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An Isolated, Antegrade, Perfused, Peroneal Nerve Anterior Tibialis Muscle Model in the Rat

A Novel Model Developed to Study the Factors Governing the Time Course of Action of Neuromuscular Blocking Agents

Ann De Haes, M.D., Martin C. Houwertjes, Johannes H. Proost, PharmD, Ph.D., J. Mark K. H. Wierda, M.D., Ph.D.

Background: **A model of an antegrade, perfused, isolated rat peroneal nerve anterior tibial muscle was developed to study potentially important factors governing the time course of action of (nondepolarizing) neuromuscular blocking agents such as concentration, blood flow, and temperature. The model allows observation of the effects of selective changes in these factors.**

Methods: **The authors isolated the anterior tibial muscle and cannulated the anterior tibial artery and vein, providing a way for single-pass perfusion with blood from a donor rat. A force transducer was connected to the tibialis anterior muscle and a stimulator was connected to the tibial nerve. The influence of intrinsic potency (EC_{90}) and muscle blood flow rate on the time course of pancuronium and rocuronium was investigated.**

Results: **The model remained stable for at least 4 h with respect to twitch height, muscle structure and function, and blood chemistry. Doubling the muscle-blood flow resulted in a significantly faster onset and offset for both pancuronium and rocuronium. Trebling the intrinsic potency (EC_{90}) was not associated with significant changes in the time course of action of the relaxants.**

Conclusion: **The authors developed and validated a model that allows us to study biophase kinetics of neuromuscular blocking agents in the anterior tibial muscle of the rat. In this model, muscle-blood flow rather than EC_{90} appears to predominantly determine the onset and offset time of nondepolarizing muscle relaxants.**

THE search continues for the ideal neuromuscular blocking agent (NMBA), which would have a rapid onset, short duration, rapid offset, and virtually no undesirable effects. The ideal agent will most likely be a nondepolarizing agent, to avoid the undesirable effects of depolarization.[1] For the search it is necessary to know the factors that influence the time course of NMBAs. Pharmacokinetic factors like plasma clearance, governing the rate of decay of plasma concentration, and the rate of equilibration between plasma and biophase (k_{e0}), are important.[2,3] Intrinsic potency, a measure of receptor affinity expressed as the concentration needed to achieve a specified degree of neuromuscular block (EC_{50} or EC_{90}), plays an important role in determining the rate of onset of neuromuscular blockade. The lower the (molar) intrinsic potency, the faster the neuromuscular blockade develops.[4–6] We used EC_{90} as a measure of the intrinsic potency (receptor affinity). It is difficult to investigate the influence of EC_{90} on the development of the neuromuscular block in the whole organism because of the presence of many confounding factors, including (re)distribution and elimination. We therefore developed an *in situ* isolated antegrade perfused nerve-muscle preparation in the rat to study the role of local factors governing the time course of action of NMBAs. Using a single-pass antegrade perfusion technique, many of the factors influencing the time course of action of NMBAs can be controlled, clearance is eliminated, and the influence of each of these variables on the time course of action can be studied independently, in contrast to *in vivo* experiments in animals with an intact circulation where the concentration of the perfusate and rate of perfusion cannot properly be controlled. The influence of processes in the biophase can be studied to reveal their influence on the time course of effect. With this anterior tibial muscle-peroneal nerve preparation we can control the perfusate concentration using a drug infusion pump with high precision. The influence of flow on the onset and the offset of the block can also be studied by using a second pump for blood perfusion.

We developed the Antegrade Perfused Peroneal nerve-Anterior Tibial muscle (APPAT) model, because the Peroneal-nerve Anterior Tibial set-up (PAT) is a reference model in NMBA research, and data are available from similar compounds obtained with it in the *in vivo* rat with an intact circulation. In another perfused nerve-muscle preparation, the isolated diaphragm model, only retrograde perfusion is possible because of the complex arterial supply.[7,8] This is an unphysiological approach by definition, which may hamper the interpretation of results.

In the current article, the APPAT model in the rat will be described and discussed. The results of our first series of experiments are presented, investigating the influence of EC_{90} and flow on the time course of action of two related steroidal NMBAs differing in receptor affinity and in pharmacokinetics in the intact rat.

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Address reprint requests to Dr. Wierda: Department of Anesthesiology, University Hospital Groningen, PO 30001, 9700 RB Groningen, The Netherlands. Address electronic mail to: j.m.k.h.wierda@anest.azg.nl.

