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LABORATORY INVESTIGATIONS

Physicochemical properties of neuromuscular blocking agents and their impact on the pharmacokinetic–pharmacodynamic relationship

J. J. Roy and F. Varin*

Faculté de pharmacie, Université de Montréal, 2900 boul. Edouard Montpetit, C.P. 6128, Succursale Centre-ville, Montréal (Québec), H3C 3J7, Canada

**Corresponding author. E-mail: france.varin@umontreal.ca*

Background. Among the factors influencing the onset of action of neuromuscular blocking agents (NMBA), the potency (EC_{50}) and the rate of equilibration between blood and the effect compartment (k_{e0}) have been highlighted. Although these descriptors are intrinsically influenced by the physicochemical characteristics of the drug, the impact of lipid solubility, molecular weight and protein binding on pharmacokinetic–pharmacodynamic (PK-PD) descriptors has not been established for most NMBA.

Methods. The octanol/phosphate buffer distribution coefficients (log *D*) of various NMBA (vecuronium, rocuronium, mivacurium isomers (*cis-cis*, *cis-trans* and *trans-trans*), doxacurium, cisatracurium, atracurium, succinylcholine) were determined. The free fraction for each drug was measured using an ultrafiltration technique. PK-PD descriptors were obtained from selected clinical studies. Correlations between physicochemical parameters (including molecular weight) and PK-PD descriptors were assessed by linear or multiple linear regression.

Results. A wide range of log *D* (−4.15 for succinylcholine to 0.75 for vecuronium) and free fraction (from 31% for vecuronium to 80% for succinylcholine) is observed for NMBA. Molecular weight combined with either lipid solubility (r^2=0.70; P=0.001) or free fraction (r^2=0.84; P<0.001) were highly correlated with potency, while for k_{e0} a greater degree of correlation was obtained when both lipid solubility and free fraction (r^2=0.74; P=0.002) were included.

Conclusions. The basic characteristics of NMBAs, namely, molecular weight, lipid solubility and protein binding, are strongly associated with the kinetics of the drug response.

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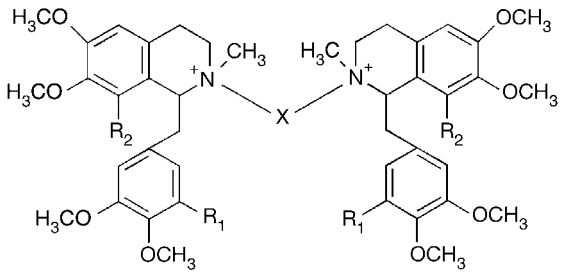
Anaesthetic drugs with a rapid onset and short duration of action are highly desirable. Thus, understanding of the factors governing their time course of action is crucial. After i.v. administration, the free fraction of a water soluble drug such as a neuromuscular blocking agent (NMBA) will generally reach the interstitial fluid mostly by filtration through intercellular junctions (gaps) but also by diffusion across endothelial cells. Once in the interstitial fluid, a NMBA will reach the neuromuscular junction, where it will bind to the nicotinic receptor and initiate its action. Intratissular diffusion is expected to be rapid for these water soluble drugs, although molecular weight has been proposed as a limiting factor in the synaptic cleft that may delay the onset of action.[1]

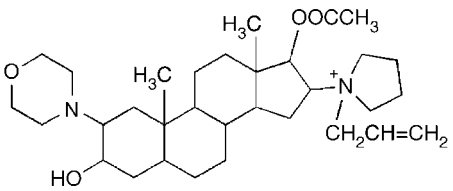
Pharmacokinetics (cardiac output, transcapillary rate of transfer, plasma clearance, and dose) as well as pharmacodynamics (potency and mechanism of action) are known to influence the profile of activity of neuromuscular blocking drugs.[2–6] In turn, the physicochemical properties of these drugs would also be expected to have an impact on the estimates of pharmacokinetic and pharmacodynamic descriptors. Currently, few data concerning the lipid solubility of neuromuscular blocking drugs are available. For a series

