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A Monte-Carlo optimal controller for muscle relaxation train-of-four

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Introduction: We designed and built a simple and robust controller for train-of-four (TOF) counts that is optimized using Monte-Carlo analysis as a performance evaluation technique. The controller is based on a lookup table of the actual TOF count and the average TOF count over the previous 2 min. The values in the lookup table were determined by minimizing simulated controller error in simulation over 100 randomly generated TOF model parameter sets. TOF simulation was achieved by extending the Bartkowski and Epstein PK/PD model [1]. The controller performance error was defined as the deviation from the desired degree of block (TOF count 1 or 2) with extra weighting for the model parameter sets that produced the highest and the lowest offset. Random noise was also introduced in the simulation used for optimization. Optimization method was a steady state genetic algorithm. The resulting controller is optimal with respect to randomly generated models (Monte-Carlo optimal), has good worst-case performance and is insensitive to the expected range of patient model variations and to noise.

Results: The performance criterion for optimization are unusual because these weight the error from the most and least sensitive patient models much higher than the rest of the models. This has the effect of minimizing the tails of the distribution of the performance over the random models at the expense of performance for more common models. This enhances controller robustness and worst-case performance at the expense of absolute controller performance. The optimized muscle relaxation controller was implemented using a laptop computer connected to a TOF Watch SX and a syringe pump. The infusion system was evaluated in 10 patients. Records were kept of relaxation and pump infusion rate.

Table 1. Actual and simulated (10 random model sets) controller performance during closed loop control

TOF count	Measured (%)	Simulation (%)
0	0.59	2.54
1	41.98	21.12
2	53.83	72.33
3	2.74	2.54
4	0.84	1.56
	$n=5543$	$n=6927$

During controller evaluation after initial bolus recovery the controller maintained 1 or 2 twitches during 96% of the time during closed loop control. The remaining 4% appear to be the noise caused by mechanical disturbance of the acceleration transducer. The block was sufficient for good surgical conditions in all cases. The controller remained stable under all conditions. Performance was similar to that expected from optimization and simulation.

Reference:

1 *J Pharmacokinet Biopharm* 1990; **18**: 335–46.

2

New fast neuromuscular blocking agent TAAC3 in the anaesthetized monkey

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Introduction: TAAC3 is a novel neuromuscular blocking agent, with short action based on metabolism in plasma. In the present study its dynamics and effect on the circulation were tested in anaesthetized *Rhesus* monkeys.

Methods: Female *Rhesus* monkeys (body weight 4.0–7.0 kg) were sedated with 10 mg kg^{-1} ketamine i.m., followed by i.v. injection of pentobarbital sodium (25 mg kg^{-1} i.v.) and subsequent infusion of 5–10 mg kg^{-1} h^{-1}. The animals' lungs were ventilated with a mixture of oxygen and nitrous oxide (2 : 3). Heart rate (provided by Biox pulse-oximeter) and blood pressure (continuous signal from a cuff placed around the tail, Finapres®) were registered. Body temperature was kept at 37–38°C. Muscle contractions of the right thumb, induced by single twitch stimulation (0.1 Hz) of the right ulnar nerve, were recorded. Incremental doses of the test drug were given to determine the ED_{90} response (90% block). This ED_{90} was injected after at least 30 min after recovery from the previous dose. After recovery from this ED_{90} for an extra hour, a dose of $3 \times ED_{90}$ was injected. Recovery parameters and effects on blood pressure and heart rate were calculated from the tracings.

Results: At the end of the experiment, the animals were allowed to recover from anaesthesia.

The results of these experiments are shown and compared to Org 9487 in Table 1.

Table 1. ED_{90} spontaneous recovery and relative changes in heart rate and blood pressure

Parameter	TAAC3 $n=6$	Org 9487 (Rapacuronium) $n=10$
ED_{90} (μg kg^{-1})	127 (15)	172 (12)
Onset (min)	1.25 (0.09)*	1.52 (0.08)
Rec 25 (min)	2.02 (0.15)*	2.81 (0.11)
Rec 50 (min)	2.46 (0.16)*	3.85 (0.21)
Rec 75 (min)	2.81 (0.21)*	4.98 (0.32)
Rec 90 (min)	3.17 (0.28)*	6.36 (0.46)
Rec 25–75 (min)	0.79 (0.09)*	2.17 (0.22)
Max/min HR (%)	1.90 (0.90)/−0.99 (0.18)	1.64 (0.40)/−0.94 (0.35)
Max/min sys (%)	6.69 (1.23)/−5.02 (2.50)	4.28 (0.79)/−4.47 (0.84)
Max/min dia (%)	5.95 (1.40)/−6.19 (2.67)	3.95 (0.95)/−4.35 (0.85)
Max/min mean (%)	6.10 (0.84)/−5.27 (2.77)	4.23 (0.93)/−3.66 (0.52)

Time expressed in minutes, mean (SEM); *$P<0.05$ vs. recovery of rapacuronium.

TAAC3 shows faster onset and shorter duration than rapacuronium at ED_{90}. Minor circulatory changes were observed, but these were not significantly different from those after rapacuronium.

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New fast neuromuscular blocking agent, TAAC3, in the anaesthetized dog

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Introduction: TAAC3 is a novel neuromuscular blocking agent with short duration of action based on metabolism in plasma. In the present study its dynamics and effect on the circulation were tested in anaesthetized dogs.

Methods: Beagle dogs (both sexes, body weight 9.5–12.0 kg) were sedated with thalamonal (0.1 mg kg^{-1}) followed by pentobarbital sodium (15 mg kg^{-1}) i.v. and subsequent mechanical ventilation of the lungs with 1.5–2.0% isoflurane in nitrous oxide/oxygen (2 : 3). Heart rate and arterial pressure were determined with an arterial line in the hind paw (*arteria femoralis*) and/or a cuff around the tail (Finapres®). Body temperature was kept between 37.5 and 38.5°C. Muscle contractions induced by single twitch stimulation (0.1 Hz) of the *n. ischiadicus* at the inside of the left hind paw were recorded by measuring the stretching force isometrically. Incremental doses of the test drug were given to determine the ED_{90} response (90% block). This ED_{90} was injected after at least 30 min recovery from the previous dose. Recovery parameters and relative effects on blood pressure and heart rate were calculated from the recordings. Blood samples before and after administration of the drug were taken for the determination of plasma histamine concentrations. At the end of the experiment, the animals were allowed to recover from anaesthesia.

Results: The results of these experiments are shown and compared to Org 9487 (rapacuronium) in Table 1.

Table 1. ED_{90} spontaneous recovery and relative changes in heart rate and blood pressure

Parameter	TAAC3 $n=6$	Org 9487 (rapacuronium) $n=4$
ED_{90} (μg kg)	240 (13)	230 (12)
Onset (min)	1.71 (0.08)	1.85 (0.23)
Rec 25 (min)	2.68 (0.05)*	3.42 (0.24)
Rec 50 (min)	3.18 (0.07)*	4.09 (0.31)
Rec 75 (min)	3.64 (0.08)*	4.88 (0.40)
Rec 90 (min)	4.05 (0.09)*	5.63 (0.48)
Rec 25–75 (min)	0.96 (0.08)	1.46 (0.22)
Max/min HR (%)	7.73 (1.19)/−3.07 (1.56)	15.40 (4.95)/−0.78 (0.40)
Max/min sys (%)	8.49 (2.20)/−27.51 (4.20)*	4.14 (2.25)/−9.78 (2.68)
Max/min dia (%)	13.87 (4.32)/−32.96 (6.25)*	8.09 (4.18)/−9.96 (1.56)
Max/min mean (%)	11.18 (2.69)/−29.74 (5.30)*	6.47 (3.08)/−10.54 (2.37)

Relative changes in percentage, mean (SEM), *$P<0.05$ vs. recovery of rapacuronium.

TAAC3 shows comparable onset but a shorter duration of action than rapacuronium in dogs at ED_{90}. After injection there is a marked transient decrease in blood pressure followed by an increase as compared to the control value

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(pre-injection). These changes are significantly more pronounced than with rapacuronium. Arterial pressure changes subsequent to TAAC3 injection were not related to histamine release.

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The use of a muscle relaxant allows a 2/3 decrease of EC_{50} of propofol needed to achieve excellent intubating conditions

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Introduction: Intubating conditions depend on the depth of anaesthesia induced by the hypnotic-opioid combination and the use of a muscle relaxant (MR) given at a dose of twice the ED_{95} or higher and after obtaining a complete block at both the laryngeal muscles and the diaphragm. The interaction between these two drug combinations remains unknown. The aim of this study was to determine the EC_{50} value (effect site concentration) of propofol needed to obtain excellent intubating conditions in 50% of patients, with or without a MR.

Methods: Forty-six ASA I–II patients were studied. Anaesthesia was induced with sufentanil ($0.3\,\mu g\,kg^{-1}$) and propofol using a target controlled infusion system. In one group ($n=25$), patients did not receive a MR, because it was not required for the perioperative period. In the other group ($n=21$), atracurium ($0.5\,mg\,kg^{-1}$) was given and the onset time of neuromuscular block assessed by the orbicularis oculi responses to train-of-four (TOF) stimulation applied to the facial nerve. Intubation was attempted after the disappearance of all the responses to TOF stimulation. The propofol effect site concentration required to obtain excellent intubating conditions was calculated in both groups according to the Up and Down Method of Dixon [1]. The EC_{50} was compared using a Mann–Whitney test and estimated using logistic regression.

Results: The EC_{50} value was significantly greater ($P<0.001$) when MR was avoided ($8.9\pm0.8\,\mu g\,mL^{-1}$, vs. ($3.3\pm0.4\,\mu g\,mL^{-1}$, mean ± SD). The rate of vasopressor administration was significantly higher when atracurium was not given ($P<0.001$) in 15/25 patients and in 1/21 patients in the groups, respectively, without and with atracurium.

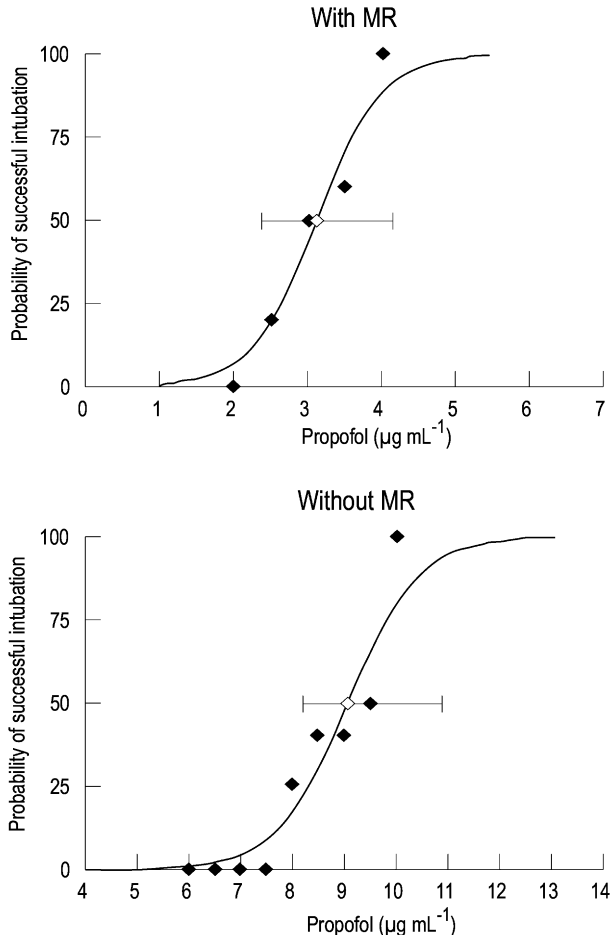

Fig. 1. Logistic regression of probability of successful intubation fitted for propofol concentration. Propofol EC_{50} was shown as open circle (means and CI).

Conclusion: The combination of hypnotic-opioid and atracurium at a dose sufficient to induce a complete block allowed a 2/3 decrease in the EC_{50} of propofol. The dose of propofol needed to be increased to obtain excellent intubating conditions without using a MR (Fig. 1), and was responsible for undesirable haemodynamic side-effects.

References:

1 *Acta Anaesthesiol Scand* 1996; **40**: 59–74.

2 McArthur JW, Colton T. *Statistics in Endocrinology*, Cambridge, MIT Rev, pp. 51–64.

5

Proliferation of two human cell-lines *in vitro* under the influence of cisatracurium with and without carboxyl esterase

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Introduction: Cisatracurium undergoes *in vivo* base-catalysed elimination reaction (Hofmann elimination). The reaction is possible due to the presence of a reverse ester at either end of the molecule. The products are laudanosine, a tertiary amine, and an electrophilic acrylate ester. Hydrolysis of the reverse ester pre-empts Hofmann elimination, while hydrolysis of acrylate esters generates an alcohol and acrylic acid, neither of which shares the electrophilic nature of the parent ester. While enzyme-catalysed hydrolysis of cisatracurium is less likely, the reaction is more likely for the first generation ester metabolites. We have previously demonstrated that cisatracurium inhibits proliferation of two human cell-lines *in vitro* and have attributed this effect to the generation of electrophilic acrylates [1]. The explanation was based on the findings that (a) mivacurium, a muscle relaxant with a similar chemical structure, but without the reverse esters, does not interfere with cell proliferation, and (b) addition of nucleophilic scavengers glutathione (GSH) or *N*-acetyl cysteine (NAC) attenuates the inhibitory effect. We postulated that, if the inhibition of cell proliferation is indeed due to acrylate esters, then the enzyme carboxyl esterase might attenuate the inhibitory effect by converting the electrophilic esters to the non-electrophilic alcohol and acid.

Methods: Hepatoma cells (HEPG2) and endothelial cells (HUVEC), harvested from veins of human umbilical cords, were isolated and cultured. Cells (2×10^4 for HEPG2 and 3×10^4 for HUVEC) were added to wells and incubated at 37°C for 72 h. The final concentration of cisatracurium was either 0 or 96 μmol and the final concentration of the enzyme either 0 or $0.4\,U\,mL^{-1}$. Eight wells for each cell line contained both the enzyme ($0.4\,U\,mL^{-1}$, added first to the wells) and the relaxant (96 μmol). At the end of incubation, the cells in each well were counted and the cell numbers expressed as percent of the mean cell number counted in wells without additives (control wells). Percent cell count in the treated wells was compared to that in control wells (defined as 100%), using Bonferroni *t*-test.

Results: When only the enzyme was added to eight wells, the cell count increased to 116% (9.8%) (mean of SD) in HUVEC cells, while in HEPG2 cells it was not changed at 101% (8.5%). When only cisatracurium was added to the wells, cell counts decreased to 10.7% (1.6%) in HUVEC and to 3.0% (2.5%) in HEPG2 cells. In both cell lines esterase increased the cell counts to 97.0% (8.4%) for HEPG2 and to 80.9% (6.2%) for HUVEC cells ($P<0.01$).