Materials and Methods

Anterior Tibial Muscle Model

Animals and Preliminary Preparation. Following approval of the Ethical Committee on Animal Experiments of the University of Groningen, male Wistar rats (fasted overnight) of 400–500 g body weight were anesthetized with intraperitoneal pentobarbitone sodium (60 mg/kg). Anesthesia was maintained with small increments (1–2 mg) of pentobarbitone sodium followed by 1–2 ml saline *via* a catheter inserted into a tail vein. Nadroparine, (Fraxiparine®, Sanofi-Synthélabo, Praha, Czech Republic) a low molecular weight heparinlike substance, was given subcutaneously (1000 international units [IE]/kg) immediately after induction and before surgical preparation. Rectal temperature was measured continuously during the experiment, and maintained at 38°C by a heating blanket connected to a temperature regulator (HSE Temperature-regulator, type 313, Hugo Sachs Electronics, March-Hugstetten, Germany).

Surgical Preparation

After a skin incision made on the lateral side of the left hind leg extending from the greater trochanter over the knee to the ankle and around the lower leg, the tendon of the anterior tibial muscle was exposed and cut near its insertion. The foot was separated from the tibia, and muscles inserted along the ventral side of the tibia were cut. The vastus lateralis and the biceps femoris muscles were separated and the lateral head of the gastrocnemius muscle and the tendon of the popliteus muscle were disconnected from the femoral epicondyle. The fascia lata was cut longitudinally, close to the ventral edge of the peroneus longus muscle. The popliteal and the anterior tibial arteries and veins were exposed distal to the sural artery and vein. Branches of the popliteal artery and vein distal to the sural artery and vein were coagulated and cut. The distal end of the anterior tibial artery and vein and its branches and those of the recurrent artery and vein not supplying the anterior tibial muscle, were ligated. The lateral crural muscles were transected along the deep peroneal nerve and removed together with the extensor digitorum and hallucis longus muscles. The deep posterior crural muscles were removed, leaving the interosseous membrane intact. Two holes were drilled distally and proximally through the tibia to insert stainless steel pins. The tendons of the superficial posterior crural muscles were fixed to the distal pin and the tendon of the biceps femoris was fixed to the proximal pin together with the lateral head of the gastrocnemius. The skin was sutured between the two pins to prevent desiccation of the tissues and the popliteal artery was ligated just distal to the sural artery. Polythene tubing (0.28 mm ID, 0.61 mm OD, Portex Fine Bore Polythene Tubing, SIMS Portex Ltd., Hythe, UK) prefilled with rat donor blood was then inserted. The popliteal vein was ligated just distal to the sural vein and polythene tubing (0.62 mm ID, 0.90 mm OD) was inserted. The rat was then transferred to a thermostatically controlled plexiglas chamber. The two transtibial pins were connected by a metal bracket to a vertical steel rod mounted on a sturdy polyvinyl chloride (pvc) base. The tendon of the tibialis anterior muscle was attached *via* an opening in the chamber to a force transducer (LB8000–25N, Maywood Instruments Ltd., Basingstroke, UK) mounted on the pvc base outside the chamber. The preparation is shown in figure 1.

Donor Procedure

Male Wistar (400–500 g) rats were anesthetized with pentobarbitone sodium (60 mg/kg) intraperitoneally. Ten minutes after intravenous injection of modified gelatin (Gelofusine®, B. Braun, Melsungen, Germany) (10 ml/kg) and nadroparine (*i.e.,* 1000 IE/kg), blood was collected from the abdominal aorta. Nadroparine (*i.e.,* 100 IE) and acetyl salicylic acid (2 mg) were added to the collected blood before transfer to an ice-cooled arterial blood reservoir. The arterial blood was continuously stirred. We will refer to this mixture as "donor blood" in this article. The donor rat dies because of exsanguination. When the reservoir becomes empty, a new donor rat is used. This provides fresh donor blood at least every 2 h.

Perfusion System

Donor blood was oxygenated (Microdialysis Pump, CMA 102, Microdialysis AB, Stockholm, Sweden) as it passed the first channel of a microinfusion roller pump (Ismatec Peristaltic Pump, IPC4, Ismatec UK Ltd., Carchalton, UK) from the reservoir through raumedic tubing (0.5 mm ID, 1.0 mm OD, Rehau, Hamburg, Germany), wound onto a holder, and was enclosed in a plexiglas chamber containing 95% O_2/5% CO_2. After oxygenation, the donor blood was warmed by a heat exchanger (Harvard thermo circulator, Harvard Apparatus Ltd., Edenbridge, UK), consisting of several turns of polythene tubing (0.40 mm ID, 0.80 mm OD), wound onto a holder enclosed in a thermostatically controlled double-walled plexiglas chamber filled with water. Oxygenated, prewarmed donor blood passed through a small filter and bubble trap and was pumped *via* a second channel of the roller pump through the arterial tubing into the popliteal artery. The arterial tubing was enclosed in thermostatically controlled double-walled silicone tubing between the second channel of the microinfusion pump and the plexiglas chamber to prevent cooling of the donor blood. The anterior tibial muscle was perfused with a flow rate of 200 $\mu l \cdot min^{-1}$. Both pumps were calibrated before every experiment. The venous return *via* the popliteal vein was collected outside the chamber and was not recirculated. The pump delivers the donor blood with a pulsatile flow, the dif-