A Benzylisoquinoliniums

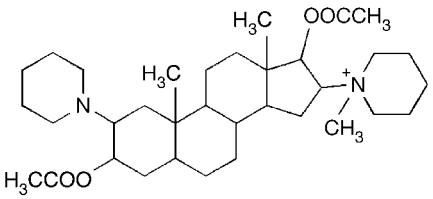


	R_1	R_2	
Atracurium	H	H	$-CH_2-CH_2-COO-(CH_2)_5-OOC-CH_2-CH_2-$
Doxacurium	OCH_3	OCH_3	$-(CH_2)_3-OOC-CH_2-CH_2-COO-(CH_2)_3-$
Mivacurium	OCH_3	H	$-(CH_2)_3-OOC-(CH_2)_2-CH=CH-(CH_2)_2-COO-(CH_2)_3-$

B Aminosteroids



rocuronium



vecuronium

C Succinylcholine chloride

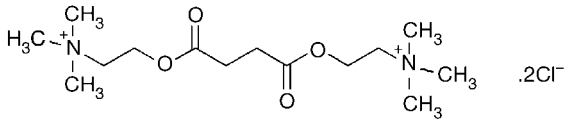


Fig 1 Chemical structures of a series of neuromuscular blocking drugs. (A) Benzylisoquinolium non-depolarizing agents; (B) aminosteroidal non-depolarizing agents; and (C) succinylcholine, a depolarizing agent.

of aminosteroidal agents, Wierda and colleagues have reported a relation between lipid solubility or protein binding and various pharmacokinetic and pharmacodynamic (PK-PD) descriptors.[7 8] However, more studies are required to enable generalization of these results to other families of compounds.

Our study aimed to characterize these physicochemical factors (lipid solubility, *in vivo* protein binding) for several neuromuscular blocking agents of differing chemical structure. A second objective was to assess the degree of association between these factors (including molecular weight), and either the potency (EC_{50}) or the plasma and effect compartment concentration equilibration rate constant (k_{e0}) obtained for these drugs in previous clinical studies.

Methods

Drugs

The structures of the various neuromuscular blocking agents used in the present study are presented in Figure 1A–C for the benzylisoquinoliniums, the aminosteroids, and succinylcholine, respectively. For the aminosteroidal family, vecuronium and rocuronium were supplied by Organon Teknika (The Netherlands). For the benzylisoquinolinium family, atracurium, cisatracurium, mivacurium isomers, and doxacurium were granted by Glaxo Wellcome Ltd (UK). Succinylcholine was purchased from Sigma (St Louis, MO, USA). Stock (100 mg %) and working solutions were stored in the refrigerator. Working solutions were prepared as required.

Distribution coefficients determination

One litre of phosphate buffer (0.048 M) was prepared by weighing dibasic sodium phosphate heptahydrate 9.70 g and monobasic potassium phosphate 1.65 g, and the pH was adjusted to 7.4 with sulphuric acid 2 M. The buffer was saturated with octanol by slow agitation for 1 h (Eberbach®, Ann Arbor, MI, USA). The two phases were allowed to stand for 1 h before separation.

The distribution coefficient of each drug was determined in octanol/buffer. Stock solutions of the test drugs were diluted in octanol-saturated buffer (referred to as the aqueous phase thereafter) to obtain a final concentration range of 100 μg ml^{-1} (doxacurium, mivacurium isomers, rocuronium, vecuronium) or 200 μg ml^{-1} (atracurium, cisatracurium, succinylcholine). Samples consisting of 400 μl of octanol and 100 μl of the aqueous phase were submitted to slow agitation for 1 h and centrifuged at 2000 *g* for 5 min. The octanol and aqueous phases were separated, evaporated (Speed Vac®, Savant Instruments Inc., Farmingdale, NY, USA) and resuspended into the HPLC mobile phase before analysis. For atracurium and cisatracurium, centrifugation was carried out at 10°C and the samples immediately stabilized with the HPLC mobile phase (pH 3). On the same day, quantitative determination of model drugs in both phases was carried out in replicate (*n*: 3–6). With the exception of succinylcholine, experiments were repeated on different days (*n*: 3–5). The distribution coefficient (log *D*) was calculated as the logarithm of the ratio obtained by dividing the total concentration (neutral and ionized forms) of the molecule in the octanol phase by that in the aqueous phase.[9]