Conclusions: The results demonstrate that, similar to the effects of GSH and NAC, carboxyl esterase either markedly attenuates the inhibitory effect of cisatracurium (HUVEC cells) or even abolishes the inhibitory effect of cisatracurium on proliferation of HEPG2 cells. We suggest that the finding strengthens the hypothesis that the electrophilic acrylate esters interact with functionally important endogenous nucleophiles and so inhibit cell proliferation. A decrease in the concentration of acrylates, due either to covalent binding of acrylates with the added nucleophiles or to hydrolysis of acrylates by carboxylesterase to non-reactive metabolites, decreases the likelihood of acrylates interacting with endogenous nucleophiles.

Reference:

1 *Br J Anaesth* 2000; **85**: 159 p.

6

Modelling of rocuronium and pancuronium in the anterograde perfused peroneal nerve anterior tibialis muscle model of the 'myasthenic rat' vs. control

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Introduction: A reduced number of acetylcholine receptors (AChR) prolongs the time course of non-depolarizing neuromuscular block [1]. We used an antegrade perfused rat peroneal nerve anterior tibialis muscle (APPAT) model to study this. AChR were reduced by experimental autoimmune myasthenia gravis (EAMG, passive transfer of antibodies) or by administration of α-bungarotoxine (α-BTX).

Methods: EAMG rats (Lewis, 200 g) were injected intraperitoneally with 20 pmol MAb35, a rat anti-AChR IgG1, 36–48 h prior to investigation [2]. After induction of anaesthesia, the model was prepared and perfusion with donor blood was started. After stabilization of the twitch, an infusion of rocuronium (roc) or pancuronium (pan) was started until 90% block was obtained. In the α-BTX rats (Wistar, 400 g), after the twitch returned to control, an infusion with α-BTX was started until slight (<10%) block occurred. After restabilization of the twitch, an infusion with the same blocker was started, again until 90% block. In the EAMG group, 30 min after the twitch returned to control, an infusion with the alternate blocker (Pan or Roc) was infused, until 90% block occurred. The perfusion and the concentration of the neuromuscular blocking agent were constant during the infusion. Data were analysed using Pk/PdFit (J. H. Proost), goodness of fit was assessed using visual inspection and statistical evaluation [3]. Data were obtained using the Sheiner model [4]. Values are presented as mean and coefficient of variation (M (% CV)).

Results: All control curves could be fitted with the Sheiner model. According to visual inspection however, fitting with the Sheiner model was not optimal for the α-BTX and EAMG groups (Table 1), resulting in larger CV.

Table 1. Onset and offset of block

	Onset (s) (75–25%)	Offset (s) (25–75%)	Ke0 min^{-1}	EC_{50} $\mu g\,L^{-1}$	Gamma
Roc					
Control	137 (21)	43 (12)	0.37 (14)	2333 (10)	7.5 (11)
α-BTX	153 (35)	143 (16)	0.21 (24)	822 (25)	3.7 (14)
Control	161 (37)	59 (17)	0.24 (8)	2387 (13)	8.5 (9)
EAMG	166 (18)	214 (35)	0.15 (27)	1093 (24)	3.6 (11)
Pan					
Control	160 (36)	51 (21)	0.33 (15)	571 (11)	8.2 (12)
α-BTX	239 (50)	160 (23)	0.16 (13)	218 (27)	4.4 (18)
Control	169 (15)	92 (23)	0.27 (22)	594 (10)	6.9 (25)
EAMG	134 (53)	188 (18)	0.15 (2)	319 (44)	4.7 (34)

Conclusions: As in myasthenic patients [5,6], the sensitivity to pan or roc is increased, and the offset times are prolonged in this model. The Sheiner model does not seem appropriate to model the time course of neuromuscular block in case of reduced number of AChR.

References:

1 *Anesthesiology* 1992; **76**: 822–43.
2 *J Neuroimmunol* 1987; **15**: 185–94.
3 *Acta Anaesthesiol Scand* 2000; **44**: 1169–90.
4 *Clin Pharmacol Ther* 1979; **25**: 358–71.
5 *Anesthesiology* 1984; **61**: 173–87.
6 *Can J Anaesth* 1992; **39**: 476–86.

7

The effects of chronic carbon dioxide exposure on the sensitivity to non-depolarizing neuromuscular blocking agents in the rat adductor and abductor laryngeal muscles

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Introduction: Differential sensitivity to non-depolarizing neuromuscular blockers has been reported between the laryngeal and peripheral skeletal muscles. These may also be unequal sensitivity to a relaxant in the adductor and the abductor laryngeal muscles. We have examined the responses of the lateral cricoarytenoid (LCA) (one of the adductor muscles of the vocal folds) and posterior cricoarytenoid (PCA) (sole abductor muscles of the vocal cords) to non-depolarizing neuromuscular relaxant after chronic exposure of carbon dioxide.

Methods: All experiments were carried out using dissected recurrent laryngeal nerve-intrinsic laryngeal muscle preparations from 11 Wistar rats, weighing 170–265 g after exposure to 14% carbon dioxide and 18% oxygen for 3 days (CO_2 group). Control animals (control group, $n = 11$) breathed room air. Preparations were bathed in glycerol-Krebs solution and after obtaining stable EMG responses of the LCA and PCA muscles by supramaximal stimulation of the recurrent laryngeal nerve for at least 30 min, the tissues were exposed to d-tubocurarine (dTc). The decreases in EMG amplitude produced by dTc were expressed as percentages of control value, and changes in the LCA and PCA muscles were compared.

Results: During exposure to 14% carbon dioxide the respiratory rates were significantly increased in the CO_2 group (120% compared to control). EMG responses in the both muscles were depressed in a concentration-dependent manner by dTc with LCA muscle being more resistant. Although there was no statistically significant differences in the degree of depression of EMG amplitude in the PCA muscles to dTc between the two groups, LCA muscle showed to be more resistant than PCA muscle at $>2.5 \times 10^{-6}$ ($mol\,L^{-1}$) in CO_2 group.

Conclusions: This study confirmed differences in sensitivity between LCA and PCA muscles to dTc. The intrinsic laryngeal muscles do not all behave similarly after chronic tachypnoea.

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Neuromuscular blocking effect of tetra-butyl ammonium compound and its interaction with vecuronium bromide in cats

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Introduction: There have been many attempts to develop an ideal muscle relaxant (MR) for rapid tracheal intubation to replace succinylcholine (SCC) because of its many undesirable side-effects, and due to some contraindication to its use, especially in infants and children.

Riker *et al.* reported that tetra-ethyl ammonium (TetA) antagonized a decamethonium induced neuromuscular (NM) block in cats [1]. Foldes *et al.* investigated that the non-depolarizing NM effect of TetA and some of its homologues which is capable of antagonizing a depolarizing MR [2].

We studied the time course of NM and cardiovascular (CV) effects of an analogue of TetA, tetra-butyl ammonium (TbuA) and the interaction between TbuA and vecuronium bromide (Vb) in cats.

Methods: Five male cats were anaesthetized with pentobarbital i.v. and their lungs mechanically ventilated to maintain a normal P_aCO_2. The sciatic nerve was stimulated with supramaximal square wave impulses of 0.4 Hz and the force of contraction of the tibial anterior muscle was recorded.

The dose responses of TbuA alone and TbuA preceded by a low dose (30% of all ED_{90} doses) of Vb were determined. The time course and circulatory effects of TbuA alone and TbuA with Vb were observed and compared.

Results: The ED_{90} values for TBuA, Vb and TBuA with Vb were $7.27 \pm 0.59\,mg\,kg^{-1}$, $14.34 \pm 0\ 9\,\mu g\,kg^{-1}$, and $4.57 \pm 0.61\,mg\,kg^{-1}$, respectively. The results indicated that TbuA with Vb enhanced the NM effect of TbuA. The onset time of TbuA and TbuA with Vb were both short at 28.8 ± 8.4 and 17.4 ± 3.2 s, respectively. The clinical duration and recovery index of $2 \times ED_{90}$ TbuA were significantly shorter than those of $2 \times ED_{90}$ Vb. The $2 \times ED_{90}$ TbuA caused slight increase of BP at the maximal block. The prior administration of Vb had no effect on BP.

Conclusion: TbuA has a non-depolarizing NM blocking effect with a remarkably short onset time compared with SCC. The cardiovascular effects of TbuA and that of the combination with Vb are minimal. Our present study suggests that TbuA would offer a new possibility for clinical use of MR, but clinical studies are required to evaluate this fully.

References:

1 *Arch Int Pharmacodyn* 1955; **330**: 90–101.
2 *Anesthesiology* 1989; **89**: A1010.

9

Monitoring of neuromuscular recovery in adults: a comparison between mechanomyography and the Datex-Ohmeda™ mechanosensor

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Introduction: Recent studies indicate that a train-of-four (TOF) ratio of >0.9 may be necessary for full recovery of pharyngeal muscle function as well as for

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airway protection mechanisms to be intact. Therefore, the use of quantitative neuromuscular monitoring is essential in order to assure adequate recovery at the end of anaesthesia. We have compared the Datex-Ohmeda™ mechanosensor (MDAT) with mechanomyography (MMG, Relaxometer 2, Groningen).

Methods: Twenty adult patients (ASA I to II) scheduled for elective surgery under general anaesthesia were enrolled into the study with their informed consent and the approval of the Ethics Committee. Neuromuscular monitors were attached to the left and right hands in accordance with a randomized protocol. Supramaximal TOF stimuli with square wave pulses of 0.2 ms duration at a frequency of 2 Hz were delivered to the ulnar nerve every 12 s via surface electrodes. Rocuronium was used as the relaxant. For the rest, the choice of the anaesthetic technique and whether or not reversal was given was left at the discretion of the attending anaesthetist. The extent to which the two monitors were in agreement was determined by the method of Bland and Altman [1].

Results:

Table 1. Mean differences (bias) and 95% limits of agreement between the MDAT and the MMG during recovery when the MDAT train-of-four (TOF) ratio was 0.70, 0.80 and 0.90

	Mean difference (MDAT–MMG)	95% Limits of agreement	
		Lower	Upper
MDAT TOF = 0.70	0.010	−0.145	0.165
MDAT TOF = 0.80	−0.008	−0.160	0.015
MDAT TOF = 0.90	−0.011	0.124	0.023

Conclusions: The limits of agreement between the MDAT and the MMG are considered as clinically acceptable. A TOF ratio of $\geq$0.9 as measured by the Datex-Ohmeda™ mechanosensor is considered as a reliable indicator of sufficient recovery from a rocuronium induced neuromuscular block.

Reference:

1 *Lancet* 1986; **1**: 307–10.

10

Comparison of rapacuronium, mivacurium and low dose rocuronium for gynaecological laparoscopy

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Introduction: Gynaecological laparoscopy is a relatively short procedure, which may be carried out on a day case basis requiring the use of agents that have a relatively rapid recovery. Rapacuronium is a new neuromuscular blocking agent that has been shown to have a rapid onset and a short duration of action with the feasibility of early reversal [1,2]. In the present study we have compared the use of rapacuronium, mivacurium and low dose rocuronium for muscle relaxation for gynaecological laparoscopies.

Patients and methods: Thirty-six patients were included in this study and anaesthetized with fentanyl 1–2 $\mu g\,kg^{-1}$, nitrous oxide in oxygen and propofol using a target controlled infusion system. Twelve patients each were randomly allocated to receive rapacuronium 1.5 $mg\,kg^{-1}$, mivacurium 0.2 $mg\,kg^{-1}$ or rocuronium 0.3 $mg\,kg^{-1}$. Tracheal intubation was attempted at 90 s and graded using a standard method and if unsuccessful, every 30 s again [3]. Onset of maximum (max) block and recovery were monitored using a TOF-Guard acceleration monitor (Biometer) delivering train-of-four (TOF) stimuli at 2 Hz every 15 s. Further smaller doses of the same relaxant were given if necessary for completion of surgery. Neostigmine 35 $\mu g\,kg^{-1}$ was given at the end of surgery if TOF ratio had not already reached 0.7. The results were subjected to analysis of variance and χ^2 tests as appropriate.

Results: The main results are given in Table 1. The onset of maximum block was significantly faster in the rapacuronium group and this group had the best intubating conditions. The intubating conditions were significantly better in the rapacuronium and rocuronium groups compared to the mivacurium group. The recovery of T_1 to 5 and 25% after the first dose of the relaxants was not significantly different. Nine, 4 and 10 patients in the rapacuronium, mivacurium and rocuronium groups required neostigmine at the end of surgery, five of these having received additional doses of relaxants. The times to reach a TOF ratio of 0.8 whether spontaneously or after neostigmine administration were not significantly different among the groups. Four patients in the mivacurium group had cutaneous flushing, one of them also having wheezing and a short lasting increase in airway pressure.

Table 1. Onset and duration of action and intubating conditions ($^*P < 0.05$ compared to the other two groups)

	Rapacuronium	Mivacurium	Rocuronium
Max block (%)	99.3 (1.56)	99.0 (2.34)	95.3 (3.94)
Time to max block (min)	1.42 (0.41)*	2.81 (1.29)	3.62 (1.53)
T_1–5% recovery (min)	8.9 (2.23)	11.0 (2.72)	10.0 (3.96)
T_1–25% recovery (min)	13.5 (2.90)	15.9 (4.31)	15.2 (4.92)
Tracheal intubation			
Excellent/good/poor	11/1/0	5/2/5*	2/8/2
TOF 0.8 (min)			
Spontaneous	38 (16.7)	27 (6.9)	30 (5.6)
With neostigmine	32 (15.1)	31 (21.1)	25 (7.6)

Conclusion: While the duration of action of rapacuronium was similar to that of mivacurium and low dose rocuronium, its use was associated with a faster onset of action and better intubating conditions.

References:

1 *Anesth Analg* 1993; **77**: 579–84.
2 *Anaesthesia* 2000; **55**: 859–63.
3 *Acta Anaesthesiol Scand* 1996; **40**: 59–74.

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Determination of propofol and remifentanil concentrations for tracheal intubation without muscle relaxant

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Introduction: Propofol in combination with remifentanil in large doses allows tracheal intubation with clinically good condition [1]. The purpose of this double blind, randomized study was to determine the effective pairs of concentrations of remifentanil and propofol for tracheal intubation using target controlled infusion.