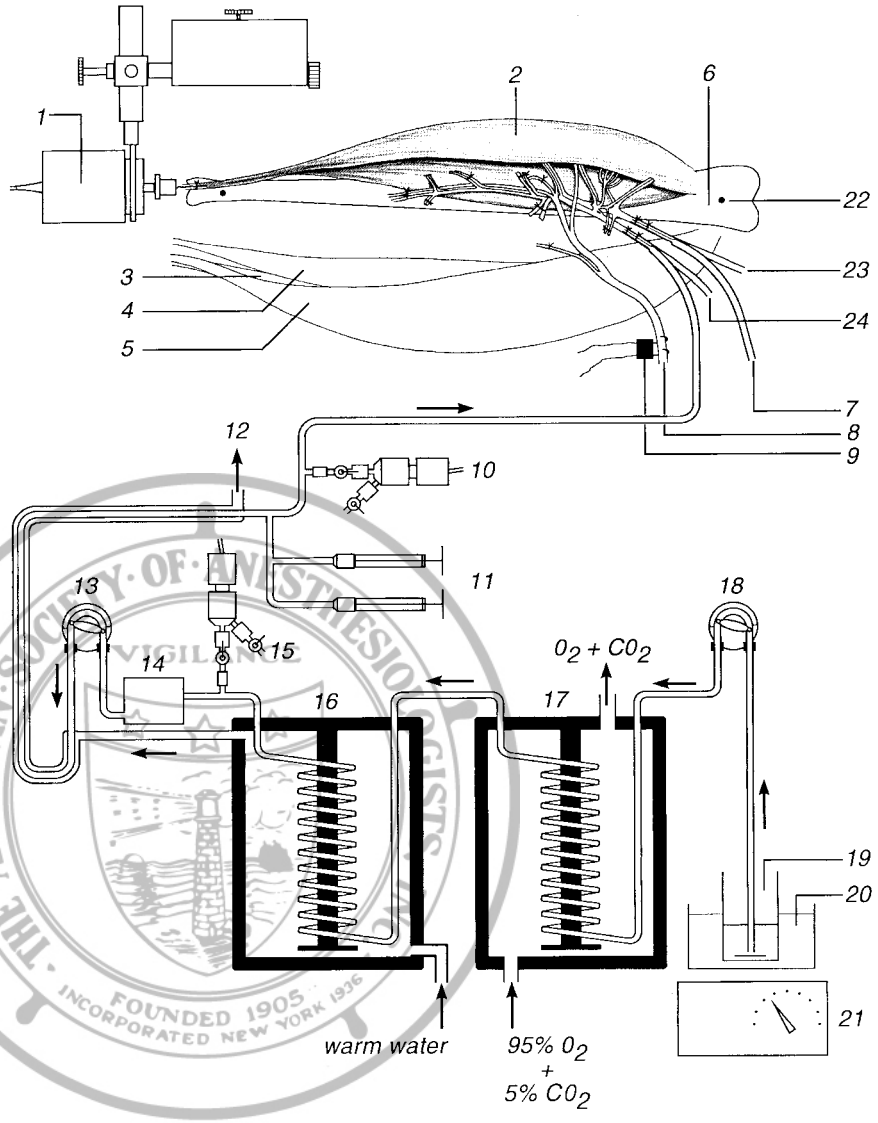

Fig. 1. This figure shows a schematic view of our APPAT model. (1) Force transducer; (2) anterior tibial muscle; (3) plantaris muscle; (4) soleus muscle; (5) gastrocnemius muscle (lateral head); (6) tibia; (7) venous outflow; (8) common peroneal nerve; (9) stimulation electrodes; (10) perfusion pressure; (11) injection of drugs; (12) water outflow; (13) peristaltic pump; (14) filter and bubble trap; (15) prepump pressure; (16) heat exchanger; (17) oxygenator; (18) peristaltic pump; (19) arterial reservoir; (20) ice; (21) magnetic stirrer; (22) stainless steel pin; (23) anterior tibial vein; (24) anterior tibial artery.

ference between systolic and diastolic pressure is approximately 12 mmHg.

