Train-of-four fade and neuromuscular block in rats: a comparison between pancuronium, vecuronium, and rocuronium

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Purpose: To clarify the relationship between neuromuscular block and train-of-four fade and to investigate the causes of these drug-dependent differences, we compared the neuromuscular block and TOF fade after pancuronium, vecuronium and rocuronium.

Methods: In 24 anesthetized rats, the sciatic nerve was stimulated, and the twitch of left tibialis anterior muscle was recorded. After T_1 (first twitch response) was kept constant at 95% block by administration of pancuronium, vecuronium, or rocuronium ($n=8$, in each), the TOF fade was measured when T_1 block was decreased to 40% and 20%. In addition, using 24 phrenic nerve-diaphragm preparations, the fade was measured when the T_1 block increased to 20% and 40% by titrating of either one of the three drugs ($n=8$, in each).

Results: In *in vivo* experiments, the fade produced by pancuronium was greater than that by vecuronium or rocuronium when T_1 block was at 40% (81 ± 9 vs 63 ± 15 and $63 \pm 6\%$, respectively) and at 20% (66 ± 13 vs 34 ± 17 and $40 \pm 6\%$, respectively). In contrast, in *in vitro* experiments, the differences did not reach significant levels among the three drugs either at 20% (32 ± 19 vs 33 ± 10 and $32 \pm 17\%$) or 40% of block (62 ± 29 vs 65 ± 14 and $55 \pm 14\%$).

Conclusions: For vecuronium and rocuronium, the results were similar *in vivo* and *in vitro*. For pancuronium, fade was greater *in vivo*. These results suggest that different neuromuscular blocking agent have different relationships between the fade and the block. *In vitro* results might not be the same as *in vivo*, possibly due to pharmacokinetic differences.

Objectif : Clarifier la relation entre le blocage neuromusculaire et l'affaiblissement de la réaction aux stimulations musculaires en train-de-quatre. De plus, comparer le bloc et l'affaiblissement du TDQ obtenu avec le pancuronium, le vécuronium et le rocuronium, et rechercher les causes des différences.

Méthode : On a stimulé le nerf sciatique chez 24 rats anesthésiés et on a enregistré la contraction (twitch T) du muscle jambier antérieur gauche. Après avoir atteint un T_1 constant (réponse à la première stimulation) avec un bloc à 95 % en administrant du pancuronium, du vécuronium ou du rocuronium ($n = 8$ dans chaque cas), l'affaiblissement du TDQ a été mesurée lorsque le bloc était réduit à 40 % et à 20 %. De plus, en utilisant 24 préparations de diaphragme-nerf phrénique, l'affaiblissement a été mesuré lorsque le bloc à T_1 a été augmenté à 20 % et à 40 % en dosant l'un des trois médicaments ($n=8$ dans chaque cas).

Résultats : Dans les expériences *in vivo*, l'affaiblissement produit par le pancuronium a été plus grand que celui qui a été provoqué par le vécuronium ou le rocuronium lorsque le bloc à T_1 était à 40 % (81 ± 9 vs 63 ± 15 et $63 \pm 6\%$, respectivement) et à 20 % (66 ± 13 vs 34 ± 17 et $40 \pm 6\%$, respectivement). Par ailleurs, dans les expériences *in vitro*, les différences n'étaient pas significatives entre les trois médicaments, que ce soit un bloc à 20 % (32 ± 19 vs 33 ± 10 et $32 \pm 17\%$) ou un bloc à 40 % (62 ± 29 vs 65 ± 14 et $55 \pm 14\%$).

Conclusion : Les résultats d'expériences *in vivo* et *in vitro* ont été similaires avec le vécuronium et le rocuronium. L'affaiblissement du TDQ a été plus important avec le pancuronium lors d'expériences *in vivo*. Ces résultats suggèrent que différents myorelaxants présentent des liens différents entre l'affaiblissement du TDQ et le bloc. Les résultats différents entre les expériences *in vitro* et *in vivo* peuvent provenir de différences pharmacocinétiques.

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TRAIN-OF-FOUR(TOF) fade is used clinically as an index of the degree of neuromuscular block produced by administration of a non-depolarizing neuromuscular blocking agent (NBA). Although it is generally accepted that the relationship between the TOF fade and neuromuscular block is constant,^{1,2} previous clinical reports have shown differences in the magnitude of the TOF fade during the recovery phase for different NBA at the same level of neuromuscular block.³⁻⁶ While non-depolarizing NBAs act mainly at the postjunctional site, some investigators have suggested that they also have a prejunctional effect that is responsible for the TOF fade.⁷⁻¹¹ Consequently, drug-dependent differences in TOF fade are believed to be a function of their prejunctional potency.^{5,10,12-14} However, Storella *et al.*¹⁵ suggested that the differences observed clinically may be due to pharmacokinetic differences among drugs rather than any pharmacodynamic difference in potency at the site of action.