Material and methods: After obtaining institutional approval and informed consent, ASA I or II patients, aged 18–55 years, were allocated randomly to three groups P1, P2 and P3 corresponding to target propofol concentrations (TC) of propofol of 4, 5 and 6 $\mu g\,mL^{-1}$, respectively. After premedication with hydroxyzine 100 mg, induction was performed using the Diprifusor® system for the propofol infusion and a Stanpump with the PK model of Minto–Schnider for remifentanil. In each group, the TC of remifentanil was set to 5 $ng\,mL^{-1}$ for the first patient; subsequent TCs were determined according to the previous patient's response to intubation. If tracheal intubation failed, according to the criteria of Viby-Mogensen *et al.*, the TC of remifentanil was increased by 0.5 $ng\,mL^{-1}$ [2]. If tracheal intubation was successful, the TC was decreased by 0.5 $ng\,mL^{-1}$. BP, heart rate, SpO_2, $ETCO_2$ and bispectral index were recorded every min for 15 min. Tracheal intubation was attempted at 10 min after equilibration of drug concentrations in the biophase. Within each group, the mean concentration of remifentanil for successful intubation was determined using the Up-and-Down method of Dixon [3]. Results were also analysed using logistic regression (SYSTAT, 7.0, SSPS Chicago, IL) for determination of ED_{50} of remifentanil. Data are expressed as mean $\pm$ SD or [95% CI].

Results: 52 patients were included allowing the determination of 5 crossover failure-to-success independent pairs of patients in each group. Mean concentrations of remifentanil in the three groups are given in the table 1.

Table 1. TC propofol in $\mu g\,mL^{-1}$. C_{mean}remi: mean concentration of remifentanil determined by Dixon's method in $ng\,mL^{-1}$. ED_{50}remi determined by logistic regression in $ng\,mL^{-1}$

	P1	P2	P3
N	16	20	16
TC propofol	4	5	6
C_{mean}remi	3.3 ± 0.8	2.9 ± 0.8	2.1 ± 0.5
ED_{50}remi	3.0 [2.9–3.1]	2.4 [2.1–2.7]	1.9 [2.3–2.4]

Mean ± SD or [95% CI].

Discussion: With propofol, a mean plasma concentration of remifentanil higher than 2 $ng\,mL^{-1}$ is needed for attaining good tracheal intubation conditions in adult patients.

References:

1 *Anesth Analg* 1998; **86**: 45–92.
2 *Acta Anaesthesiol Scand* 1996; **40**: 59–74.
3 McArthur JW, Colton T. *Statistics in Endocrinology*, Cambridge, MIT Rev, pp. 51–64.

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Interactions between non-depolarizing muscle blockers (NMB) at the nicotinic acetylcholine receptor (n-AchR)

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Introduction: Neuromuscular blocking agents (NMBs) act as competitive antagonists at the n-AchR in the neuromuscular junction (NMJ). Administration of certain combinations of two NMBs can lead to unexpected potentiation and prolongation of the blocking effect, increasing the risk for adverse outcomes due to residual muscle block at the end of an anaesthetic [1]. The underlying mechanism for this potentiation is unknown. Different affinities of NMBs to n-AchR in the NMJ, especially for drugs with different molecular structures (aminosteroids vs. benzoisoquinolines) may be an underlying cause. Two alternative hypothesis exist: (1) potentiation is a purely postsynaptic effect due to altered affinities of NMBs for the two binding sites formed by α-subunits of the postsynaptic n-AchR, or (2) potentiation arises from combined pre- and postsynaptic n-AchR effects. Using a postsynaptic model we tested the hypothesis that the effect of a combined application of two NMBs of the same structural class would be additive, while combining NMBs of the two different classes would show potentiation.

Methods: Adult n-AChR (mouse muscle) were heterologously expressed in frog oocytes (*Xenopus laevis*) by cytoplasmic injection of cRNAs encoding α, β, δ and ϵ subunits. Functional channels were activated with 10 μmol acetylcholine (ACh), alone and in solutions containing various concentrations of the NMBs The resulting currents were recorded using a whole cell two-electrode voltage clamp technique. The concentration–response relations from measurements on 5–6 oocytes were fitted to a logistic function by means of an iterative, non-linear least-squares program, which derived the 50% inhibitory concentrations (IC_{50}) and Hill coefficients for single drug applications (using GraphPad Software). Subsequently dose response curves were obtained for combinations of two drugs mixed in a ratio of $0.5 \times IC_{50}$ values of each single agent. Isobolographic analysis was used to define the type of interaction between NMBs [2].

Results: IC_{50} values for single drug application were 5.9 (95% confidence interval 4.9–6.9), 9.9 (8.4–11.4), 10.5 (7.6–13.4) and 43.0 (33.6–52.4) nmol for pancuronium (PAN), vecuronium (VEC), mivacurium (MIV) and d-tubocurarine (d-TC), respectively. None of the tested combination showed an effect that was significantly different from additivity, as the theoretical IC_{50} values for additivity were within the 95% confidence interval of the experimentally determined IC_{50} (Table 1).

Table 1. IC_{50} for different relaxant combinations

Combinations	IC_{50} (nmol)	Confidence interval (95%)	Theoretical IC_{50} (nmol) for additivity
PAN/VEC	8.5	7.1–9.9	7.9
VEC/MIV	12.0	9.7–13.3	10.2
MIV/d-TC	21.6	15.9–29.5	26.3

Conclusions: Using an oocyte expression model the rank order of potency of the tested NMBs was PAN > VEC > MIV > d-TC, which correlates with their known *in vivo* efficacy. Neither the combined application of two NMB from one structural class nor a combination from both classes (aminosteroid plus benzoisoquinoline) had a more than additive effect. This supports the hypothesis that presynaptic action contributes to the mechanism of potentiation.

References:

1 *Anaesthesia* 1998; **53**: 872–8.
2 *Life Sci* 1989; **45**: 947–61.

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Potentiation of mivacurium blockade by low dose of pancuronium: a pharmacodynamic – pharmacokinetic study

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Introduction: Low dose of pancuronium significantly potentiates mivacurium blockade [1]. With the use of the isolated arm technique, our hypothesis was that this potentiation might be due to an increase in the bioavailability of mivacurium secondary to the inhibition of plasma cholinesterase activity of pancuronium [2].

Methods: After written informed consent 20 patients ASA I–II were randomized into 2 groups, group mivacurium, M ($n = 10$) and group pancuronium + mivacurium, PM ($n = 10$). All patients had fentanyl and propofol for induction of anaesthesia and were intubated without muscle relaxants. The M group received mivacurium 0.12 mg kg^{-1}, while the PM group received pancuronium 15 μg kg^{-1} followed 3 min later by mivacurium 0.1 mg kg^{-1}. Anaesthesia was maintained with isoflurane and N_2O. Neuromuscular characteristics were recorded using a force transducer. Arterial blood samples were withdrawn at 30, 60, 90, 120 s and at 4 and 10 min. Cholinesterase activity (BChE) was measured for all patients before induction of anaesthesia and 3 min after injection of pancuronium in the PM group.

Results: No difference was noticed in patient characteristics. BChE activity was within normal range for all patients and decreased by 25% in the PM group ($P < 0.05$). Duration to 25% recovery was significantly longer in Group PM compared to group M (38(12) vs. 16(5) min; $P < 0.01$). The plasma concentration of active *cis–trans* and *trans–trans* isomers of M was significantly higher in the PM group from 60 s until 10 min (Table 1).

Table 1. Plasma concentrations (ng mL^{-1}, SD) of *cis–trans* and *trans–trans* isomers; $P < 0.001$ group PM vs. group M

	Group M ($n = 10$)		Group PM ($n = 10$)	
	Cis–trans	*Trans–trans*	*Cis–trans*	*Trans–trans*
30 s	816 ± 15	1654 ± 300	749 ± 160	1366 ± 270
60 s	369 ± 130	785 ± 230	634 ± 120*	1187 ± 135*
90 s	153 ± 80	373 ± 150	378 ± 90*	500 ± 142*
120 s	31 ± 10	257 ± 100	93 ± 25*	345 ± 115*
4 min	10 ± 2	71 ± 20	88 ± 15*	100 ± 24*
10 min	0	0	4 ± 2*	15 ± 10*

Conclusion: The early rate of decay of the plasma concentration of mivacurium is decreased in the presence of low dose of pancuronium. This is probably due to inhibition of plasma cholinesterase activity induced by of pancuronium.

References:

1 *Anesthesiology* 1996; **84**: 562–5.
2 *Anesth Analg* 2000; **91**: 732–5.

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Neuromuscular effects of rapacuronium during sevoflurane and isoflurane anaesthesia

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Introduction: Rapacuronium is a new aminosteroidal muscle relaxant with a rapid onset, a short duration of action and the possibility of early reversal [1]. Volatile anaesthetic agents, and sevoflurane in particular, have been shown to prolong the time course of other short and intermediate acting muscle relaxants [2,3]. It has also been shown that potent volatile anaesthetics can delay antagonism of neuromuscular block [4]. The present study was designed to compare the effects of two commonly used volatile anaesthetic agents on neuromuscular blocking effects and reversibility of rapacuronium.

Methods: Forty patients (ASA I to III) had anaesthesia induced with fentanyl 1–2 μg kg^{-1} and propofol 1–3 mg kg^{-1}. They were randomly allocated to maintenance of anaesthesia with 1.5 MAC (end-tidal) of sevoflurane (S) or isoflurane (I) ($n = 20$ each) in 66% N_2O in O_2. Onset and recovery of neuromuscular block to various end-points was measured by stimulation of the ulnar nerve using a supramaximal train-of-four (TOF) stimulation at 2.0 Hz every 10 s and recording the force of contraction of the adductor pollicis muscle. Neuromuscular responses and end-tidal concentration of volatile anaesthetics were allowed to stabilize for 8–10 min prior to administering a bolus dose of rapacuronium 1.5 mg kg^{-1}. Within each maintenance group half the patients were allowed to recover spontaneously (Groups IS and SS) while the block in the other half was reversed (Groups IR and SR) using neostigmine 50 μg kg^{-1} with glycopyrrolate 10 μg kg^{-1} at recovery of T_1 (first response of TOF) to 10%. The data were subjected to anova and post-tests.

Results: There were no significant differences in the onset times 53 (1.7) and 59 (2.5) s, respectively, for sevoflurane and isoflurane) or in times to recovery of T_1 to 10% (14.6 (1.2) and 14.7 (0.8) min, respectively). The times to 25%

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recovery of T_1 were not significantly different among the groups, averaging between 15 and 20 min. The time to spontaneous recovery of TOF ratio to 0.8 was significantly longer in patients receiving sevoflurane (76.5 compared to 54.9 min; $P = 0.0204$). These times were significantly shortened within each anaesthetic group by administration of reversal to 29.2 and 24.9 min, respectively. The difference between the groups receiving neostigmine was not significant. T_1 25–75% recovery index (RI) was also significantly shortened by administration of neostigmine but the times for comparable groups within each anaesthetic group were not different. Eleven out of 20 patients had not attained a TOF value of 0.8 within 15 min of neostigmine administration.

Table 1. Onset and recovery of neuromuscular block (Mean (SD))

	IS	SS	IR	SR
T_1 25% (min)	18.8 (5.0)	20.6 (8.6)	15.0 (3.7)	14.9 (4.5)
TOF 0.8 (min)	54.90 (15.72)	76.54 (21.04)	24.9 (8.08)	29.20 (11.49)
$T_1$25%–75% RI (min)	15.03 (5.91)	15.17 (7.61)	5.48 (3.85)	4.03 (2.53)
No. attaining TOF 0.8 within 15 min	–	–	5	6

Conclusion: Spontaneous recovery from rapacuronium induced neuromuscular block is longer in the presence of sevoflurane compared to isoflurane but reversibility is similar with the two anaesthetics.

References:

1 *Anesth Analg* 1993; **77**: 579–84.
2 *Anesth Analg* 1998; **87**: 936–40.
3 *Can J Anaesth* 1999; **46**: 29–33.
4 *Anesth Analg* 1995; **80**: 1175–80.

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Chemical chelation as a novel method of NMB reversal characterization of the org 25969 NMB complex

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Introduction: Synthetic host molecules, such as cyclodextrins, have been used extensively as pharmaceutical excipients to increase water-solubility, metabolic stability and bioavailability of lipophilic drugs. Compared with other cyclic host molecules, cyclodextrins are usually more water-soluble and biologically well tolerated. The increasing commercial availability of cyclodextrins plus their favourable supramolecular chemistry properties makes them interesting targets for development as medicinal agents. It was postulated that chemical chelation of neuromuscular blocking agents (NMBs) by a host molecule, such as cyclodextrin would promote dissociation of the latter from nicotinic acetylcholine receptors (nAChR) on the muscle, leading to the reversal of neuromuscular blockade.

Methods: The interaction of Org 25969 with rocuronium has been studied using nuclear magnetic resonance spectroscopy (NMR), single crystal X-ray analyses and isothermal titration microcalorimetry.

Results: The cyclodextrin-based host molecule was observed to form a tight 1:1 complex with the NMB guest. Both NMR and X-ray crystallography were used to study the geometry and conformation of the host–guest complex, with the NMB molecule intercalated in the cyclodextrin ring.

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Chemical chelation as a novel method of NMB reversal – discovery of Org 25969

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Introduction: Formation of an inclusion complex (*encapsulation, chemical chelation*) is part of the well-known area of supramolecular chemistry or host-guest chemistry. To date, many cyclic organic compounds, e.g. cyclophanes, cyclic oligosaccharides, cyclic peptides, calixarenes, crown ethers, aza crown ethers, etc. are known to be capable of forming an inclusion complex with another molecule, organic or inorganic.

One of the few therapeutic uses of small molecule synthetic hosts is the use of chemical chelators such as EDTA and desferoxamine in the treatment of heavy metal poisoning. Synthetic host molecules, especially cyclodextrins, have also been used as pharmaceutical excipients to increase water-solubility, metabolic stability and bioavailability of lipophilic drugs. Compared with other cyclic host molecules, cyclodextrins are usually more water-soluble and biologically well-tolerated. The increasing commercial availability of cyclodextrins plus their favourable supramolecular chemistry properties makes them interesting targets for development as medicinal agents.

Methods: Our hypothesis was that chemical chelation of neuromuscular blocking agents (NMBAs) by a host molecule would promote dissociation of the latter from nicotinic acetylcholine receptors (nAChR), leading to the reversal of neuromuscular blockade. Since this mechanism of action does not involve direct activation of cholinergic systems, it may circumvent the undesired side-effects attendant with acetylcholinesterase (AChE) inhibitors such as neostigmine. Hence, there should be no need for the concomitant use of a muscarinic acetylcholine receptor (mAChR) antagonist such as atropine or glycopyrrolate. The use of chemical chelators as reversal agents for NMBAs also has the potential advantage that they could be used to reverse the action of both depolarizing and non-depolarizing NMBAs, because of this lack of involvement of nAChRs in the mechanism of action. In addition, they may be safely employed for the reversal of 'profound (or complete) block'.

Results: Org 25969 is a synthetic cyclodextrin-based host molecule that forms tight host–guest complex with rocuronium and as a result reverses rocuronium-induced neuromuscular block. The reversal produced by Org 25969 is not only fast and highly efficient, but also without any visible cardiovascular side-effects.