Support

Prewarmed water was pumped by a thermostatic circulator through the outer layer of the heat exchanger and the silicone tubing, to warm and maintain the donor blood perfusate at 37°C. The temperature inside the plexiglas chamber was maintained at 29°C, using a thermostatically-controlled lamp. Pressure between the two channels of the microinfusion roller pump was measured *via* a side branch of the filter and bubble trap and was maintained at 10 mmHg by adjusting the pump pressure of the first channel. Perfusion pressure was measured (Pressure Transducer, HP 78342A, Hewlett Packard, Boeblingen, Germany) in one branch and muscle relaxants were infused *via* another branch of the arterial tubing. The trachea was intubated and artificial ventilation maintained with room air, delivered by an infant ventilator (MK3, Hoek Loos, Schiedam, The Netherlands) at a frequency of 90 breaths/min and a tidal volume of 20 ml·kg^{-1}. The twitch tension of the left tibialis anterior muscle elicited by supramaximal square wave stimuli of 0.2 ms duration and applied to the common peroneal nerve at 0.1 Hz (Grass S88, Grass Instruments, Quincy, MA) was measured by means of the force transducer (Harvard Isometric Transducer, Harvard Apparatus, Ltd., Edenbridge, UK) and recorded by the muscle relaxation monitor (MK2, Research Group for Experimental Anesthesiology and Clinical Pharmacology, Groningen, The Netherlands[9]). Preload was measured continuously with the force transducer and was kept constant at approximately 15g.

Validation

A series of experiments was carried out to validate the model. We tested the viability of the model without the use of NMBAs for 4 h and examined neuromuscular function by measuring the twitch height and donor blood homeostasis by analyzing blood gases, hematol-

ogy, and blood chemistry. On two occasions the entire arterial donor blood volume was collected in two successive 2-h periods from the arterial tubing for hematologic, chemical, and blood gas analysis. The perfused tibialis anterior muscles and the contralateral muscles were taken out and histologic samples were examined by the pathology department. We used the stability of the twitch height together with the stability of pressure (preload) as a variable for the perfusion quality. We chose a basal flow of 200 $\mu l \cdot g^{-1} \cdot min^{-1}$, because the muscle function and the pressure were stable at that rate, and subsequently, we could decrease and increase that rate by a factor of two without problems with perfusion pressure or preparation stability. The flow rates applied are within published reference values, which vary considerably, from 40 $\mu l \cdot g^{-1} \cdot min^{-1}$ to 1700 $\mu l \cdot g^{-1} \cdot min^{-1}$.[10–12]

Experiments

Sequence of Events. In each experiment, the same basic procedure was followed. After stabilization, either pancuronium (0.025 $\mu g/\mu l$) or rocuronium (0.1 $\mu g/\mu l$) was infused into a branch of the arterial tubing by a second infusion pump at approximately 5 $\mu l \cdot min^{-1}$. The rate of the pump was adjusted to achieve a stable neuromuscular block of 90%, stable block being defined as no changes in the neuromuscular block for at least 2 min. Having determined this, the pump was stopped, and 30 min after the twitch height returned to the control value, the infusion was started again, with a constant flow at the rate previously determined with the first infusion. In pilot experiments, we investigated intervals of 15, 30, and 60 min after the twitch height returned to normal, before we restarted an infusion with rocuronium or pancuronium. The time interval did not affect onset and offset of neuromuscular block. We also took blood samples at those times to show that rocuronium and pancuronium were already under the limit of detection. Furthermore, we used a pharmacokinetic-pharmacodynamic (PK–PD) modeling procedure to simulate the receptor occupancy by neuromuscular blocking agents and we found that 30 min after the twitch height returned to normal, receptor occupation is $\ll 1\%$. The rate of development of neuromuscular block was measured. The onset index (or rate of onset) was defined as the time elapsed from 75% twitch height to 25% twitch height. At a stable 90% neuromuscular block, the infusion of the NMBA solution was discontinued and replaced by saline (0.9% NaCl). The recovery index during the washout of the NMBA was measured; recovery index (or offset index or rate of offset) was defined as the time elapsed between 25% and 75% twitch height. The peroneal nerve was stimulated every 10 s throughout the entire experiment.

Experiments

We conducted the following two experiments: (1) Influence of blood flow on the rate of onset and offset of the neuromuscular block; (2) Influence of potency on the rate of onset and offset of the neuromuscular block.