The present study was undertaken to determine whether drug-dependent differences exist between the TOF fade and neuromuscular block, and if drug-dependent differences do exist, to identify differences in the prejunctional potency of NBAs at the neuromuscular junction. *In vivo* and *in vitro*, neuromuscular preparations from rats were used to create a clinical and a steady-state model, respectively. We compared the TOF fade produced by three drugs, pancuronium, vecuronium, and rocuronium, in these two different models.

Materials and methods

Male Sprague-Dawley rats weighing between 300 and 450 g were used in all experiments. The experimental protocol was approved by our Institutional Animal Care and Use Committee.

IN VIVO EXPERIMENT

Twenty-four rats were anesthetized with 30 mg·kg⁻¹ pentobarbitone and 500 mg·kg⁻¹ urethane, *ip*, if necessary, followed by 15 mg·kg⁻¹ and 250 mg·kg⁻¹, respectively. Tracheostomy was performed, and the lungs of each rat were ventilated with O₂ 100% adjusted to maintain the arterial CO₂ tension at about 40 mmHg. The left external jugular vein and the internal carotid artery were cannulated for administration of the NBAs and to record the arterial blood pressure, respectively. Rectal temperature was maintained between 36 and 37°C with an incubator. The sciatic nerve was dissected from the gluteal space and ligated, and bipolar platinum electrodes were attached to the peripheral portion of the nerve. The distal tendon of the left tibialis anterior muscle was isolated from the

surrounding tissue and connected to a force transducer (TB-611, Nihon Kohden, Tokyo, Japan) at a resting tension of 30 g. TOF stimulation (SEN-7103 stimulator, Nihon Kohden), consisting of four supramaximal pulses, was applied to the sciatic nerve by the bipolar platinum electrodes every 20 sec. The duration and the frequency of the pulses were 0.2 msec and 2 Hz, respectively. Neuromuscular block was monitored by measuring the T₁ (first twitch response in TOF stimulation) and TOF fade, which were calculated, as follows:

$$\text{T1 block (\%)} = 100 \times (C - T_1) / C$$

$$\text{Fade (\%)} = 100 \times (T_1 - T_4) / T_1$$

Where C is the control tension before the NBA is added, and T₄ is the tension produced by the last twitch response in the TOF stimulation.

Rats were assigned to one of three groups according to NBAs: pancuronium, vecuronium, or rocuronium (n = 8, in each). Following stabilization of responses and determination of the control tension, a continuous infusion of a NBA was started. The infusion rate was adjusted to maintain the T₁ block at about 95%. The infusion rate was kept constant at this T₁ block for at least 30 min, after which the infusion was discontinued. During recovery from neuromuscular block, the TOF fade was determined when the T₁ block reached the point nearest 40% and 20%. In addition, the recovery index (time required for the T₁ block to recover from 75% to 25%) was recorded.

IN VITRO EXPERIMENT

Twelve rats were anesthetized with halothane and sacrificed. The right and left hemidiaphragms with accompanying phrenic nerves were dissected out to yield 24 phrenic nerve-hemidiaphragm preparations. Each preparation was placed vertically in an individual organ bath filled with 100 ml modified Krebs' solution¹⁶ with the following composition: NaCl 113 mM, KCl 4.7 mM, CaCl₂ 1.4 mM, NaHCO₃ 25 mM, MgSO₄ 0.9 mM, KH₂PO₄ 1.2 mM, and glucose 11.5 mM. The solution in the bath was aerated with 95% O₂/5% CO₂, and its temperature and pH were maintained at 37°C and between 7.38 and 7.42, respectively. The tendon of the hemidiaphragm was connected to a force transducer to record muscle contraction, and the muscle was stretched to a resting tension of 10 g. TOF stimulation using the same pattern and interval as described in the *in vivo* experiment was applied to the phrenic nerve.

As in the *in vivo* experiment, rats were assigned to one of three groups: pancuronium, vecuronium, or

rocuronium groups ($n = 8$, in each). Each group included equal numbers of left and right hemidiaphragms. After measurement of baseline tension, the concentration of the NBA in the bath was increased gradually until T_1 block reached about 20%. Initial doses of pancuronium, vecuronium, and rocuronium were 1.5, 3.0, and 8.0 μM , respectively. The initial doses were determined with other preparations in a similar experimental model in a preliminary investigations. The doses were adjusted by adding the agent or modified Krebs' solution. At least 30 min was allowed to establish a pseudo-steady-state condition for the drug concentration between the bath and the preparation, and the TOF fade was measured. Then the drug concentration was increased until the T_1 block reached about 40%, and after 30 min, the TOF fade was measured again. The solution in the bath was replaced with 100 ml of modified Krebs' solution without NBAs, and recovery from neuromuscular block was monitored.