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Org 25969 reverses rocuronium-induced neuromuscular blockade in the cat without important haemodynamic effects

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Introduction: The novel reversal agent Org 25969 reverses neuromuscular block by formation of complexes with steroidal neuromuscular blocking agents (NMBs). Since this mechanism does not involve direct interactions with receptors or enzymes, Org 25969 has potentially less cardiovascular side-effects. To evaluate this, we studied the effects of Org 25969 on rocuronium-induced steady-state block of the *tibialis* muscle in the anaesthetized cat.

Method and results: The sciatic nerve was stimulated (supramaximal voltage, 0.25 ms duration at 10 s intervals) in anaesthetized cats, to produce contractions of the tibialis muscle. Rocuronium was administered as a bolus of 0.2–0.3 mg kg^{-1} i.v., followed by an infusion of a 0.6 mg mL^{-1} solution at a rate adjusted to produce a steady block of twitch tension of about 90%. After 5 min of stable 90% block, the infusion was switched off, a volume of saline equivalent to the volume of vehicle used for test compound was given, and the muscles allowed to recover spontaneously. One hour after full recovery, a stable 90% block was re-induced for 5 min. The infusion with rocuronium was stopped and 1 mg kg^{-1} Org 25969 was given i.v.

Org 25969 caused rapid reversal of neuromuscular block. After bolus injection of saline, 75% recovery occurred after 6.2 min. This was reduced to 1.3 min after injection of Org 25969 (Fig. 1). No significant changes in heart rate and blood pressure were observed.

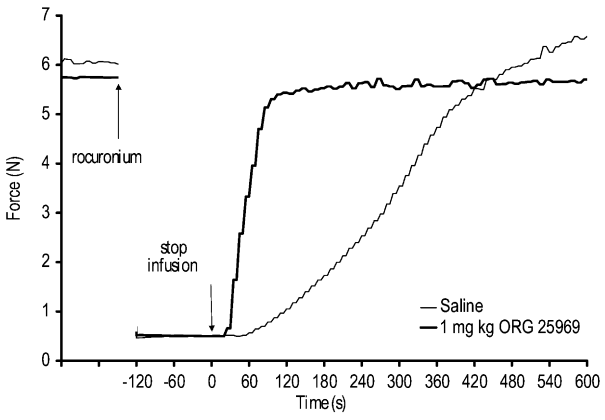

Fig. 1. Reversal of M. tibialis block ($n = 3$).

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Org 25969 causes selective reversal of neuromuscular block induced by steroidal NMBs in anaesthetized guinea pigs

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Introduction: Complex formation between steroidal neuromuscular blocking agents (NMBs) and the novel reversal agent Org 25969 results in rapid reversal of block in the isolated mouse hemi-diaphragm preparation. In this study, we have studied this in the anaesthetized guinea pig.

Method: Male Dunkin–Hartley guinea pigs (body weight: 610–870 g) were anaesthetized with pentobarbital 30 mg kg^{-1} and urethane 900 mg kg^{-1} i.p. After tracheotomy, the animals were artificially ventilated. Catheters were placed in both jugular veins and a catheter was placed in the carotid artery for continuous monitoring of arterial blood pressure and for taking blood samples for blood gas analysis.

The sciatic nerve was stimulated (supramaximal rectangular pulses of 0.5 ms duration at 0.1 Hz using a Grass S88 Stimulator.

The force of gastrocnemius contractions was measured using a force displacement transducer (Grass FT03). Body temperature was maintained at 37–38°C by using an electrical heating pad. Muscle contractions, blood pressure and heart rate were recorded on a multi-channel recorder (Grass, model D7)

After a bolus injection, an infusion of the NMB was started to obtain a steady state 90% neuromuscular block. The infusion was stopped and spontaneous recovery allowed to occur. After complete recovery, the procedure was repeated, and, after the infusion of muscle relaxant was stopped, an intravenous bolus injection of 1 mg kg^{-1} Org 25969 was given. The results are summarized in Table 1.

Results:

Table 1. 25–75% recovery index

	Spontaneous recovery (min)	Recovery after 1 mg kg^{-1} Org 25969 (min)
Rapacuronium	2.1 ± 0.4	0.3 ± 0.0*
Rocuronium	2.5 ± 0.3	0.3 ± 0.0*
Pancuronium	7.4 ± 1.8	0.3 ± 0.0*
Vecuronium	12.9 ± 3.1	0.4 ± 0.0*
Succinylcholine	4.2 ± 0.7	4.4 ± 0.4
D-Tubocurarine	4.5 ± 0.4	5.7 ± 1.2
Atracurium	7.2 ± 1.2	9.3 ± 2.4
Mivacurium	34.4 ± 6.2	16.3 ± 4.6

Mean ± SEM; $n = 4$; *$P < 0.05$ vs. spontaneous recovery.

Conclusion: Org 25969 caused rapid reversal of neuromuscular block induced by steroidal NMBs. After administration of all steroidal NMBs, a 90% recovery of twitch height was obtained within one minute after i.v. injection of Org 25969. However, Org 25969 is less active against neuromuscular block induced by non-steroidal NMBs. Injection of 1 mg kg^{-1} Org 25969 did not cause significant changes in heart rate or blood pressure.

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Neuromuscular blockade induced by steroidal NMBs can be rapidly reversed by Org 25969 in the anaesthetized monkey

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Introduction: Org 25969 is a novel reversal agent, whose action is based on the formation of complexes with steroidal neuromuscular blocking agents. Org 25969 has been shown to reverse neuromuscular block of single twitches in the guinea pig and the cat. In this study, we evaluate the action of Org 25969 with train-of-four (TOF) stimulation in the anaesthetized monkey.

Method: Female *Rhesus* monkeys (body weight 4.0–7.0 kg) were sedated with ketamine 10 mg kg^{-1} i.m., followed by pentobarbital sodium (25 mg kg^{-1} i.v.) and subsequent infusion at 5–10 mg kg^{-1} h^{-1}. The animals' lungs were ventilated with a mixture of oxygen and nitrous oxide (2 : 3). Heart rate and blood pressure were determined with pulse oximetry and with a cuff placed around the tail. Body temperature was kept at 37–38°C. Muscle contractions induced by TOF stimulation (0.07 Hz) of the ulnar nerve of the right thumb were recorded. After a bolus injection of rocuronium bromide or vecuronium bromide, an infusion was started to reduce the first twitch contraction of the train-of-four to approximately 10% of its baseline value. After a steady-state block had developed, the infusion was stopped and the preparation was allowed to recover spontaneously. This procedure was repeated again, but at the very time the infusion was stopped, 1 mg kg^{-1} Org 25969 was given i.v. Recovery parameters were calculated from the recordings.

At the end of the experiment, the animals were allowed to recover from anaesthesia.

Results: The results of these experiments are shown in Table 1.

Table 1. Neuromuscular recovery times (min) with and without Org 25969; values are mean and SEM; $n = 4$; *$P < 0.05$ vs. spontaneous recovery

TOF ratio	Spontaneous recovery	After 1.0 mg kg^{-1} Org 25969
Rocuronium		
0.50	7.4 (0.8)	0.5 (0.1)*
0.75	10.2 (1.0)	0.9 (0.1)*
0.90	14.5 (1.1)	1.9 (0.5)*
Vecuronium		
0.50	12.7 (0.6)	1.5 (0.2)*
0.75	17.4 (1.0)	2.4 (0.2)*
0.90	23.1 (1.8)	4.4 (0.6)*

Org 25969 caused rapid reversal of both rocuronium- and vecuronium-induced neuromuscular block. No signs of re-curarization were observed. Injection of Org 25969 did not have significant effects on blood pressure or heart rate. After the recovery from anaesthesia, the animals showed no signs of abdominal discomfort, as seen after neostigmine.

Conclusion: Org 25969 is a fast and effective reversal agent for steroidal NMBs with no important cardiovascular actions and lack of side-effects associated with anticholinesterase agents.

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Org 25969 causes selective reversal of neuromuscular blockade induced by steroidal NMBs in the mouse hemi-diaphragm preparation

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Introduction: Org 25969 is a novel reversal agent (RVA), which acts by the formation of complexes with neuromuscular blocking agents (NMBs). In this study, we evaluate the selectivity of Org 25969 for several steroidal and non-steroidal NMBs.

Method: Male mice (20–30 g) were humanely killed and each hemi-diaphragm with its phrenic nerve was placed on a tissue holder in a tissue bath filled with a modified Krebs–Henseleit buffer at 37°C, bubbled with 95% oxygen and 5% carbon dioxide. The phrenic nerve was stimulated continuously using a Grass S88E stimulator (rectangular pulses of 0.2 ms every 20 s at a supra-maximal voltage of 2.5 V) and isometric force was recorded using Grass FT03 transducers and a Grass 79D recorder. In preliminary studies, the ED_{90} doses for all NMBs had been determined and no spontaneous reversal was observed after administration of the NMBs.

After a stimulation period of at least 30 min, a 90% blocking dose of NMB was administered. Twenty minutes later, increasing doses of Org 25969 were administered at intervals of 10 min. The results are summarized in Table 1.

Table 1. Reversal of neuromuscular block; (Mean ± SEM; $n = 4$)

	90% Block by NMB at (μmol)	50% Reversal by Org 25969 at (μmol)	Maximum reversal (%)	At (μmol)	50% Ratio RVA/NMB
Rocuronium	3.60	1.2 ± 0.8	95.1 ± 2.3	3.6	0.3 ± 0.2
Rapacuronium	28.00	12.5 ± 1.8	64.2 ± 32.4	28.0	0.4 ± 0.1
Vecuronium	0.85	0.8 ± 0.1	90.6 ± 5.1	1.4	0.9 ± 0.2
Pancuronium	0.90	1.2 ± 0.3	60.4 ± 8.7	1.8	1.3 ± 0.3
Mivacurium	8.00	–	0.0 ± 0.0	160.0	–
Atracurium	45.00	–	4.7 ± 4.7	1400.0	–
Succinylcholine	45.00	–	24.5 ± 8.8	900.0	–

Results: All steroidal NMBs could be effectively reversed. Org 25969 was more effective against rocuronium and rapacuronium than against vecuronium

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and pancuronium. In contrast, the reversal of the block, induced by non-steroidal NMBs, was less effective, within the concentration range of Org 25969 used. We also studied the effects of Org 25969 and neostigmine on 90% block and profound block induced by rocuronium (3.6 and 10.8 μmol, ED_{90} and $3 \times ED_{90}$ dose, respectively). Twenty min after induction of a 90% block, both Org 25969 (3.6 μmol) and neostigmine (7.0 μmol) caused reversal of neuromuscular blockade (97.9 ± 2.8% and 74.4 ± 9.5%, respectively, $n = 4$). However, after obtaining profound block with $3 \times 90\%$ blocking dose of rocuronium, only Org 25969 (10.8 μmol) was able to reverse the block (61.9 ± 8.8%), whereas neostigmine was ineffective, even at higher concentrations (7.0–9.0 μmol).

Conclusion: Org 25969 is a novel reversal agent, which effectively reverses neuromuscular blockade induced by steroidal NMBs. Org 25969 is less effective against non-steroidal NMBs. In contrast to neostigmine, Org 25969 can reverse profound block induced by steroidal NMBs.

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Onset of the neuromuscular block and the slope of dose-response line simulated with an empty or a receptor-filled effect compartment

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Introduction: Pharmacodynamic models simulate neuromuscular block (NMB) based on the time course of the free concentration of the muscle relaxant [D] in an effect compartment, and D required for the half-maximal NMB (IC_{50}) using the Hill equation. Most models consider an effect compartment of a negligibly small volume or containing a negligibly small amount of the relaxant. Only a few models consider binding of D to the postsynaptic receptors. Inclusion of binding to the receptors offers distinct advantages in pharmacodynamic simulations. The goal of the present study was to examine the roles of the volumes assigned to the effect compartment as well as of binding of D to the postsynaptic receptors in these compartments.

Methods: The decay of the plasma concentrations of the hypothetical muscle relaxant D was postulated to follow an arbitrarily selected triexponential function and the transport from plasma into the effect compartment and back via diffusion. Binding of D to the receptors, if present, decreases the free concentration of D in the effect compartment. In turn, the binding was simulated with two rate constants: one for the formation of the relaxant–receptor complex, $k_{association}$, the other for dissociation, $k_{dissociation}$. The ratio $k_{dissociation}/k_{association}$ defines the equilibrium dissociation constant K_D and, hence, the affinity of the receptors for D and IC_{50}. The small volume of the effect compartment was calculated as the sum of all the synaptic spaces in muscle ($=1.6 \times 10^{-6}$ L kg^{-1}), while the large volume was that of the interstitial space in muscle ($=6 \times 10^{-2}$ L kg^{-1}). The total molar amount of receptors was calculated per kg body weight based on the published number of receptors in a single human end plate (2.1×10^{7}). Receptor concentrations were calculated assuming a uniform dilution of the receptors in the effect compartments. Hence, the small volume is associated with a high (7.75×10^{-5} mol), and the large volume with a low (2×10^{-9} mol), receptor concentration. NMB was calculated either from the fractional receptor occupancy by D or from (D) when no receptors were present. The equations were solved numerically using the program MATHEMATICA. In all models, ED_{50} produced NMB_{50} at 4.58 min.

Results: The models containing empty effect compartments, of either a small or a large volume, projected onset times to peak submaximal NMB as independent of the magnitudes of the NMB and shallow slopes for the dose-response lines. Only the projection by the model consisting of a small volume of the effect compartment filled with the receptors differed in that the onset times were inversely correlated with the magnitude of the NMB and the slope of the dose-response line was steep. The projections differ widely and are suitable to be tested in clinical experiments. Using several MRs, clinical studies designed to estimate the onset times to peak submaximal NMB and the slopes of the dose-response lines can, hence, provide information about the optimal assumptions regarding the volume of the effect compartment and the receptor concentration in it.

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Anaphylaxis to muscle relaxants in Northern Ireland

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Introduction: Neuromuscular blocking agents (NMBs) are the commonest among anaesthetic agents as a cause of anaphylactic reactions. We report on the recent cases of anaphylaxis to NMBs in Northern Ireland.

Methods and results: Thirty cases of suspected anaphylaxis to NMBs were reported to the Regional Immunology Service in Belfast during 1998–2000, 14 of whom showed positive responses to skin prick testing (SPT) to one or more of these agents (Table 1). Rocuronium and suxamethonium showed the highest number of positive tests.

Table 1. Frequency (number of cases) of positive SPTs

	Primary reaction*	Cross-reactivity†	No. of positive SPTs
Atracurium	2	2	4
Mivacurium	0	2	2
Pancuronium	0	1	1
Rocuronium	5	2	7
Vecuronium	0	2	2
Suxamethonium	7	0	7

*Cases where the agent was the cause of the anaphylactic reaction; †cases where the agent was not implicated in the reaction but demonstrated a positive SPT.