Protein binding determination

Informed consent was obtained from each subject before blood donation. Five healthy adults, four women and one man, mean age 43 (5.5) (range 35–48) yr, American Society of Anesthesiologists status I, who were not taking any medication were recruited. Volunteers fasted for at least 12 h before blood sampling. Blood samples (70 ml) were collected in sodium citrate enriched tubes and centrifuged (2000 *g* for 15 min). Plasma was separated into five aliquots of approximatively 6 ml, flash frozen on dry ice and stored at −70°C.

In vitro plasma protein binding was determined for rocuronium, cisatracurium, and succinylcholine. A method similar to that described by Cameron and colleagues[10] was used on thawed samples. For succinylcholine, non-specific plasma esterases were inhibited by the addition of echothiophate (20 μl of echothiophate 0.2% per ml of plasma). Plasma samples were spiked extemporaneously by the addition of model drug at concentrations varying from 2000 ng ml^{-1} (cisatracurium and rocuronium) to 20 000 ng ml^{-1} (succinylcholine). These concentrations were representative of the *in vivo* exposure and fell within the range of the analytical assay. Spiked plasma samples were immediately flash frozen for determination of total concentration. Aliquots of blank plasma were also analysed. Free concentration (*fu*) of model drug was determined at room temperature in duplicate as follows: 1 ml plasma was applied onto microseparation devices having a molecular weight cut-off of 30 000 (Centrifree, Amicon Micropartition System, Beverly, MA). The microseparation devices were centrifuged at 1200 *g* for 5 min. A precise aliquot (50 or 100 μl) of the ultrafiltrate was then transferred into 1 ml pre-acidified blank plasma and flash frozen until HPLC analysis. The unbound fraction was calculated by dividing drug concentration in the ultrafiltrate by total drug concentration.

HPLC analysis

Drug concentrations were determined using HPLC-UV for doxacurium,[11] HPLC-fluorescence for atracurium, cisatracurium, and mivacurium,[12 13] HPLC-EC for rocuronium and vecuronium,[14] and tandem mass spectrometry for succinylcholine.[15]

PD descriptors

In order to assess the degree of correlation between commonly used physicochemical parameters and the PK-PD relationship, the effect compartment concentration corresponding to 50% block (EC_{50}), often referred to as the potency factor, and the equilibration rate constant between plasma and the effect compartment (k_{e0}) were chosen as descriptors. In order to minimize the inherent variability of clinical studies, mean values published by our group for these model drugs, vecuronium,[14 16] rocuronium,[17] mivacurium,[18] doxacurium,[11 19] cisatracurium,[12 20] atracurium,[21 22] and succinylcholine[23] were used to establish correlations (Table 1). In studies where isoflurane was used as the anaesthetic agent, a correction factor of 1.7 was applied to the EC_{50} value to account for its well-established potentiating effect.[17] In six studies[14 17 18 19 22 23] intensive arterial blood sampling was carried out for 2 min after the administration of a bolus dose of the NMBA and at frequent intervals thereafter. Monitoring of neuromuscular function was carried out using single twitch or train-of-four stimulation, in agreement with good clinical research practice standards.[24]

Statistical analysis

Linear regression and multiple linear regression were used to evaluate the degree of association between some dependent variables, EC_{50} and k_{e0}, and the independent variables, namely molecular weight, partition coefficient, and the free fraction of individual drugs. The analysis consisted of the examination of the coefficient of determination (r^2) and coefficient of multiple determination (r^2) in the evaluation of the linear regression and multiple regression, respectively, where r^2 was used as a measure of the goodness of fit of the regression surface. A Partial *F*-test and a Multiple Partial *F*-test were done to evaluate the relevance of adding a

Table 1 Physicochemical and PK-PD parameters of neuromuscular blocking agents. *Values obtained for mivacurium pharmacological concentration (sum of *cis-trans* and *trans-trans* isomers); †values reported by Cameron *et al.*;[10] ‡this value was later corrected for the potentiating effect of isoflurane