To investigate the influence of blood flow on the time course of the neuromuscular block, we changed the blood flow supplying the anterior tibial muscle. The rate of infusion of rocuronium and pancuronium was adjusted to maintain a constant concentration in the perfusate entering the muscle in each rat. After determining the rate of infusion needed to produce a stable 90% block, the recordings were started. We started with a flow of 200 μl/min and a constant rate of NMBA infusion. At a stable 90% block, the NMBA infusion was stopped. As soon as the twitch height returned to its control value, blood flow to the muscle was increased from 200 μl/min to 400 μl/min. After 30 min, a constant rate NMBA infusion at twice the previous rate (in order to maintain the same concentration of the NMBA in the blood) was started again until a stable 90% block was obtained. The NMBA infusion was stopped again, and after the twitch height returned to normal, the flow was decreased to 200 μl/min. After 30 min, the NMBA infusion was restarted until a stable 90% block was again obtained and then again stopped. The experiment was terminated after the twitch height had returned to its control value.

Six rats were studied with rocuronium and 6 rats with pancuronium. The time course of neuromuscular block was recorded throughout the experiment: from the start of the first NMBA infusion until the twitch height returned to normal after the last NMBA infusion.

Pharmacokinetic-pharmacodynamic Modeling

Pharmacokinetic-pharmacodynamic modeling was carried out using the PkPdFit program (written by J. H. Proost, Pharm.D., Ph.D., Department of Pharmacokinetics ad Drug Delivery, University of Groningen, Groningen, the Netherlands). The effect compartment concept[13,14] was used as link model to describe the relation between perfusate concentration and effect compartment concentration and characterized by the rate constant k_{e0}. The NMBA concentrations in perfusate were calculated according to the following example. At the low flow setting, pancuronium (0.025 $\mu g/\mu l$) is infused at 5 μl/min into a perfusate flow of 200 μl/min. This makes a total perfusate flow of 205 μl/min and a pancuronium flow of 0.125 μg/min, resulting in a pancuronium concentration of 610 μg/l (0.832 μM). After replacing the NMBA infusion by saline, the concentration was assumed to drop to zero. Because of the technical setup, the actual time of arrival of NMBA in the artery lags behind the start of the infusion pump at time zero. Therefore a time lag between time zero and the rise of the perfusate concentration was assumed, and this time

Table 1. Time Course of Rocuronium and Pancuronium at Different Flow Rates

		200 μl/min (1)	400 μl/min	200 μl/min (2)	200 μl/min
Rocuronium	Onset (s)	125 (38)	104 (24)†‡	141 (9)	134 (17)§
	T_{50} (s)	248 (20)	180 (22)†‡	288 (20)	266 (17)§
	Offset (s)	60 (9)	53 (9)‡	70 (20)	63 (11)§
Pancuronium	Onset (s)	138 (30)	112 (26)‡	179 (29)*	159 (27)§
	T_{50} (s)	252 (40)	180 (38)†‡	291 (33)*	271 (35)§
	Offset (s)	52 (11)	44 (5)‡	56 (6)	54 (7)§

Mean onset and offset indices (s) and times from start of infusion to 50% neuromuscular block (T_{50}, s) of rocuronium and pancuronium at different flow rates (n = 6). The numbers in brackets = SD. The onset and offset indices depict the times from 25% to 75% and 75% to 25% neuromuscular block respectively. 200 μl/min (1), 200 μl/min (2) and 200 μl/min represent the first and second infusion and the mean values of the first and second infusion respectively.

† statistically significant ($P < 0.05$) differences between the first infusion at 200 μl/min and the infusion at 400 μl/min; ‡ statistically significant ($P < 0.05$) differences between the infusion at 400 μl/min and the second infusion at 200 μl/min; * statistically significant ($P < 0.05$) differences between the first infusion at 200 μl/min and the second infusion at 200 μl/min; § statistically significant ($P < 0.05$) differences between the infusion at 400 μl/min and the mean of the two 200 μl/min infusions.

lag was estimated during the fitting procedure. After this time lag, the perfusate concentration was assumed to rise almost instantaneously to the calculated concentration, and was maintained during a time period equal to the actual duration of the NMBA infusion, followed by an almost instantaneous drop to zero.

Pharmacodynamic modeling involved a sigmoid E_{max} model to describe the relation between the effect compartment concentration and the effect, characterized by the steepness γ and EC_{50}. For each NMBA and for each of the three consecutive experiments, the twitch height-time data of identical experiments were analyzed by PK/PD modeling using an iterative Bayesian two-stage procedure[15,16] that minimizes the sum of squared differences between measured and predicted twitch height. This method provides more precise estimates of the mean values and standard deviations (SD) of the model parameters than the traditional standard two-stage approach. The fit was evaluated by visual inspection of the measured and calculated data, by the degree of minimization of the hysteresis loop (systematic aberrances), by the residual SD, and by the Akaike Information Criterion (AIC) value.