Positive reactions to rocuronium were observed in 7 (5 female, 2 male) cases. In 5 of these, SPT findings were consistent with the anaesthetic history, whereas in the other two the anaesthetic history and SPT results indicated reactivity to succinylcholine with possible cross-reactivity or true allergy to rocuronium. The clinical grade of reaction to rocuronium, on the Ring and Messmer scale, ranged from II to IV [1]. All patients made a full recovery.

Laboratory findings indicated elevated serum mast cell tryptase values (measured at 1–6 h post-reaction) in all 7 rocuronium cases with raised urinary methylhistamine in 6/7 cases. Skin prick testing for rocuronium was performed using the 10 mg mL^{-1} solution and at 1 : 10 dilution and resulted in significant weal formation in all patients at both concentrations. Three of seven patients were SPT positive for rocuronium alone. Positivity was recorded for one additional drug in 3 patients and for 3 other NMBs in one patient.

These cases highlight some points of interest: firstly, concern has been expressed at the rate of 'false positive' skin prick tests when rocuronium is used at 1 : 10 or greater concentration [2]. This may occur with intradermal testing but has not been our experience with skin prick testing. Secondly, rocuronium is now the most common non-depolarizing muscle relaxant to be implicated in anaphylactic reactions in Northern Ireland. The reasons for this are unclear but it may be a reflection of a greater use of this agent [3]. Whether this reflects increased allergenic potential or changing patterns of usage is unclear.

References:

1 *Lancet* 1977; **26**: 466–9.
2 *Br J Anaesth* 2000; **84**: 108–11.
3 *Br J Anaesth* 2001; **86**: 678–82.

23

Immediate reactions to rocuronium

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Introduction: Anaphylactoid reactions to rocuronium have been reported, although the mechanisms, specific or non-specific, are debated. We present a series of 12 patients who reacted within minutes of injection of rocuronium and who had biochemical and allergological testing demonstrating an IgE-mediated mechanism.

Methods: Patients were studied from 1996 through 2000 in a single region of France. All had a reaction 1–5 min after injection of rocuronium and other anaesthetic drugs. Blood was withdrawn 5–100 min after the onset of the reaction, plasma histamine (RIA, Immunotech/Beckman Coulter) and mast cell tryptase (UniCAP, Pharmacia) concentrations were measured, and quaternary ammonium specific (QAS) IgE was evaluated through the binding to a choline analogue bound to Sepharose® [1]. Five weeks to 20 months thereafter, intradermal skin tests were performed on the back of patients with serial dilutions of all the administered drugs, as well as prick tests with latex, measurements of QAS and latex IgEs, and *in vitro* histamine release.

Results: None of the 12 patients (M = 4, F = 8, age = 41.2 ± 12.8 year) had a history of a previous reaction to anaesthetics. Only one had a history of allergy and one patient had never been anaesthetized before. The reactions were life-threatening in 10 patients (grade 4 = 1; grade 3 = 9) and moderate in 2 (grade 2 = 2). Plasma histamine concentration was increased in all the patients (mean ± SD = 1950 ± 3837 nmol L^{-1}; range: 16–13420 nmol L^{-1}; normal

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value <6 nmol L^{-1}), as were tryptase (100 ± 67 μg L^{-1}; range : 22–229 μg L^{-1}; normal value <12 μg L^{-1}) and QAS-IgE (percent binding: 14.4 ± 9.2%; range: 4.3–30.5%, normal value <2.5%). Rocuronium bromide solution (10 mg mL^{-1}) was diluted 1 : 100–1 : 10 000. Intradermal skin tests, performed on the back, were positive (diameter of oedema = 9 mm) to the dilution 1 : 10 000 in eight patients, to 1 : 1000 in 2 and to 1 : 100 in two. All the patients also had positive skin tests to at least one other neuromuscular blocking agent. QAS-IgEs were persistently present in all patients (range: 4.5–30%). *In vitro* histamine release was positive to rocuronium in six patients. All the tests were negative to latex and to the other administered drugs.

Conclusion: All the patients released histamine during the reaction, and mast cell was involved in the reaction, as indicated by increases in the tryptase concentrations. All the patients, and even the one, who had never received neuromuscular blocking agents, were sensitized to quaternary ammonium ions before the reaction, as shown by the positivity of specific IgE. Thus, rocuronium appears responsible for the observed reactions through an IgE-mediated mechanism.

Reference:

1 *Allergy* 1991; **46**: 452–8.

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Intubation-associated vocal cord dysfunction – a randomized, placebo-controlled trial

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Introduction: Tracheal intubation may be performed with or without neuromuscular blocking drugs. Only limited data comparing the incidence of postoperative vocal cord dysfunction after intubation with or without a neuromuscular blocking agent (NMBA) is available. The aim of this study was to evaluate whether NMBAs as part of an induction regimen influences postoperative vocal cord function.

Methods: After Ethics Committee approval and informed consent, 30 patients were randomized to receive atracurium (Atr) 0.5 mg kg^{-1} (Group A, $n = 15$) or saline (Sal, Group B, $n = 15$). Anaesthesia was induced with fentanyl 2 μg kg^{-1} followed 5 min later by propofol 2.0 mg kg^{-1} over 10 s. Thereafter, Atr or Sal were administered over 5 s, followed 3 min later by tracheal intubation. The same experienced anaesthetist blinded to the treatment intubated all patients. Tube size (men: ID 8.5 mm, women: ID 7.5 mm), type of tube (Woodbridge, Mallinckrodt), use of an introducer, use of lidocaine gel and no stomach tube were standardized. Intubating conditions were evaluated according to the standard scheme [1]. In addition, difficult intubation was graded according to Cormack and Lehane [2], patients of grades 3 and 4 being excluded from the study. Anaesthesia was maintained with remifentanil 0.25 μg kg^{-1} min^{-1} and isoflurane 0.4–0.6% (end-tidal) in oxygen/air. Hoarseness was recorded as 0 = none, 1 = mild, 2 = moderate, 3 = severe [3]. All patients were asked for presence and severity of hoarseness in the PACU and on postoperative days 1, 2 and 3. Larynx and vocal cords were evaluated by stroboscopic examination by an experienced ENT-physician. Patients with pre-existing abnormalities of the larynx and vocal cords were excluded. Sequelae of tracheal intubation such as oedema, erythema, granuloma, haematoma of the vocal cords and arytenoid luxation were recorded 24 and 72 h after anaesthesia and after recovery from anaesthesia. Statistical analysis was performed using Mann–Whitney *U*-test (demographic data), Fisher's exact test, or anova and statistical significance assumed at $P < 0.05$.

Results: Patient characteristics and duration of anaesthesia were comparable between groups. One patient in group B was excluded due to laryngospasm requiring rescue succinylcholine, in addition, one patient in group A was excluded because of major study protocol violation. Therefore, we report the results of 28 patients. Intubation was possible in all patients, but diaphragmatic reaction to intubation was present significantly more often in group B ($P < 0.01$). The incidence of postoperative hoarseness and vocal cord dysfunction are shown in Table 1.

Table 1. Frequency of postoperative hoarseness and vocal cord dysfunction (*n* or median and range); *$P < 0.05$ vs. group A. †$P = 0.052$ vs. Group A

	Group A ($n = 14$)	Group B ($n = 14$)
Hoarseness in PACU:		
Incidence	2	6
Severity	0 (0-1)	0 (0-2)
Cumulative incidence	2	9†
Vocal cord sequelae:		
Incidence	1	7*

Conclusions: These preliminary data show that an induction regimen without NMBA while allowing tracheal intubation in most patients is associated with an increased incidence of intubation-associated morbidity.

References:

1 *Acta Anaesthesiol Scand* 1996; **40**: 59–70.
2 *Anaesthesia* 1984; **39**: 1105–11.
3 *Anesthesiology* 1987; **67**: 419–21.

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Phonomyography of the corrugator supercilii muscle: signal characteristics and best recording site

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Introduction: The contraction of skeletal muscles creates low frequency sounds. Few quantitative methods exist to measure neuromuscular blockade at the corrugator supercilii muscle, which follows that of the larynx. This study investigated the acoustic signal characteristics and determined neuromuscular blockade of mivacurium at the corrugator supercilii muscle.

Methods: Twelve patients were included in the study. After induction of anaesthesia and insertion of a laryngeal mask, a small condenser microphone (frequency range: 2.5–10 kHz) was placed on 6 different areas on the forehead (Fig. 1a) and the peak-to-peak response after single twitch supramaximal stimulation of the facial nerve was measured. Then the microphone was placed where the response was largest and mivacurium 0.2 mg kg^{-1} was administered. Lag time, onset time, peak effect and time to reach 25, 75 and 90% of control signals were determined using single twitch stimulation (0.1 Hz), additionally TOF stimulation was applied every minute. The signals were digitized and Fast-Fourier-transformation was applied to determine peak frequencies and the power–frequency relationship at different stages of neuromuscular blockade.

Results: In all 7 women and 5 men, the best response was obtained just above the middle portion of the eyebrow (Fig. 1b, area 2) as biphasic signals of a mean duration of 502 ± 137 ms with a mean maximum amplitude of 2.37 ± 0.67 mV. In all patients, a stimulation of the contralateral *corrugator supercilii* muscle produced an inverted signal and its amplitude was 34% of the signal of the ipsilateral *corrugator supercilii*.

Peak frequency was in a range 2.5–5 Hz. Signal recording up to 40 Hz detected 90% of total signal power. After mivacurium 0.2 mg kg^{-1}, lag time was

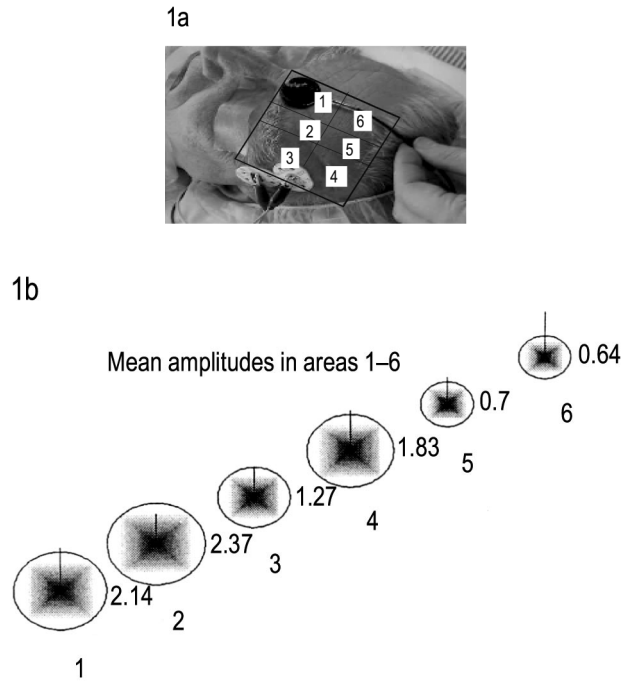

Fig. 1. (a) Positions of condenser microphone on the forehead; (b) amplitudes at the six sites.

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40 ± 22 s, peak effect of 91 ± 4% was reached after 140 ± 32 s. Recovery to 25%, 75% and 90% of control was reached after 6.9 ± 1.7 min, 13.4 ± 2.9 min and 15.2 ± 3.8 min, respectively. Peak frequencies did not change with intensity of blockade during onset and offset. Recording of offset of neuromuscular blockade was terminated when train-of-four stimulation evoked a T4/T1 ratio of ≥1.

Conclusions: Phonomyography can be used to measure NMB quantitatively at the corrugator supercilii muscle. The best signal is recorded above the middle portion of the eyebrow. Most of the signal power is below hearing range (<20 Hz).

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Flow and not potency (EC_{90}) is related to rate of onset/offset of neuromuscular block

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Introduction: We developed an antegrade perfused rat peroneal nerve tibialis anterior muscle model (APPAT) to study potentially important factors governing the time course of effect of neuromuscular blocking agents. Potency (EC_{90}) has been shown to be inversely related to the onset time [1], whereas a relationship between blood flow and time-course of effect could not be shown in man [2].

Methods: We isolated the tibialis anterior muscle of Wistar rats and cannulated the artery and vein supplying the muscle, providing a way for single-pass perfusion with blood from donor rats (flow 200 μL min^{-1}). The peroneal nerve was stimulated (single twitch, 0.1 Hz, square wave stimulation of 0.2 ms) and the muscle was connected to a force transducer. After stabilization of the twitch, a constant infusion, maintaining a blood concentration resulting in 90% depression of the twitch (EC_{90}), with either rocuronium (R) or pancuronium (P) was started. After a stable twitch depression was obtained, the infusion was stopped and after the twitch allowed to return to its control value. The rate of blood flow was then doubled. After 30 min, a second infusion with R or P with the same concentration was started, again until a stable 90% twitch depression was obtained and the infusion then stopped. The time course of action was recorded through the entire experiment. The onset index was defined as time from 75% twitch height to 25% twitch height, and the recovery index, vice versa.

Results: The EC_{90} (SD) was 1034 (167) and 3274 (256) μg L^{-1} for *P* and *R*, respectively.

Flow and not EC_{90} had a significant influence on the onset index. (P_{slow} vs. P_{fast}, R_{slow}, vs. R_{fast} were 159 vs. 112, and 134 vs. 90 s, respectively; Fig. 1) and also on the recovery index (P_{slow} vs. P_{fast}; R_{slow} vs. R_{fast} were 54 vs. 44, and 64 vs. 53 s, respectively).

Conclusion: In this APPAT model, which excludes interference of pharmacokinetic factors such as plasma clearance we found that flow and not potency (EC) had a significant influence on onset and recovery index of the neuromuscular block.

References:

1 *Eur J Anaesthesiol* 1995; **12** (Suppl. 11): 45–54.
2 *Br J Anaesth* 1997; **79**: 24–8.

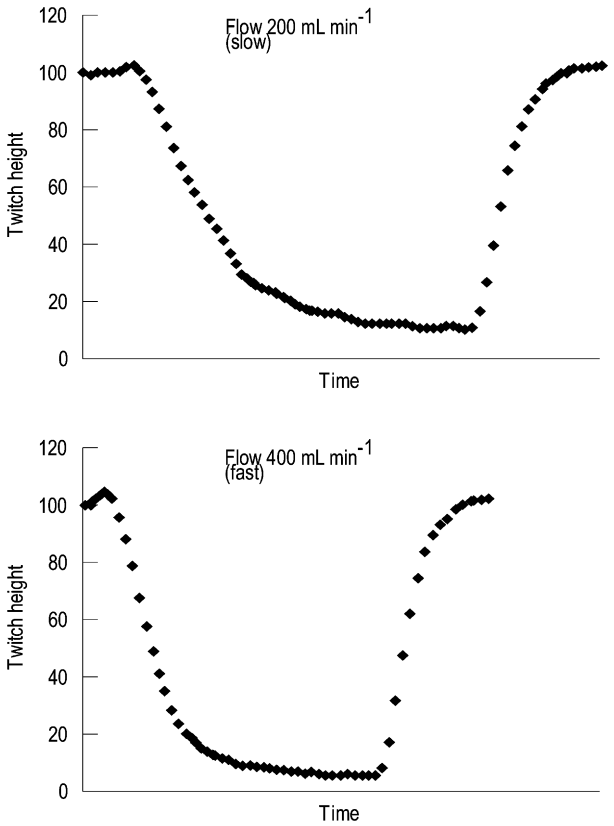

Fig. 1. Blood flow vs. onset and offset of block.