	Physicochemical properties		Pharmacodynamic parameters					
	MW Base	Free fraction (% SD)	Authors	Anaesthetics	Nerve stimulus	EC_{50} (ng ml^{-1})	EC_{50} (nmol ml^{-1})	k_{e0} (min^{-1})
Vecuronium	558	31 (4)†	Alloul and colleagues, 1996[16]	Isoflurane	TOF	154‡	0.276‡	0.085
			Ducharme and colleagues, 1993[14]	Isoflurane	Single twitch	122‡	0.219‡	0.086
Rocuronium	546	54 (4)	Dragne and colleagues, 2002[17]	Propofol	Single twitch	1008	1.847	0.127
				Isoflurane	Single twitch	592‡	1.085‡	0.09
Mivacurium*	1029	70 (3)†	Laurin and colleagues, 2001[18]	Balanced	Single twitch	130	0.126	0.101
Doxacurium	1035	53 (6)†	Zhu and colleagues, 1997[19]	Propofol	TOF	129	0.125	0.053
			Gariépy and colleagues, 1993[11]	Isoflurane	TOF	54‡	0.052‡	0.051
Cisatracurium	933	62 (9)	Tran and colleagues, 1998[20]	Propofol	TOF	153	0.164	0.054
			Bergeron and colleagues, 2001[12]	Propofol	TOF	157	0.168	0.057
Atracurium	929	63 (7)†	Donati and colleagues, 1991[44]	Isoflurane	TOF	454‡	0.489‡	0.068
			Ducharme and colleagues, 1995[22]	Isoflurane	Single twitch	431‡	0.464‡	0.043
Succinylcholine	290	80 (7)	Roy and colleagues, 2002[23]	Propofol	Single twitch	734	2.531	0.058

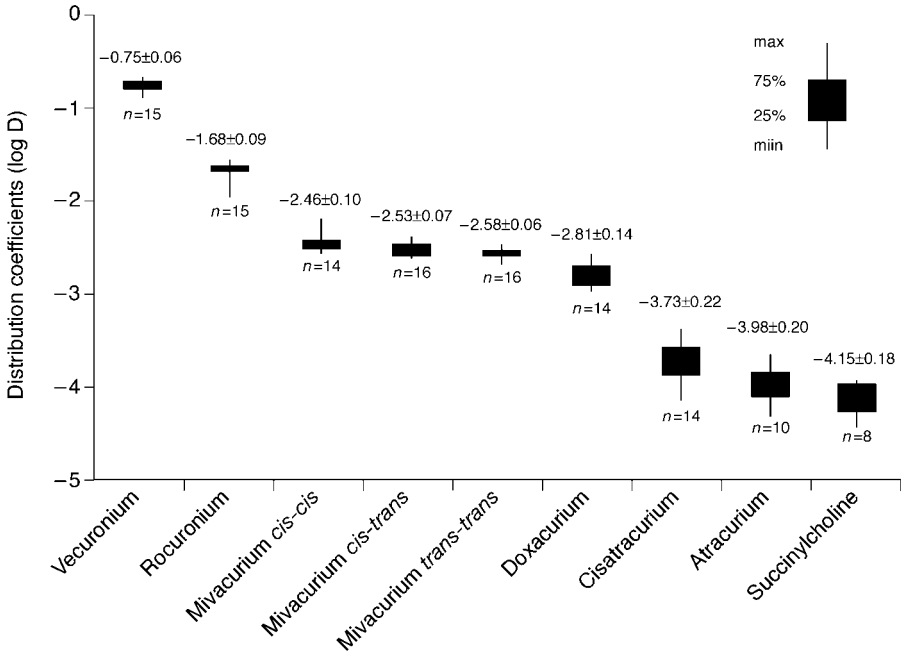


Fig 2 Quartile distribution of the distribution coefficients (log *D*) for various neuromuscular blocking drugs. The number of samples studied are given (*n*). Results are also expressed as mean log *D* (SD).

cofactor to the regression. The level of significance was set at 0.05. Data are presented as mean (SD).