Statistical Analysis

Groups were compared using the Wilcoxon signed ranks test for paired values (comparison of increased values of the same animal) or the Mann–Whitney U test (comparison between unrelated values); a P value of ≤ 0.05 was considered significant. Data are presented as mean values with SDs between brackets, unless stated otherwise.

Results

Validation

The APPAT model remained viable for at least 4 h with respect to twitch height in the absence of a NMBA. Chemical analysis of blood samples remained in the normal range[17–19] and did not change significantly during the experiment on two rats. Binding of rocuronium and pancuronium to the tubing was tested experimentally and was found to be virtually absent. Histologic examination after 4 h revealed no abnormalities. Specifically, there was no degeneration or regeneration, no fatty degeneration or fibrosis, nor any signs of inflammation. The nuclei were in their normal position at the border of the muscle cells.

Experiments

The average concentrations needed to reach a 90% block of the twitch height (EC_{90}) were calculated to be 5.37 (0.42) μM for rocuronium and 1.41 (0.23) μM for pancuronium. The onset and offset times are summarized in table 1, as well as the time from the start of infusion until 50% neuromuscular block was reached (T_{50}). An example of a typical registration is shown in figure 2.

The rate of onset and rate of offset as well as T_{50} were significantly faster when the blood flow was increased, for both rocuronium and pancuronium. The onset ($P = 0.1$) and offset ($P = 0.1$) indices were not significantly different when rocuronium was compared with pancuronium.

Pharmacokinetic-pharmacodynamic Modeling

The results of the PK–PD modeling procedure are summarized in table 2. There is a significant difference in the k_{e0} values when the results of the 400 μl/min experiments are compared to the 200 μl/min values, both for rocuronium and pancuronium. There is no significant

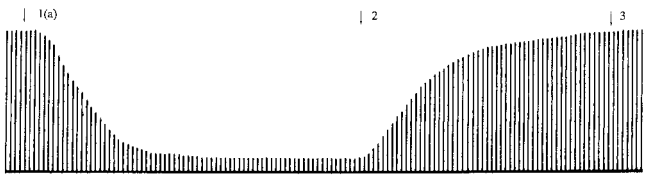

Fig. 2. This is a sample of a registration that we get after an experiment. (1) start infusion of the neuromuscular blocking agent; (2) stop infusion of the NMBA at 90% depression of the twitch height; (3) twitch height is back to original height: start of 30 min interval in which no further intervention is made.

Table 2. PK-PD fitting results of rocuronium and pancuronium at different flow rates

		200 μl/min (1)	400 μl/min	200 μl/min (2)	200 μl/min
Rocuronium	k_{e0} (min^{-1})	0.34 (0.01)	0.37 (0.06)	0.29 (0.08)	0.31 (0.06)§
	EC_{50} ($\mu M.1^{-1}$)	3.54 (0.37)	3.27 (0.22)†	3.51 (0.22)	3.52 (0.30)§
	Gamma	6.9 (0.7)	6.6 (0.4)	7.2 (0.8)	7.0 (0.7)
Pancuronium	k_{e0} (min^{-1})	0.34 (0.06)	0.43 (0.08)†‡	0.31 (0.03)	0.32 (0.05)§
	EC_{50} ($\mu M.1^{-1}$)	0.96 (0.18)	0.91 (0.13)‡	0.99 (0.14)	0.97 (0.15)§
	Gamma	7.4 (1.0)	6.8 (0.4)	7.5 (1.0)	7.4 (1.0)

Results are given as mean values (SD). 200 μl/min (1), 200 μl/min (2) and 200 μl/min represent the first and second infusion and the mean values of the first and second infusion respectively.

† statistically significant ($P < 0.05$) differences between the first infusion at 200 μl/min and the infusion at 400 μl/min; ‡ statistically significant ($P < 0.05$) differences between the infusion at 400 μl/min and the second infusion at 200 μl/min; * statistically significant ($P < 0.05$) differences between the first infusion at 200 μl/min and the second infusion at 200 μl/min; § statistically significant ($P < 0.05$) differences between the infusion at 400 μl/min and the mean of the two 200 μl/min infusions.

K_{e0} = rate of equilibration between plasma and biophase; PK–PD = pharmacokinetic-pharmacodynamic.

difference between the γ or k_{e0} of the rocuronium and pancuronium experiments, when we compare the results of the 200 μl/min experiments of pancuronium with those of rocuronium ($P = 0.22$ and $P = 0.85$ respectively). The same holds for the 400 μl/min experiments ($P = 0.2$ and $P = 0.11$ respectively) and for all results together too ($P = 0.09$ and $P = 0.6$ respectively). Figure 3 shows a typical fit of the pancuronium 400 μl/min group.