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Effects of lidocaine on the neuromuscular action of cisatracurium

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Background: Lidocaine is useful as a local anaesthetic and antiarrhythmic agent perioperatively. It may augment neuromuscular block for both non-depolarizing and depolarizing muscle relaxants. Cisatracurium is a muscle relaxant, which is an isomer of atracurium. We investigated the interaction of cisatracurium and lidocaine *in vitro*.

Methods: Forty male Sprague Dawley rats (100 g) were divided into four groups (control, lidocaine 1, 10 and 100 μg). The animals were anaesthetized with 40 mg kg^{-1} pentobarbital. The hemidiaphragm with phrenic nerve was dissected and mounted within 5 min in a bath containing 100 mL Krebs solution with oxygenation at 32°C. The phrenic nerve was stimulated at supramaximal intensity by a Grass S88 stimulator through an SIU5 isolation unit. The twitch height was measured by precalibrated Grass FT88 force displacement transducer and recorded with Grass 79 polygraph. After stabilization of the twitch response, cisatracurium 100 μg was added to the Krebs solution. When stable 3–5 twitch inhibition was obtained after 10 min, saline 1 mL or lidocaine 1, 10 or 100 μg was added to the solution. The twitch response was measured again after 10 min. The data were analysed by repeated measures ANOVA.

Results: There was a significant depression of the twitch response in lidocaine 10 μg and 100 μg groups compared with control and lidocaine 1 μg groups. The lidocaine 100 μg group showed a significantly greater depression compared with 10 μg group (Table 1).

Table 1. The changes of twitch height after added lidocaine 0, 1, 10 and 100 μg (%); Values are mean ± SD. Values after added lidocaine are significantly reduced compared to values after cisatracurium. $^{*}P < 0.05$ compared with control and lidocaine 1-μg groups. †$P < 0.05$ compared with control and lidocaine 10-μg groups

	Initial	10 min after cisatracurium	10 min after lidocaine
Control	100	92.1 ± 4.7	80.9 ± 16.1
Lidocaine (1 μg)	100	92.1 ± 4.2	74.6 ± 16.7
Lidocacine (10 μg)	100	88.5 ± 7.8	43.0 ± 21.8*
Lidocaine (100 μg)	100	88.9 ± 6.9	19.7 ± 17.3*†

Conclusion: We conclude that lidocaine will increase the sensitivity to cisatracurium in the hemidiaphragm preparation of the rats. Lidocaine may thus be a cause of possible recurarization.

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Some syringe pumps are inaccurate when used with a high rate request frequency

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Introduction: Four syringe pumps in use at our hospital were being considered for use in a closed loop muscle relaxation system. To evaluate them for suitability we tested two pumps with internal stepper motors (Becton Dickinson DPS, Becton Dickinson Pilot Anesthesia) and two pumps with DC motors (Graseby 3400, IVAC P4000).

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Methods: We controlled the syringe pumps by a computer via the syringe pump RS232 serial connection. The computer requested an infusion rate from the syringe pump at 0.1 Hz and records were kept of infused volume. Infused volume was calculated from syringe plunger movement by the pump's internal volume measuring potentiometer. Because the test conditions involve the syringe pump's internal volume measuring potentiometer as a volume reference, the results are not dependent on syringe inaccuracies or placement.
Results: Under the test conditions, the syringe pumps that we tested with internal DC motors do not move the syringe plunger sufficiently to deliver the requested drug infusion rate. At an infusion request rate of 0.1 Hz, the actual infusion rate was significantly lower than the requested infusion rate (Fig. 1).

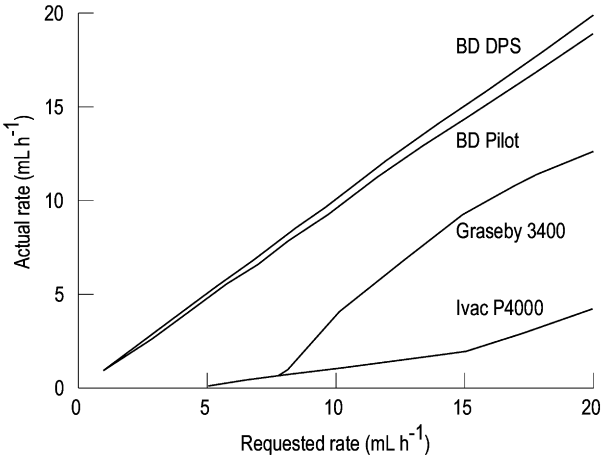

Fig. 1. Actual infusion rate vs. requested rate for a rate request frequency of 0.1 Hz for the pumps tested.

Conclusion: It is concluded that depending on the type of syringe pumps, infusion rate and rate request frequency, there can be a difference between requested infusion rate and the delivered infusion rate. This has important consequences when using infusion rate data to calculate average infusion rate for and for choosing a syringe pump suitable for a closed loop control system. Of the pumps tested, syringe pumps with internal stepper motors perform better than syringe pumps with internal DC motors. If an infusion device is to be used in an application with frequent rate changes, the device should be tested to assume that it is capable of providing the required accuracy under the conditions of use.

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Alkoxy-acetoxy benzyl quaternary derivatives of tropinyl diesters. Ultrashort acting non-depolarizing muscle relaxants

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Introduction: During our search for new, ultrashort acting muscle relaxants we synthesized and pharmacologically evaluated two series of tropinyl diesters containing (a) 4-acetoxy-3,5- dimethoxybenzyl, and (b) 4-acetoxy3-methoxybenzyl quaternizing groups.

Methods: (a) Chemistry: Alkoxy-acetoxybenzyl halides were synthesized from the corresponding hydroxy-methoxy benzaldehydes in three steps, followed by quaternization of various tropinyl diesters prepared previously. (b) Pharmacology: Anaesthetized and tracheostomized rats and juvenile pigs were set up for electromyographic (anterior tibial muscle) recording of the evoked neuromuscular responses. Drug administration was via a cannulated jugular vein. Heart rate and blood pressure changes were measured using a cardiotachometer and pressure transducer. Cumulative ED_{50} neuromuscular blocking (NMB) doses, onset and recovery index (RI) (25–75% twitch recovery) and degree of cardiac vagal block (CVB) were determined. Mean values and SEM were calculated.
Results: Four selected agents were tested in pigs and showed adequate potency (183–343 $\mu g\,kg^{-1}$ ED_{50} NMB), a rapid onset, and short RIs (0.5-1.0 min). CVB ranged from 26–65%. For comparison, values for rocuronium were: ED_{50} NMB: 350 $\mu g\,kg^{-1}$, onset 1.5 min, RI: 3.2 min, CVB: 47% (Table 1).
Conclusion: Potent quaternary tropinyl diesters with ultrashort non-depolarizing NMB action could be produced by introduction into the aralkyl quaternizing moiety of a hyrolyzable acetyl ester group. The number of methoxy groups adjacent to the acetoxy group did not alter the pharmacological profile.

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Di-acetoxy benzyl quaternized ultrashort acting tropinyl diester muscle relaxants

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Introduction: We have previously observed that acylation of the benzyl group that quaternizes the tropine diesters improves their pharmacological profile as ultrashort acting non-depolarizing neuromuscular blocking agents. Having an additional acetoxy substituent on the benzyl group further improves the profile in some aspects.
Methods: We quaternized bis-tropine diesters of several selected diacids with diacetoxybenzyl halides and tested them in anaesthetized, tracheotomized and ventilated adult rats and juvenile pigs by neurally evoked compound electromyographic (*m. tibialis ant.*) response. Some compounds were further tested in higher animal species. Drugs were administered via a cannulated jugular vein. Heart rate (tachometer) and blood pressure (transducer) changes were also measured. Neuromuscular blocking potency (NMB ED_{50}), onset, and 25–75% recovery index (RI), and cardiac vagal block (CVB) were determined.
Conclusion: The acyl substituent (on the benzyl quaternizing group) that contributes desirable pharmacological profiles to these bistropinium diesters also promotes ultrashort duration of action by being readily hydrolysable. Having two acetoxy substituents appears to further enhance these advantages in several instances. Accordingly, TAAC3 has been selected to undergo extensive study for potential human trials.

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Skin testing in patients sensitized to neuromuscular blocking agents

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Introduction: Skin tests are considered the gold standard for the diagnosis of neuromuscular blocking agent (NMBA) allergy. However, NMBAs may elicit false positive skin tests, especially at high concentrations, as well as false

Table 1. Neuromuscular blocking profile in the rat (mean (SEM))

	NMB ED50 ($\mu g\,kg^{-1}$)		Onset (min)		RI (min)		CVB* (%)		*n*	
	a	b	a	b	a	b	a	b	a	b
Glutaryl	717 (52)	487	0.5 (0.1)	0.5	0.7 (0.1)	0.4	50 (8)	55	3	4
Sebacoyl	280 (20)	350	0.4 (0.05)	0.6	0.5 (0.05)	0.4	99 (1)	95	4	3
Undecanoyl	500 (50)	500	0.4 (0.05)	0.55	0.5 (0.05)	0.4	99 (1)	95	4	3
1	280 (64)	400	0.7 (0.1)	0.55	0.8 (0.1)	0.5	65 (12)	85	6	3
2	367 (83)	365	0.4 (0.05)	0.6	0.5 (0.05)	0.5	20 (10)	20	3	4
3	400 (58)		0.7 (0.1)		0.8 (0.05)		75 (9)		3	
4	337 (65)	270	0.5 (0.05)	0.6	0.6 (0.1)	0.5	40 (11)	30	4	7
5	200 (54)	150	0.7 (0.05)	0.55	0.8 (0.2)	0.5	30 (5)	25	7	7
Rocuronium	450 (40)		1.0 (0.1)		1.3 (0.2)		60 (8)		5	

a, 4-acetoxy-3,5-dimethoxybenzyl quaternaries; b, 4-Acetoxy-3-methoxybenzyl quaternaries; 1, *trans*-3-hexene-1,6-dicarbonyl; 2, *trans*-cyclobutane-1,2-dicarbonyl; 3, cyclopentane-1,1-dicarbonyl; 4, cyclohexane-1,3-dicarbonyl; 5, *trans*-5-norbonene-2,3-dicarbonyl. *CVB at 80% NMB.

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Table 1. Neuromuscular blocking profile in the rat and the pig

	NMB ED_{50} ($\mu g\ kg^{-1}$)	Onset (min)	RI (25–75%) (min)	CVB (at 80% NMB)	*n* (%)
Rat					
TAAC2	148 (17)	0.70 (0.1)	0.50 (0.05)	26 (15)	5
TAAC3	232 (31)	0.75 (0.1)	0.55 (0.04)	7 (2)	9
TAAC4	450 (20)	0.75 (0.1)	0.60 (0.1)	35 (12)	4
TAAC5	188 (24)	0.75 (0.1)	0.55 (0.1)	77 (4)	5
TAAC8	159 (15)	0.50 (0.1)	0.5 (0.05)	90 (6)	5
TAAC9	273 (18)	0.80 (0.1)	0.5 (0.05)	98 (2)	5
TAAE	84 (22)	0.65 (0.1)	0.5 (0/05)	32 (5)	11
TAAR4	218 (15)	0.70 (0.1)	0.45 (0.05)	5 (3)	10
TAAR613	240 (35)	0.65 (0.1)	0.5 (0.1)	28 (11)	9
Rap	950 (162)	0.75 (0.2)	0.65 (0.8)	92 (8)	6
Pig					
TAAC2	450 (76)	0.9 (0.02)	0.9 (0.1)	75 (3)	3
TAAC3	230 (35)	0.95 (0.1)	1.1 (0.1)	41 (2)	6
TAAR4	275 (44)	0.68 (0.1)	0.85 (0.1)	43 (9)	7
TAAR613	225 (31)	0.8 (0.1)	0.86 (0.1)	54 (5)	6
TAAE	197 (42)	0.65 (0.1)	0.85 (0.1)	42 (5)	7

(3,4-diacetoxybenzyl tropanium)-O-CO-(X)-CO-O-(3,4-diacetoxybenzyl tropanium), where-in X = (CH2)3 in TAAC3 (CH2)4 in TAAC4, etc., and X = 2,3-norborn-5-ene in TAAE, cyclobutane-1,2- in TAAR4, and cyclohexane-1,3- in TAAR613. Rap = rapacuronium. CVB = cardiovagal block.

negative tests if dilution is too high. It is now possible to measure specific IgEs and allergy mediators during the reaction. From a cohort of patients who had reacted during an anaesthetic and had plasma histamine, mast cell tryptase and quaternary ammonium (QAS) specific IgEs were measured, we selected patients with positive specific IgE to evaluate skin testing to NMBAs.

Methods: From 1993 to 2000, blood samples were obtained from 133 patients within the first hour(s) following an anaphylactoid reaction during anaesthesia. Plasma histamine was measured by radioimmunoassay (Immunotech, France) and tryptase by radioimmunoassay until 1997 and by fluoroimmunoassay thereafter (RIACT and UniCAP, Pharmacia, Sweden). QAS-specific IgEs were measured using a choline analogue bound to Sepharose®. Four weeks or more later, the allergologist performed intradermal skin tests on the back of patients using 0.05 mL of serial dilutions of the administered NMBA, and of all the other injected drugs. Prick tests with latex were also performed. A wheal diameter of more than 9 mm, with a surrounding flare, 15 min after injection, was considered positive. QAS-IgEs were measured a second time in these patients.

Results: 51 patients (17 male and 34 female) aged 46 ± 11.9 year (mean ± SD) (range 17–67 year) had increased QAS-IgEs on the day of the reaction (percent binding to anti-human IgE: 15.3 ± 8.5%, range 2.7–32.2%, normal values <2.5%). The administered NMBAs were: suxamethonium ($n = 18$), rocuronium ($n = 13$), atracurium ($n = 11$), vecuronium ($n = 6$), pancuronium ($n = 2$), and mivacurium ($n = 1$). Reactions were life threatening in 38 cases (5 cardiac arrests) and moderate in 13. Reactions appeared after induction of anaesthesia in 48 cases, and later than 30 min in 3 patients who received atracurium. Histamine concentration was increased in 42 patients and borderline in 2, tryptase was increased in 41 and borderline in 4; five patients had normal values for both. 48 patients had positive skin tests to the administered NMBA. The highest dilution (lowest concentration) eliciting skin positivity was recorded. Twenty-eight patients were positive for dilution of 1:1000 or higher (succinylcholine 13, rocuronium 10, atracurium 3, vecuronium 2) and 20 for 1:100 dilution (succinylcholine 3, rocuronium 3, atracurium 7, vecuronium 4, mivacurium 1) and for 1 : 10 dilution (pancuronium 2). Three patients had negative tests: 2 had increased histamine concentration and borderline tryptase following a moderate reaction to succinylcholine and one had a delayed reaction 1 h after atracurium injection, with normal histamine and tryptase. The four other patients with normal mediator concentrations had positive skin tests to 1 : 100 dilution of vecuronium ($n = 2$), atracurium ($n = 1$) or mivacurium ($n = 1$). QAS-IgEs remained positive in all the patients. No patients had positive skin tests to the other administered drugs or to latex.