Results

In Figure 2, the distributions of the physicochemical parameter log *D* with octanol/buffer for each of the neuromuscular blocking agents are presented.

Amongst the model drugs, the most lipophilic were the singly charged aminosteroidal compounds, namely, vecuronium and rocuronium. With a log *D* of −4.15, succinylcholine was found to be the least lipid soluble neuromuscular blocking agent, being almost 10 000 times less soluble in octanol than vecuronium. The overall coefficient of variation was less than 6%, indicating a high degree of day-to-day reproducibility.

In Table 1, the free fraction of drug in plasma measured for cisatracurium, rocuronium, and succinylcholine is presented. The results given for the other neuromuscular blocking agents (atracurium, mivacurium, doxacurium, and vecuronium) were published previously by our laboratory using a similar approach.[10]

We tested if a correlation existed between k_{e0} and different characteristics of the neuromuscular blocking drugs (molecular weight, log *D*, or free fraction). For the k_{e0}, log *D*, as a single independent variable, proved to yield the highest

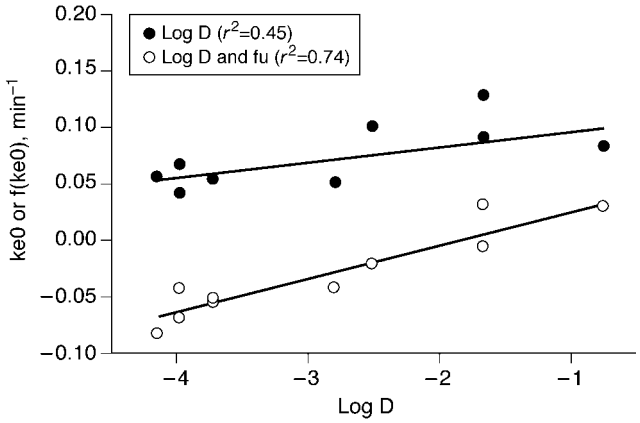


Fig 3 The linear dependence of k_{e0} on log D for a series of neuromuscular blocking agents is illustrated. The correlation improves when the free fraction is included as a covariate in the multiple linear regression. The y axis represents the k_{e0} value for the linear regression, and a function of k_{e0} (f(k_{e0})) for the multiple linear regression, where k_{e0} is adjusted to account for the free fraction. The lines represent the predicted values.

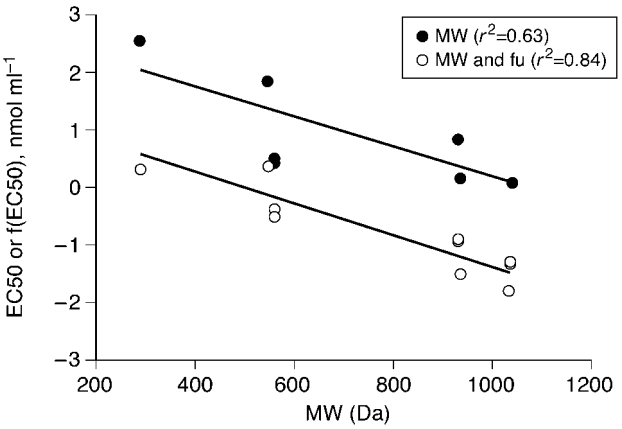


Fig 4 The linear dependence of EC_{50} on molecular weight is illustrated. The correlation improves when the free fraction is included as a covariate in the multiple linear regression. The y axis represents the EC_{50} value for the linear regression, and a function of the EC_{50} (f(EC_{50})) for the multiple linear regression, where the EC_{50} is adjusted to account for the free fraction. The lines represent the predicted values.

association for our model drugs. A poor correlation was found between k_{e0} and the free fraction (r^2=0.07) or molecular weight (r^2=0.16). In Figure 3, the linear dependence of k_{e0} on log D for a series of neuromuscular blocking agents is illustrated (r^2=0.45, P=0.016). The addition of the free fraction as a covariable results in a significant improvement in the determination coefficient (r^2=0.74, P=0.002). The Multiple Partial F-test and the Partial F-test gave P values smaller than 0.001, which supports the addition of the free fraction as a cofactor in our regression. The equation describing this multiple regression is:

$$k_{e0}=0.0567+(0.0300*\log D)+(0.00173*fu)$$

The substitution of the free fraction by molecular weight did not improve the degree of association (r^2=0.49).