Discussion

A comparison of results obtained with pancuronium and rocuronium revealed that flow rather than intrinsic potency is related to the onset index and offset index time of pancuronium and rocuronium.

The time course of action of NMBAs is likely to be governed by more than a single factor and may depend on the type of NMBA. For the majority of NMBAs, the time course appears to be governed by their pharmacokinetics[2,3,20] and the concentration-effect relation can be satisfactorily described by the Sheiner model.[13] A rapid decrease in the concentration of a muscle relaxant in plasma (because of a high rate of elimination or a fast distribution) results in rapid equilibration, and thus in a short onset time.[2,3,20,21] Intrinsic potency (receptor affinity) may also affect the time course of action[2,22] by the phenomenon of "buffered diffusion". For a muscle relaxant with a high affinity for the acetylcholine receptor (AChR), one would expect a high degree of binding and a low free concentration in the biophase. A less potent relaxant will have a lower affinity for the AChR, while the concentration of free drug in the biophase will be higher, and equilibrium in the biophase will be reached faster, resulting in a faster onset. Consequently, the drug will be washed away more rapidly, because of the greater gradient for redistribution to plasma, which depends on the concentration of unbound drug.[2,22] How-

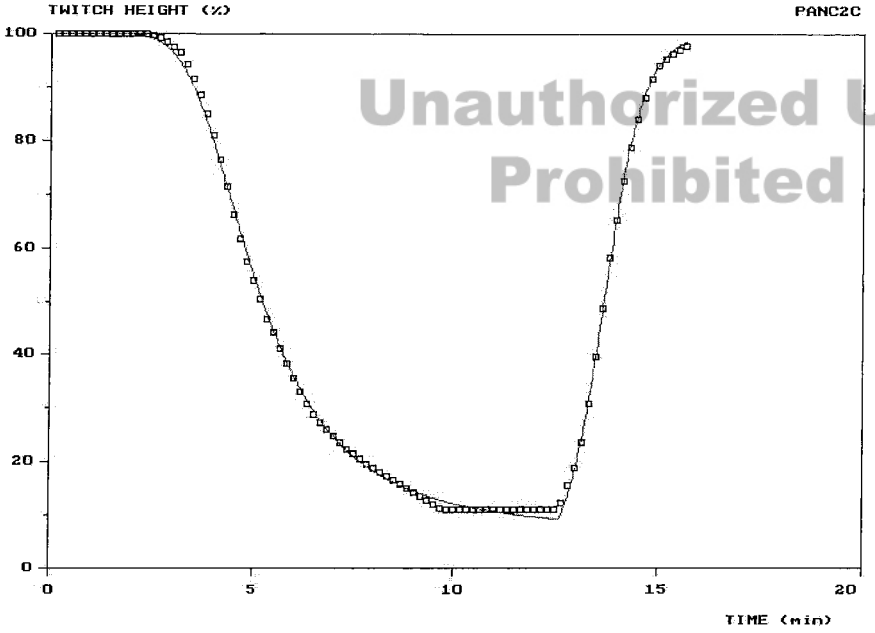

Fig. 3. This figure shows a representative fit, as was obtained after the described PK/PD fitting procedure.

ever, convincing experimental evidence for the influence of receptor affinity on time course of action is still lacking. Physicochemical properties of the NMBA, such as the degree of lipophilicity, seem to underlie both the relation between time course and plasma clearance as well as the relation between lipophilicity and affinity, and therefore seem to affect the time course at two levels, *i.e.*, pharmacodynamics and pharmacokinetics.[2,23] Protein binding may also influence the time course. Finally, the onset of action is also affected by the cardiac output, circulation time to the muscle, and muscle blood flow. All these affect the complex variable k_{e0}.[3] In the current model, we eliminated variations in the cardiac output and circulation time by controlling the blood flow to the tibialis anterior muscle.