Conclusion: In this cohort of reactors with persistently positive quaternary ammonium specific IgEs, skin tests to the administered NMBA appeared positive in 94% of subjects. For NMBAs able to elicit non-specific histamine release at high concentrations, it appears necessary to consider skin testing at 1 : 100 dilution to enhance the sensitivity, despite the possible false positive tests. False negative results were suspected in two cases of reaction to suxamethonium. This study shows that skin tests are not 100% reliable and should be discussed in the light of specific IgE and mediator measurements.

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The effect of mivacurium on isolated rat tracheal muscle

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Introduction: Neuromuscular blocking drugs are designed to specifically block nicotinic cholinergic receptors at the neuromuscular junction, but many bind to muscarinic cholinergic receptors on ganglia, nerve endings, and smooth muscles, thereby altering parasympathetically mediated airway calibre and heart rate.

Methods: We studied the effects of mivacurium on the tension of tracheal smooth muscle by using an isolated rat tracheal preparation. The cumulative effect of acetylcholine after pretreating the tracheal smooth muscle with mivacurium at different concentrations, as well as the effect of L-NAME and propranolol on the tension of tracheal smooth muscle after pretreating with mivacurium were investigated.

Results and conclusions: Mivacurium shifted the acetylcholine dose-response curve to the right. L-NAME and propranolol had no effect on the tension of tracheal smooth muscle after pretreating with mivacurium. We have found that mivacurium relaxes isolated rat tracheal smooth muscle and this effect has no relationship to beta-adrenoceptors or nitric oxide.

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Potency of rapacuronium, its metabolite Org 9488, and other non-depolarizing muscle relaxants on recombinant muscle-type acetylcholine receptors

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Introduction: Rapacuronium (RAP) is a recently introduced non-depolarizing muscle relaxant (NMB) characterized by fast onset and short duration of action. Its 3-desacetyl metabolite, Org 9488, also exerts neuromuscular-blocking effects, which may become apparent after prolonged maintenance of relaxation with rapacuronium. The aim of this study was to compare the relative potency of RAP and ORG 9488, as well as Rocuronium (ROC), Vecuronium (VEC), Pancuronium (PAN), and D-Tubocurarine (D-TC) on fetal and adult isoforms of muscle-type achetylcholine receptors (n-AchR) using the *Xenopus laevis* oocyte expression system.

Methods: Nicotinic acetylcholine receptors (mouse fetal/γ-extrajunctional form and mouse adult/ϵ-junctional form) were expressed separately in *Xenopus* oocytes by cytoplasmic injection of cRNAs encoding α, β, γ and δ subunits (fetal) or α, β, δ and ϵ subunits (adult). Functional channels were activated with 10 μmol acetylcholine (ACh), alone and in solutions containing various concentrations of the NMBs. The resulting currents were recorded using a whole cell two-electrode voltage clamp technique. Data represent results from 4-6 oocytes for each NMB. Percentage inhibition of control-currents was plotted against drug concentrations (log mol). The concentration–response relations were fitted to a logistic function by means of an iterative, non-linear least-squares program, which derived the 50% inhibitory concentrations (IC_{50}) and Hill coefficients [1].

Results: All tested NMBs reversibly inhibited ACh-activated inward currents in the tens of nmolar range (Table 1). Org 9488 was a more potent inhibitor than RAP. The rank order of potency for the adult n-AChR subtype was PAN > VEC > MIV > ROC > d-TC > ORG 9488 > RAP. The fetal n-AChR was more potently inhibited than was the adult receptor type by RAP and Org 9488, and less inhibited by d-TC.

Table 1. Inhibition of Ach activated inward currents

	IC50 (nmol)		Hill coefficient	
Antagonist	γ	ϵ	γ	ϵ
Rapacuronium	66.2	100.0	1.00	1.01
Org 9488	14.9	45.8	0.77	0.59
d-Tubocurarine	82.0	43.0	1.12	0.78
Rocuronium	20.9	22.4	1.04	1.03
Mivacurium	9.1	10.5	0.99	0.97
Vecuronium	7.6	9.9	1.22	1.06
Pancuronium	3.4	5.9	1.1	0.93

Conclusions: Org 9488 is an active metabolite of rapacuronium and appears in our experiments to be more potent than rapacuronium itself. This finding is

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consistent with modelled drug concentrations of the two compounds at the effect site in patients [2]. With the oocyte expression model, the rank order of potency of the tested NMBs for the ε-nAChR correlates well with their known *in vivo* efficacy. Differences in potency at the two isoforms of the n-AChR, as observed here for RAP, ORG 9488, and d-TC have been reported previously for other steroidal NMBs [3]. These data provide further validation of the oocyte expression system for pharmacodynamic studies of NMBs.

References:

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34

Curare in medicine: in search of a disease (a historical perspective)

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Introduction: With the availability of the jungle drug curare in Europe, doctors were eager to identify possible therapeutic indications for its use. We recapitulate on a variety of historic therapeutic trials using curare (Table 1).

Table 1. Historical therapeutic trials with curare

Year	Author	Place	Disease or condition	Mode of application of curare	Number of patients	Outcome	Ref.
1812	W. Sewell	London	Rabies	Not reported	Not reported	Not reported	[1]
1858	L. A. Sayre	New York	Tetanus	Topical	1	Died	[2]
1859	M. Vella	Turin, Italy	Tetanus	Topical	3	2 died 1 recovered	[3]
1878	D. Drummond	Newcastle	Chorea	Injection	2	1 recovered	[4]
1906	A. Läwen	Leipzig, Germany	Strychnine poisoning	With artificial respir. only	Suggestion		[5]
1912	A. Läwen	Leipzig, Germany	Surgery	Injection	Not reported	Positive	[6]
1941	A. E. Bennett	Omaha, USA	Electro shock therapy	Not reported	>640	Positive	[7]
1946	F. Johnson	USA	Dysmen.	i.v.	Not reported	Not reported	[8]

Results and conclusion:
The use of curare for treatment of the above-mentioned disorders, given our knowledge of curare today, was reasonable, since these were basically spastic in nature. Nevertheless, most of these attempts failed because: (a) artificial respiration was not an established therapeutic modality, or its necessity was not appreciated (b) ineffective modes of application were used despite better knowledge at the time, or (c) owing to its mechanism of action the use of curare could only be limited to the symptomatic treatment of disease rather than represent a therapeutic principle. Thus, after more than a century's efforts, curare has made its clinical impact as a pharmacological tool improving the technique of anaesthesia and intensive care and not as a drug with any therapeutic value of its own.

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35

Simulations with two binding sites at a receptor: single twitch, neuromuscular block and its reversal

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Introduction: Pharmacodynamic models for non-depolarizing muscle relaxants (MRs) simulate the neuromuscular block (NMB) based on the concentration of a relaxant in a postulated effect compartment. Therefore, the models can simulate neither the twitch strength in the absence of a MR nor the reversal of the NMB. Our goal was to devise a model based on the physiologic and pharmacologic concepts of release of acetylcholine (ACh) from the motor nerve terminal, its degradation and binding to the postsynaptic receptors, and a competition of a MR with ACh for binding to the receptors. Twitch strength was simulated as a function of the receptors occupied by ACh at two binding sites.

Methods: Several parameters in the model were collected from the literature (receptor number at a single motor end plate, the number of ACh molecules released by the first stimulus, the volume of the synaptic cleft). Free ACh was postulated to be hydrolysed with $t_{½} = 1$ ms. The association and dissociation rate constants for binding of ACh or the MR to either site at a single receptor approximated the reported values. We postulated that the affinities of both ACh and the MR for site$_1$ were 10 times higher than for site$_2$. The equilibrium dissociation constants for site$_1$ were 10^{-7} and 10^{-5} mol for the MR and ACh, respectively. Formation and decay of the following 8 complexes were considered (A = ACh, D = MR, R = receptor): ARØ, ØRA, ARA, DRØ, ØRD, DRD, ARD, and DRA. The twitch strength was simulated using the concentration of the doubly occupied activated receptor [ARA] and the constants$_A$ and [ARA]$_{50}$ in the Hill equation. Both constants were derived from the model by iterative adjustments of their values to fit the theoretical logit NMB vs. log [D] line using [D] in the Hill equation. The value of (D was set to 4.4 and IC$_{50}$ was defined by postulating that at IC$_{50}$ 87.5% of the receptors are occupied by D (3 complexes). The concentrations of the 8 complexes were obtained by numerical integration of 8 differential equations at 0.01 ms intervals using the program MATHEMATICA. Reversal from different levels of NMB was attempted either by decreasing the rate of ACh hydrolysis or by a combination of a decreased rate of hydrolysis and an increased stimulus-induced release of ACh.

Results: The theoretical logit NMB vs. log [D] line yielded an IC$_{50}$ of 4.5×10^{-7} mol. This line was well simulated by the twitch model, with A of 7.6 and [ARA]$_{50}$ of 4.37×10^{-8} mol. The value of IC$_{50}$ and its 95% confidence interval derived from the twitch model were 4.5×10^{-7} and $(4.2–4.8) \times 10^{-7}$ mol, respectively. The maximal twitch strength without MR was 0.996 at 0.13 ms after the stimulus, when 0.1% of the receptors were occupied by ACh at both sites and 73% of the released ACh molecules were bound to the receptors in the form of 3 complexes. Starting from NMB$_{50}$, either a 95% or a 99% inhibition of ACh hydrolysis simulated the recovery of the twitch to less than its full strength. On the other hand, a nearly full twitch strength (0.993) could be achieved by postulating a milder inhibition of ACh hydrolysis (50–87.5%) together with an increase in the stimulus-induced release of ACh (30–20%). Simulations of the reversal from a deeper NMB (NMB$_{60}$ to NMB$_{90}$) approached reported clinical results only when the dual mechanism of action of the reversal agent was postulated. With further refinements, the model may be applied to simulate twitch fade.

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Studies on a new rapid onset and short-acting neuromuscular blocking agent, TAAC3, in anaesthetized cats

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Introduction: TAAC3, bis[*N*-(3,4-diacetoxybenzyl)-tropinium-α-*yl*] glutarate dibromide, is an interesting rapid onset, short-acting neuromuscular blocking agent (NMBA). Studies were carried out to investigate its NM blocking potency, time course profile, and autonomic and cardiovascular effects in anaesthetized cats.

Methods: Female cats were anaesthetized with medetomidine and anaesthesia maintained with α-chloralose/pentobarbital sodium and artificially ventilated with room air. Twitches of the *tibialis* anterior and *soleus* muscles were induced by electrical stimulation of the sciatic nerve

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(0.1 Hz, 0.25 ms duration). Arterial pressure was recorded from a catheter in a carotid artery and heart rate was measured by integrating the pressure signal. Autonomic selectivity was determined by measuring the effects of the drug on vagus nerve-stimulated bradycardia and cervical sympathetic nerve-induced contractions of the nictitating membrane. NM and autonomic blocking potencies were determined by injecting different doses of the test compound at approximately 30-60 min intervals, i.e. using doses producing between ~15 and 85% block of the responses. NM blocking profiles and effects on arterial pressure and heart rate were determined at doses producing ~90% twitch block.

Results: TAAC3 did not produce muscle fasciculations or pre-block twitch augmentation. Ninety percent NMB doses of TAAC3 produced rapidly developing and extremely short-lasting NM block (Table 1). Following higher ($3 \times 90\%$ NMB) doses, the time to complete block was approximately halved, without markedly affecting the recovery of neuromuscular function. By contrast, NM block produced by rapacuronium recovered more slowly and was markedly longer lasting at the higher dose. Estimates of autonomic effects gave a vagal/NMB dose ratio of 3–4 and nictitating membrane/NMB dose ratio of 10. The results indicate a potential for changes in heart rate and arterial pressure.

Table 1. NM blocking time course profiles of TAAC3 and rapacuronium in anaesthetized cats (mean and SEM)

Compound	Dose (mg kg^{-1})	Onset (min)	Duration of 100% block (min)	25–75% Recovery (min)	Duration 90% (min)
~ED_{90}					
TAAC3 ($n=8$)	0.24	1.6 ± 0.1	–	0.7 ± 0.1	3.2 ± 0.3
Rapacuronium ($n=7$)	0.30	1.9 ± 0.1	–	1.5 ± 0.1	5.0 ± 0.2
~$3 \times ED_{90}$					
TAAC3 ($n=4$)	0.77	0.8 ± 0.1	2.5 ± 0.2	0.6 ± 0.1	4.6 ± 0.3
Rapacuronium ($n=3$)	0.90	0.9 ± 0.1	8.5 ± 1.8	2.4 ± 1.0	15.0 ± 3.8

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The efficacy and safety of priming principle with vecuronium in parturients

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Introduction: Vecuronium is an intermediate acting muscle relaxant, which has minimal placental transfer and adverse effect on the neonate [1]. Although these advantages make it a desirable skeletal muscle relaxant for Caesarean section, the precise clinical evaluation in pregnant women has been poorly documented. The current study was designed to investigate the efficacy and safety of the priming principle using vecuronium in parturient and non-pregnant patients.

Methods: After receiving approval from the Institutional Review Board we obtained written informed consent from 41 healthy patients undergoing Caesarean section (C-group) and 41 patients undergoing gynaecological surgery (G-group). The ages ranged from 23 to 47 years in both groups. Anaesthesia was induced with propofol and fentanyl i.v., and maintained with 50% N_2O in O_2 and 0.7% sevoflurane. Neuromuscular block was evaluated using the evoked compound electromyogram. The single dose dose-response curves and dose–duration relationships (after 90 $\mu g\,kg^{-1}$) were determined in 35 patients each in the two groups. The remaining 6 patients in each group received 10 $\mu g\,kg^{-1}$ of vecuronium as a priming dose followed 4 min later 90 $\mu g\,kg^{-1}$. Measurements included recording the onset time and clinical duration (recovery to 25%). Dose-response curves were graphically compared as a log-probit plot, the curves were statistically analysed for parallelism and ED_{50} and ED_{90} estimated. Student's *t*-test was used for other statistical analysis.