Molecular weight, as a single independent variable, proved to have the highest degree of association with the EC_{50} of our model drugs (Fig. 4). A poor degree of association was found between the EC_{50} (nmol ml^{-1}) and the free fraction (r^2=0.11) or log D (r^2=0.002). The linear dependence of the EC_{50} on molecular weight is illustrated (r^2=0.63, P=0.002). The addition of log D as a covariable results in an improvement in the determination coefficient (r^2=0.77, P=0.001, data not presented). The Multiple Partial F-test and the Partial F-test gave P values smaller than 0.05, which supports the addition of the log D as a cofactor in our regression. The equation describing this multiple regression is:

$$EC_{50}=2.429-(0.00307*MW)-(0.271*\log D)$$

If we substitute the free fraction to log D as a covariable for the molecular weight, the coefficient of determination for the EC_{50} (nmol ml^{-1}) using multiple linear regression is somewhat better (r^2=0.84, P<0.001, Fig. 4).

$$EC_{50}=1.409-(0.00281*MW)+(0.0275*fu)$$

Discussion

Our results demonstrate a very strong association between physicochemical characteristics such as lipid solubility, protein binding, and molecular weight and some PK-PD parameters, namely the EC_{50} and k_{e0} values, that are most often used as descriptors for the onset time of neuromuscular blocking agents.

The onset time reported for a neuromuscular blocking agent in a clinical study will vary according to methodological factors such as the type of anaesthesia, the dose administered, the type of stimulation (single twitch *vs* train-of-four response), and the clinical end-point used for onset time determination (peak effect or fixed degree of twitch depression) during neuromuscular monitoring.[4 25] As the clinical protocols used to obtain our reference values were not identical, we elected to establish correlations between the drug's physicochemical parameters and the k_{e0} or EC_{50} values instead of the onset times, as the former parameters should be dose independent and can be adjusted to account for methodological differences (e.g. use of inhalation agents).

Circulatory factors are recognized to have a direct impact on k_{e0} values that may, in turn, alter the onset of action of a drug.[11 26 27] These results suggest that, for a given NMBA and under identical clinical settings, inter-individual variations in k_{e0} become sufficiently large to distinguish the impact of k_{e0} on onset times from that of other confounding factors. Alternatively, onset times may vary without any detectable changes in k_{e0}.[19 26] Furthermore, an equilibration delay similar to that of other NMBAs was reported recently for succinylcholine.[23] These findings suggest that the limiting factors for k_{e0} and onset times are different, and that their net effects may differ.

Three physicochemical properties of a drug (partition coefficient, protein binding, and molecular weight) affect

its transfer from blood to tissue. As neuromuscular blocking agents are large ionized molecules, their transcapillary rate of transfer in muscle tissue is expected to be mostly pore-restricted (intercellular gaps of 10–20 nm).[28] However, the relative contribution of the large number of smaller pores is uncertain. The molecular span of many NMBAs is approximately 1–2 nm between both quaternary amines.[29] Therefore, molecular size and weight are not expected to be limiting factors for the transcapillary filtration of NMBAs.[30] Accordingly, no correlation was observed between the k_{e0} value and molecular weight for our series of NMBAs.

Diffusion across the endothelial membrane is not expected to be important for ionized drugs such as neuromuscular blocking agents.[31] According to Fick's law, the rate of diffusion would depend on the solute's unbound concentration, its partition coefficient, and its degree of ionization. However, membrane permeability is also related to the surface occupied by the pores in different endothelia and will differ according to the size and shape of each molecule.[32 33] These physicochemical factors are thus expected to have a direct impact on the onset times of NMBAs.