Our experiments revealed that the rate of onset and offset of neuromuscular block of rocuronium and pancuronium were not significantly different (table 1), in spite of their different time course in intact animals and man. This indicates the predominant role of pharmacokinetics (*i.e.*, mainly renal and hepatic function) in determining their time course of action *in vivo*. The similarity of the time course of rocuronium and pancuronium in our model indicates that receptor affinity has no clinically relevant effect on the time course of action of NMBAs. At first glance, this finding contradicts earlier reports. Kopman *et al.*[4] showed that molar potency, expressed as ED_{95}, is predictive for the speed of onset of neuromuscular block, expressed as the time needed to reach 50% of the maximum effect. However, both onset time and ED_{95} are markedly affected by pharmacokinetic factors including clearance, which may explain, at least in part, the observed relation.[2,22] The relatively fast onset of gallamine and *d*-tubocurarine (according to Kopman[4] not as fast as rocuronium, however) cannot be explained by their rapid clearance. However, for many NMBAs including rocuronium and rapacuronium, the rapid decrease of the plasma concentration is likely to be a factor related to fast onset. Wright *et al.*[24] demonstrated that the rapid onset of rapacuronium was caused by its high rate constant k_{e0}, and suggested that this high value for k_{e0} was caused by its low intrinsic potency. We do not have a satisfactory explanation for the discrepancy between these findings and our results. The differences may be related to the different experimental conditions. In clinical studies, as well as in intact animals, PK–PD modeling is applied in a situation where the plasma concentration of the NMBA is changing continuously. In our experimental setup, the NMBA concentration changed momentarily from zero to the EC_{90}, and, after reaching steady-state, dropped to zero. Moreover, differences between muscle types, differences in the muscle blood flow, and species differences may play a role.

We found that blood flow (perfusion rate) has a significant effect on the onset and offset indices. The mean offset index for rocuronium in the tibialis anterior model (at a flow of 200 μl/min) was 63 s, and for pancuronium 54 s (see table 1). For reference: in the rat *in vivo*, the offset index after a two times ED_{90} dose of rocuronium was 132 s (unpublished results, Johannes H. Proost et al., Research Group for Experimental Anesthesiology and Clinical Pharmacology, University Hospital Groningen, the Netherlands, January 13, 1993). For pancuronium, the offset index after a ED_{90} dose was 156 s.[25] Goat *et al.*[26] found a significant variation in the onset time of gallamine, when the blood flow through the femoral artery in the dog was varied. There was no significant change on the offset time. Abdulatif *et al.*[27] reported that the different pharmacodynamic profiles of mivacurium in man at the adductor pollicis and orbicularis oculi muscles were not related to a difference in blood flow. Our results differ fundamentally from their studies, because we did not study the behavior of the NMBA under the same conditions. We controlled the perfusate concentration of the neuromuscular blocking agent at any given time, and our results are not confounded by kinetic variables such as clearance and distribution, except for biophase kinetics and potential elimination in the perfusate. The differences in onset index associated with variations in flow can be explained by the following mechanism: if the blood flow is high, more molecules of the neuromuscular blocking agent arrive at the neuromuscular junction in a given time-interval. As the NMBA diffuses from the capillary to the interstitial fluid, the decrease in its concentration will be more pronounced at higher perfusion rates. As a result, the concentration gradient between blood in the capillary and biophase is larger, so that more molecules will leave the perfusate to enter the biophase, and maximum block is achieved faster. The same holds for the offset index: when we stop the infusion with neuromuscular blocking agent, the concentration in the perfusate entering the muscle drops almost immediately to zero. Because of diffusion back to perfusate the concentration within the capillary decreases less rapidly. The higher the perfusion rate, the lower the concentration in the capillary, thus providing a greater concentration gradient between biophase and perfusate. This will accelerate the wash-out, thus providing a faster offset.

The viability of the nerve-muscle preparation is good, as can be concluded from donor blood gas analysis, donor blood chemistry, histology, and twitch height, all of which remained stable throughout the experiment for at least 4 h. This period excludes the time needed for the preparation (*i.e.*, 6 h).

Another isolated perfused nerve-muscle model described in literature is the isolated diaphragm preparation.[7,8] However, this model allows only venous perfusion of the muscle because of the complex diaphragmatic arterial supply. Drugs must be injected through the vein, in retrograde flow, which may alter capillary perfusion and hence may affect the results. In

the APPAT-muscle model in the rat, we maintain the physiologic flow direction. It is a single-pass model. The roller-pump supplies the donor blood with a pulsatile flow, the difference between systolic and diastolic pressure being approximately 12 mmHg. Although blood supply may still differ somewhat from normal physiologic conditions, our setup may be the best available approach so far.

In conclusion, our experiments revealed that the time course of action of rocuronium and pancuronium were similar in spite of a difference in intrinsic potency (EC_{90}) by a factor of 4. Doubling the flow resulted in a faster onset and offset of the neuromuscular block.

To study this, we developed an *in situ* antegrade perfused isolated tibialis anterior muscle preparation in the rat. Using this model, the time course of action of NMBAs can be determined under stable conditions over a period of at least 4 h.

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