Results: The dose response curves in the two group were parallel and yielded statistically similar values. ED_{50} and ED_{90} of vecuronium were 26.6 and 43.7 $\mu g\,kg^{-1}$ in the C-group, and 28.4 and 45.5 $\mu g\,kg^{-1}$ in the G-group, respectively. There were no significant differences between dose–duration relationships in the two groups. Onset time was significantly shorter in the C group. The onset time was also significantly shorter in the primed groups than in the 90 $\mu g\,kg^{-1}$ bolus injection groups. However, the clinical duration was similar in the four groups. The priming dose caused significant incidence of symptoms of muscle weakness especially in the parturients.

Conclusions: Our results suggest that the similar doses of vecuronium can be used in parturients and non-pregnant patients. The priming principle should be used with caution in parturients because of muscle weakness after the administration of priming dose.

Reference:

1 *Anesthesiology* 1984; **60**: 569–73.

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Repetition of PTC stimulation at the adductor pollicis does not affect final recovery of atracurium blockade

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Introduction: Post-tetanic count (PTC) remains a valuable method for assessing profound neuromuscular blockade [1]. However subsequent response to repetitive stimulation might be altered due to post-tetanic facilitation (PTF). To avoid PTF it has been advocated to limit the interval of stimulation to 6–10 min [2]. However, the impact of PTF on clinical recovery (TOF ratio higher than 0.7) has not been evaluated. In addition, some new devices such as the TOF Watch SX® allow repetitive PTC (50 Hz) stimulation as often as every 2 min. Therefore, we assessed the effect of repetitive PTC stimulation (every 3 min) on the neuromuscular recovery of atracurium blockade).

Methods: After informed consent 15 ASA I-II patients scheduled for peripheral surgery under general anaesthesia and requiring tracheal intubation were enrolled into the study. Anaesthesia was induced with sufentanil and propofol, and atracurium 0.5 $mg\,kg^{-1}$ administered to facilitate tracheal intubation. Neuromuscular characteristics were assessed at the adductor pollicis by TOF watch accelerometers on both arms. After onset of maximum block, PTC was recorded every 3 min in one arm and TOF responses every 15 s in the other arm. Between each PTC stimulation the accelerometer returned to a TOF mode every 15 s and if a response from TOF stimulation was detected the repetitive PTC was stopped. The following parameters were recorded: onset of maximum block, mean time of PTC stimulation, the maximum number of responses to PTC, time of the first TOF response, time to T_1 25% recovery, and times to reach TOF ratios of 0.7, 0.8 and 0.9. TOF ratios during recovery were compared using paired *t*-tests.

Results: All patients had complete block in both arms. The mean duration of PTC stimulation was 25 ± 10 min, the first response of the TOF in the PTC stimulated arm appeared on average 1 min before in the other arm. No significant difference was noticed for subsequent responses nor for clinical recovery (TOF ratio higher than 0.7).

Conclusion: Repetitive PTC stimulation every 3 min does not alter the final clinical recovery of TOF stimulation during atracurium block when assessed by accelerography.

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Succinylcholine with or without rocuronium pretreatment for ambulatory anaesthesia – a randomized, prospective, controlled trial

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Introduction: Due to its rapid onset and ultra-short duration of action succinylcholine (Suc) is still commonly used to facilitate tracheal intubation in ambulatory anaesthesia [1]. However, Suc-induced postoperative myalgia is a frequent problem [1]. Rocuronium (Roc) is efficacious in preventing Suc-induced fasciculation. However, its effectiveness in reducing muscle pain is controversial [2] and side-effects such as muscle weakness have not been investigated systematically. We studied the incidence and severity of fasciculation and myalgia from Suc in outpatients – with and without pretreatment with Roc (0.06 $mg\,kg^{-1}$) and assessed muscle weakness and other side-effects. This data were compared with a control group not receiving Suc.

Methods: After Ethics Committee approval and informed consent, 120 patients undergoing ambulatory anaesthesia were randomized to 1 of 3 treatment groups as follows ($n=40$ each): Group A: Roc 0.06 $mg\,kg^{-1}$ followed 4 min later Suc 1.5 $mg\,kg^{-1}$; Group B: saline (Sal) followed 4 min later by Suc 1.5 $mg\,kg^{-1}$; Group C: Sal, followed 4 min later by Roc 0.6 $mg\,kg^{-1}$. Induction regimen was standardized and pretreatment was administered in a double-blind fashion. At time 0, fentanyl 1–2 $\mu g\,kg^{-1}$ and pretreatment were given; 3 min later, the patients were asked for signs of muscle weakness, i.e.

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diplopia, heavy eyelids, difficulty in swallowing or speaking and dyspnoea by an investigator blinded to the patient's group assignment. At 4 min, anaesthesia was induced with thiopentone 4–7 mg kg^{-1} and Suc (groups A and B) or Roc (group C), followed 60 s later by tracheal intubation. Anaesthesia was maintained with remifentanil 0.25 $\mu g\,kg^{-1}\,min^{-1}$ and desflurane 2–3% (end-tidal) in oxygen/air. Fasciculations were recorded as 0 = absent, 1 = mild, 2 = moderate, 3 = severe. The incidence of dysrhythmia and bradycardia was noted. Myalgia was assessed as: 0 = none, 1 = slight, 2 = moderate, 3 = severe [3]. Postoperative care and pain management were standardized. All patients were questioned on presence and severity of myalgia in the PACU and 24 h postoperatively (by telephone). Data were subjected to Mann–Whitney *U*-test (demographic data), Fisher's exact test or anova and statistical significance was assured at $P < 0.05$.

Results: Precurarization with Roc reduced the incidence and severity of fasciculation significantly. However, it did not have any effect on the incidence and severity of myalgia, but regularly led to signs of muscle weakness (90%) (Table 1). Cardiovascular side-effects, e.g. bradycardia and dysrhythmia, requiring atropine i.v. were only observed in the Suc-groups ($n = 3$ in each).

Table 1. Fasciculations and myalgia after succinylcholine with or without rocuronium pretreatment; values are numbers (%) or median and 25–75 percentiles (severity). *$P < 0.001$ vs. A/C. †$P < 0.001$ vs. A

	Group A ($n = 40$)	Group B ($n = 40$)	Group C ($n = 40$)
Fasciculation			
Incidence	1 (2.5)	32 (80)*	0 (0)
Severity	0 (0)	2 (1–2)*	0 (0)
Myalgia at 24 h			
Incidence	7 (17.5)	11 (27.5)	7 (17.5)
Severity	0 (0)	0 (0–1)	0 (0)
Muscle weakness			
Incidence	36 (90)	9 (22.5)†	6 (15)†

Conclusions: Pretreatment with Roc was associated with muscle weakness before loss of consciousness, without reducing the incidence of myalgia. In view of these results, the clinical value of pretreatment is questionable.

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Ulinastatin antagonizes the neuromuscular blocking effect of d-tubocurarine in the intrinsic laryngeal muscles in rat preparations

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Introduction: It has been reported that ulinastatin (a protease inhibitor purified from human urine; UTI) delays the onset of neuromuscular block produced by non-depolarizing muscle relaxants and hastens the recovery of neuromuscular block in anaesthetized patients [1]. This study investigated the comparative effects UTI on the lateral cricoarytenoid (LCA) (one of the adductor muscles of the vocal folds) and posterior cricoarytenoid (PCA) (sole abductor muscles of the vocal cords) to non-depolarizing neuromuscular relaxant in rat preparations.

Material and methods: After approval by our Animal Use Committee, all experiments were carried out using dissected recurrent laryngeal nerve-intrinsic laryngeal muscle preparation in 11 rats. The preparations were perfused continuously with modified Krebs solution and contractions of the LCA and PCA muscles by supramaximal stimulation of the recurrent laryngeal nerves were recorded. After obtaining stable evoked EMG responses from both muscles for at least 30 min, the tissues were exposed to d-Tubocurarine (d-Tc) 6.0×10^{-7} (mol L^{-1}) with UTI 0 (control), 5000, and 20 000 units dL^{-1}. The decrease in EMG amplitude produced by dTc was expressed as a percentage of control values.

Results: EMG amplitudes of LCA and PCA were significantly decreased to 31% and 17% of control value after dTc 0.6 μmol, respectively. Although no changes in LCA amplitudes were demonstrated after application of two doses of UTI, PCA responses were significantly increased after 20 000 units dL^{-1} (30% of control).

Conclusions: This study confirms the difference in sensitivity between adductor and abductor laryngeal muscles, and unequal sensitivity to a protease inhibitor, UTI in the intrinsic laryngeal muscles was demonstrated.

Reference:

1 *Anesth Analg* 1999; **89**: 1565.

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Evaluation of an Iterative Two-Stage Bayesian technique for population pharmacokinetic analysis of rich data

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Introduction: Population pharmacokinetics has focused mainly on the analysis of sparse data from a relatively large group of subjects, i.e. only a few blood samples are taken from each individual. The analysis of rich data from a relative small group of subjects, the traditional study design in phase I/II studies, has got much less attention in literature. The Standard Two-Stage (STS) method is still regarded as a golden standard for the analysis of rich data. An Iterative Two-Stage Bayesian (ITSB) technique for the assessment of population parameters was evaluated for its performance to study designs with rich data collection, using clinical data and Monte Carlo simulations.

Methods: Data were obtained from clinical studies with rapacuronium, 3-desacetyl-rapacuronium, rocuronium and mivacurium. ITSB and STS analysis was performed using the program MW\Pharm 3.50 (written by the author, and available from MediWare BV, Groningen, The Netherlands). The applied ITSB procedure was similar to that described by Mentré and Gomeni [1] and by Bennett and Wakefield [2].

In the Monte-Carlo simulations, the study design was similar to that of the corresponding clinical study. Individual pharmacokinetic parameters were randomly drawn from a population as defined by mean and standard deviation (SD). The 'true' plasma concentration values at predefined time points were calculated using the individual pharmacokinetic parameters, and the 'measured' plasma concentrations were randomly drawn around the 'true' values, assuming a specified level of assay error. For each example, 100 data sets were generated. The generation of the Monte Carlo data and the analysis was performed using MW\Pharm 3.50. The accuracy and precision of the calculated means and SDs of model parameters was evaluated by comparing their mean error (ME) and root mean squared error (RMSE), respectively.

Results: ITSB analysis of the clinical data resulted in mean parameters close to those obtained by STS, except in cases where STS analysis could not be applied satisfactorily in each patient. Interindividual SDs obtained by ITSB were consistently smaller than those after STS.

In the Monte-Carlo studies, bias and precision of the population means and SDs were satisfactory, and were markedly better than for STS. In particular, STS overestimated interindividual SDs consistently.

Conclusion: ITSB is a valuable technique for population pharmacokinetic analysis of rich data, and is superior to STS.

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Estimation of potency of rapacuronium in elderly subjects

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Introduction: It is known that the duration of action of muscle relaxants may be prolonged in the elderly [1]. Rapacuronium is an aminosteroid muscle relaxant with a rapid onset and a short duration of action whose effect has also been shown to be prolonged in the elderly [2,3]. It is not known if this is due to a difference of relative potency of rapacuronium in this age group. The aim of this study was to estimate the potency of rapacuronium in elderly patients and to compare the results with those obtained previously in a group of adults aged 18–40 year [4].

Methods: The study was performed under a DDX from the Medicines Control Agency. Twenty-two ASA grade I–III patients aged 65 or over and undergoing surgery under general anaesthesia were included in the study. Patients with renal or liver dysfunction, on concurrent medications known to interact with muscle relaxants and those, whose body weight deviated by more than 30% of their ideal, were excluded. Anaesthesia was with fentanyl 1–2 $\mu g\,kg^{-1}$ and propofol 1–2 mg kg^{-1} followed by a propofol infusion.

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Patients: lungs were ventilated with 66% nitrous oxide in oxygen and ventilation adjusted to maintain an end-tidal carbon dioxide concentration of 35–40 mmHg. Neuromuscular block was monitored by stimulation of the ulnar nerve transcutaneously at the wrist in a single twitch mode at 0.1 Hz and recording the force of contraction of the adductor pollicis muscle using a force displacement transducer and a neuromuscular function analyser. The skin temperature over the adductor pollicis muscle was maintained above 32°C by wrapping the arm in cotton wool. Patients were randomly allocated to receive rapacuronium 0.25, 0.5, 0.75 or 1.0 mg kg^{-1} as a single bolus dose. Maximum block was allowed to supervene after each dose irrespective of the time taken to attain it. The response data were arc–sine transformed, regression analysis carried out, dose-response lines constructed and $ED_{50\ and}$ ED_{95} estimated. The dose-responses curves were subjected to analysis of covariance.

Results: The average age of the patients was 74 (6) years (mean and SD) compared to 30 (6) years in the adult subjects studied previously. The degree of block increased in a dose dependant manner in both population groups. Analysis of the dose response curves showed that they did not differ in their slopes, and the separation between them was not significantly different. The calculated ED_{50} and ED_{95} in the elderly and adult groups are shown in Table 1. Our results show that there are no differences in the potency of rapacuronium between the adults and the elderly, a finding previously reported with other relaxants [5]. Any differences in duration of action are therefore likely to be due to kinetic factors.

Table 1. ED_{50} and ED_{95} (95% confidence limits)

	Elderly	Adults*
ED_{50} (mg kg^{-1})	0.37 (0.33–0.42)	0.36 (0.30–0.42)
ED_{95} (mg kg^{-1})	0.78 (0.70–0.86)	0.79 (0.67–0.93)

*Data from Breslin *et al.* [4].

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Postoperative neuromuscular function following anaesthesia maintained by volatile inhalational agents

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Introduction: A prospective, observational study was carried out to examine the association between volatile anaesthetic agent administration and neuromuscular function in the immediate post-operative period. On each of two consecutive days, all patients in a tertiary hospital who underwent general anaesthesia for elective surgery were studied. Neuromuscular function was assessed on arrival in the recovery room electromyographically using a Datex Relaxograph (Datex NMT 221) in the uncalibrated mode.

Results: Almost half (48%: 17) of patients who received a volatile agent, but no neuromuscular blocking drug (Group 1) had TOF ratios <1.0. However, the lowest TOF values in this group were 0.91 and 0.92. A significant negative correlation ($r = -0.8$) was present between neuromuscular function and 'dose' of volatile agent administered.

In patients who received a neuromuscular blocking agent in addition to a volatile agent (Group 2), 88% (14) patients had a TOF < 10.0 and 25% (4) had a TOF < 7.0. In 9 of the 16 patients in Group 2 a neuromuscular monitor was used. No association was demonstrated between use of a peripheral nerve stimulator and the degree of residual weakness in the immediate post-operative period (Fisher exact test). The two lowest TOF ratios in this group (0.24 and 0.27) were obtained in patients in whom a nerve stimulator was used intraoperatively.

Conclusion: This study shows that even when neuromuscular blockers are not administered, postoperative weakness can occur in inverse relation to the dose of volatile agent administered. However, the residual weakness is much more apparent when a muscle relaxant is used along with a volatile agent.

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