For many years, organic solvent/aqueous partitioning systems have been used as predictors for biological membrane partitioning of anaesthetic agents.[34] The log *D* coefficient seemed more appropriate for neuromuscular blocking agents because these drugs are fully ionized at pH 7.4. The log *P* value would have required that the degree of ionization at a given pH be considered as it assumes that only the neutral form of the compound is the partitioning species.[35 36] Recently, the lipid solubility of a series of aminosteroidal neuromuscular blocking agents and related investigational compounds was documented using the octanol-buffer solvent as the partitioning system.[7 37] For vecuronium, the partition coefficient was similar to ours (−0.77 *vs* −0.75) but not for rocuronium (−0.80 *vs* −1.68). In our study, rocuronium was found to be more hydrosoluble than vecuronium and this would be compatible with its higher polarity (Fig. 1B).

Although NMBAs are intrinsically very polar, individual log *D* values vary considerably. Indeed, almost a four log scale difference in lipid solubility is observed between succinylcholine and vecuronium. Overall, benzylisoquinoliums were found to be largely more water soluble (log *D* values less than 2) than the aminosteroidal series, probably because of their double positive charge.

Wierda and Proost[7] found a positive correlation (r^2=0.866) between lipid solubility and the k_{e0} values of a series of aminosteroidal compounds administered to anaesthetized patients. Lipid solubility was found to be the primary variable responsible for the time course of action but this relation was significantly improved when the free fraction was also taken into account. Our findings are in agreement with their study. Moreover, these results suggest that this association between lipid solubility and k_{e0} could be applied to most neuromuscular blocking agents.

In our study, the free fraction varied considerably, that is from 31% (vecuronium) to 80% (succinylcholine). It is generally agreed that in a series of aminosteroidal compounds, protein binding will increase as lipid solubility increases.[7 37] A definite relation between both factors was observed for our study drugs (r^2=0.67, P=0.002), and that independently of the chemical class. Adding the free fraction as a co-factor greatly improved the degree of association between log *D* and k_{e0}.

There is substantial evidence to confirm that drug potency plays a major role in the onset of action of a neuromuscular blocking agent. An inverse relationship between onset times and molar potency (ED_{50}) has been established for a series of aminosteroidal neuromuscular blocking agents in anaesthetized patients.[4 38] This observation was supported by *in vitro* animal preparations,[39] and further explained by a kinetic-dynamic model.[40] According to Lee,[30] the inverse correlation between NMBA potency and lipid solubility (r^2=0.812) reported by Wierda and Proost[7] for the aminosteroidal series could be coincidental with other factors such as steric hindrance. Indeed, physicochemical characteristics such as the large molecular size and the quaternary ammonium structure of NMBAs may have a definite impact on the onset of these drugs. These observations are reinforced by the limited space available for diffusion within the synaptic cleft (30–50 nm).[41] In turn, the repetitive binding to the acetylcholine receptor is enhanced because the nerve terminal presents a physical barrier to diffusion out of the cleft.[42] In our study, where molecules from different chemical families were tested, the best predictive factor for the molar potency was molecular weight. This finding is in support of Lee's hypothesis and the analysis made by Ramzan,[1] who first proposed molecular weight as a determinant of the speed of onset for a series of NMBAs. Inclusion of succinylcholine in our series of model drugs allowed a greater range of molecular weight to be studied. Its deletion from the series had very little impact on the multiple linear regression for the descriptors k_{e0} (r^2=0.77, P=0.003) and EC_{50} (r^2=0.79, P=0.002). However, it is possible that its different mechanism of action could be a confounding factor.

In our study, the linear dependence of drug potency on molecular weight was improved by the addition of either lipid solubility or protein binding as a covariable. Indeed, a linear correlation was observed previously between the molecular weight and the partition coefficient of a series of organic cations.[43] Thus, the added value of *fu* or log *D* to the predictive value of the EC_{50} would be consistent with Wierda and colleagues's observations.[7 37] In their study, the range of molecular weight was too small to be considered as an independent variable and was therefore not tested. The fact that, in our study, lipid solubility was not found to be a major determinant of potency but a synergistic factor to molecular weight supports Lee's hypothesis.

In conclusion, the basic characteristics of NMBAs, namely, molecular weight, lipid solubility and protein binding, are strongly associated with the kinetics of drug response.

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