions at rates that are of a similar order to those at which they are formed, as is actually observed (Sheridan & Lester 1977).

This activity contrasts with the commonly held perception of drug–receptor complexes as discrete, stable, physically identifiable, molecular entities-evoked by use of such terms as receptor-binding. Instead, in reality, the ion-pair drug-receptor complex is a transient species-the expression of a very fast dynamically reversible equilibrium. It is, therefore, fast dynamic equilibration that drives the receptors to recapture dissociated molecules, albeit fleetingly, in competition with mass-action-induced leakage away from the receptor within the synaptic cleft.

*Differential retarding effects*

It is difficult to use literature data to test the concept of structure-related differential receptor offset retarding effects because duration of action (which doubtless has been recorded) has not always been reported for groups of non-metabolizable compounds. In practice, however, ion-pair bonds are reinforced (or otherwise) by other bonding or anti-bonding forces between antagonist and receptor which are also determined by the chemical structure of the antagonist. Change in the contribution of any one such factor within a series of compounds should, therefore, be reflected both in potency and in duration, irrespective of metabolism. This is evident in the parallel changes in structure, potency and duration seen in recent studies of pancuronium, vecuronium and their respective desacetoxy derivatives (Table 1; Bowman et al 1988).

Firstly, 3,17-di-desacetoxypancuronium, **1c**, and 3,17-di-desacetoxyvecuronium, **2c**, which lack centres for esterase metabolism, differ only in their respective 2-NMe and 2-NH functions. Because **2c** is fully protonated at physiological pH, and the acetylcholine receptor is known to have a hydrophobic bonding sub-centre (Stenlake 1963, 1979; Kharkevich & Skoldinov 1986a), the enhanced hydrophobic bonding capacity conferred by the 2-*N*-methyl group accounts for the higher potency and longer duration of **1c** compared with that of **2c**.

Secondly, each group of parent, mono- and didesacetoxy compounds has the same steroidal skeleton, the same quaternary ammonium (or ionizable tertiary amino) substituents in the same positions and with the same stereochemical configuration. Potency falls progressively in each group as the number of acetoxy groups falls from two to one to zero, in reflection of the innate C=O · · · · H–N hydrogen-bonding capacity of acetoxy groups to enhance the stability of the antagonist–receptor complex. The fall in potency in the vecuronium group (**2a–c**) is paralleled by the expected incremental decrease in duration. In the pancuronium group, 3-desacetoxypancuronium, **1b**, similarly shows the expected decrease in duration, but loss of the second acetoxy group in 3,17-didesacetoxypancuronium, **1c**, is anomalous, further reducing potency, but not duration, possibly because of unusually rapid liver uptake of the 3-desacetoxypancuronium, **1b**,

similar to that of 17-desacetoxypancuroniun (Sugrue et al 1975; Agoston et al 1980b).

Given that supporting evidence for this anomaly is forthcoming, the corollary is that all ion-pair events between ionized acids and bases and their receptors are determined similarly. Repetitive binding has been shown to apply to bases such as atropine (Thron & Waud 1968) and tetrodotoxin (Colquhoun et al 1972). It is reasonable to expect, therefore, that a differential receptor-offset retarding effect will also apply in any series of acids or bases. Such a conclusion would, however, be largely of academic interest, because, more often than not, high potency and extended action, rather than limited duration, is the prime requirement of most therapeutically useful compounds.

## Potency and Metabolism of Short-acting Agents

*Potency versus metabolism*

Typical short-acting compounds, subject to a combination of rapid plasma esterase metabolism and non-enzymic base-catalysed degradation in-vivo, give rise to flattened potency-duration profiles (Fig. 1). These profiles are dominated by the effects of metabolism but show small, though distinct, increases in duration with increasing potency. Comparison of the plots in Fig. 1 suggests, however, that for all series there is an optimum potency, beyond which any further increase in potency is only attainable at the expense of an increase in duration. It appears, therefore, that ultra-short non-depolarizing neuromuscular block is only attainable in compounds of moderate to low potency; a requirement that, fortunately, also favours rapid onset.

Two other factors relating to plasma carboxyesterase and non-enzymic metabolism, respectively, also affect duration.

*Plasma carboxyesterase metabolism*

Plasma esterase hydrolysis also is necessarily influenced indirectly by neuromuscular blocking potency. Thus the higher the potency the lower the concentration applied, and the greater the proportion of receptor-complex-bound agent in the effective biophase. The higher the potency, therefore, the lower the concentration of unbound antagonist in the plasma compartment in which hydrolysis occurs. Accordingly, breakdown by plasma esterase hydrolysis in any series of compounds is reduced as potency increases.

Furthermore, optimum structures for potency and enzymic hydrolysis are also different. For example suxethonium is hydrolysed faster than its more potent analogue suxamethonium (Foldes et al 1956). Mivacurium is metabolized rapidly (Savarese 1990) whereas metabolism of its more potent analogue, doxacurium, is very slow (Basta et al 1986). Homologous bis-*N*-1-adamantyl diesters (Kharkevich & Skoldinov 1986b; Kharkevich et al 1989) and bis-thiazolium diesters (Stenlake 1993; Stenlake et al 1993) also show dissimilar structural requirements for potency and esterase hydrolysis. Because high potency not only disfavours plasma esterase hydrolysis but also reduces antagonist concentration in the plasma compartment, it is highly unlikely that short action, sufficiently short to warrant replacement of suxamethonium, can be achieved in a highly potent non-depolarizing compound metabolized solely by plasma esterase hydrolysis.

*Non-enzymic metabolism*

Non-enzymic base-catalysed metabolism such as Hofmann elimination (Stenlake et al 1981, 1992; Welch et al 1994; Dhar et al 1996) or the base-catalysed decomposition of bis-thiazolium di-esters (Stenlake 1993; Stenlake et al 1993) is also concentration-dependent. It differs from enzymic metabolism, however, in that it is non-saturable and is not confined to the plasma compartment. In consequence, high concentration in the receptor biophase owing to high potency should speed decomposition therein, hasten receptor offset, and shorten duration. It follows that compounds with the potential to undergo fast, non-enzymic, base-catalysed decomposition under physiological conditions of pH and temperature, either alone or in combination with plasma esterase hydrolysis, offer the best chance of leading to moderate-to-high-potency short-acting non-depolarizing neuromuscular blocking agents.

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