Statistical analysis

Results are expressed as the mean $\pm$ SD. Statistical analysis was performed by one-way analysis of variance with Scheffé's multiple comparison. Differences were considered significant at $P < .05$.

Results

IN VIVO EXPERIMENT

The actual T_1 block during the drug infusion were almost 95% in three groups (Table I). The actual T_1 block at the time the TOF fade was measured for the 40% and 20% data points were considered acceptably close to 40% and 20% block to permit comparison among groups. At both points, the TOF fade in the pancuronium group was greater than in the vecuronium or rocuronium groups. These differences between drugs (pancuronium *vs* vecuronium and rocuronium) were observed not only at 20 and 40% block (Figure). The difference did not reach to significant level in the TOF fade between the vecuronium and rocuronium groups at either 40% or 20% of T_1 block ($P = .95$). The recovery index of the pancuronium group was longer than those of the other two groups. The difference did not reach to significant level in the recovery indices between the vecuronium and rocuronium groups ($P = .65$). All subjects recovered over 90% of initial control twitch.

IN VITRO EXPERIMENT

The actual T_1 block at the time the TOF fade was measured for the 20% and 40% data points were considered acceptably close to 20% or 40% to permit comparisons between groups. At either degree of T_1 block,

FIGURE Recovery of T_1 *vs* TOF fade. A: Pancuronium; B: Vecuronium; C: Rocuronium.

the differences did not reach significant levels in the TOF fade between groups ($P = .98, .60$, respectively, Table II). Thus, for vecuronium and rocuronium, the

TABLE I Data *in vivo* experiment

	<i>Pancuronium</i>	<i>Vecuronium</i>	<i>Rocuronium</i>
Infusion rate ($\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	6.4 ± 3.0	$64 \pm 22^*$	$140 \pm 45^{*\dagger}$
Actual T1 block (%) during drug infusion	95.8 ± 1.7	95.6 ± 2.2	96.7 ± 0.6
Actual T1 block (%)			
20% data points	20.2 ± 0.9	19.4 ± 1.4	20.6 ± 0.9
40% data points	40.0 ± 0.4	39.9 ± 2.9	39.7 ± 2.2
TOF fade (%)			
20% block	66.3 ± 13.4	$33.8 \pm 16.7^*$	$39.9 \pm 9.6^*$
40% block	81.1 ± 9.4	$63.1 \pm 15.0^*$	$62.8 \pm 6.3^*$
Recovery index (sec)	520 ± 270	$85 \pm 33^*$	$160 \pm 32^*$

Values are mean $\pm$ SD; n = 8 for each group.

* $P < .01$ vs pancuronium.

$\dagger P < .01$ vs vecuronium.

TABLE II Data *in vitro* experiment

	<i>Pancuronium</i>	<i>Vecuronium</i>	<i>Rocuronium</i>
Concentration (μM)			
20% block	1.6 ± 0.4	$3.0 \pm 0.4^*$	$7.4 \pm 1.5^{*\dagger}$
40% block	1.8 ± 0.5	$3.6 \pm 0.5^*$	$8.8 \pm 1.4^{*\dagger}$
Actual T1 block (%)			
20% data points	20.4 ± 1.1	20.2 ± 0.7	20.7 ± 2.2
40% data points	39.9 ± 1.1	39.8 ± 0.9	41.4 ± 2.4
TOF fade (%)			
20% block	32.0 ± 18.5	32.9 ± 9.5	31.7 ± 16.8
40% block	62.1 ± 28.9	64.5 ± 13.9	54.6 ± 14.3

Values are mean $\pm$ SD; n = 8 for each group.

* $P < .01$ vs pancuronium.

$\dagger P < .01$ vs vecuronium.

results were similar *in vivo* and *in vitro*. In contrast, for pancuronium, fade was greater *in vivo*. The T1 recovered to over 90% of initial control twitch in all preparations following replacement of the organ bath with modified Krebs' solution without NBA.

Discussion

Train-of-four fade is not necessarily different *in vivo* and *in vitro*, but the differences between drugs *in vivo* might not be found *in vitro*. In previous studies in humans, it was noted that TOF fade depends not only on the drug but also on the size of a single bolus dose.^{3,12} To exclude this dose-dependent effect, we collected data *in vivo* during recovery from a uniform degree of neuromuscular block. Given that the T₁ block was stable for at least 30 min prior to data collection, a pseudo-steady-state for drug distribution between plasma and muscle is assumed to have been

reached. Although the *in vitro* experiment showed no drug-dependent differences in TOF fade at a given degree of neuromuscular block, TOF fade was greater with pancuronium than with vecuronium or rocuronium *in vivo*.

It has been suggested that acetylcholine (ACh) released at the neuromuscular junction stimulates prejunctional receptors and facilitates the mobilization of ACh from reserve stores to the immediately available store. This is why the output of ACh can keep up with the demands of repetitive stimulation.¹⁰ A block by non-depolarizing NBAs would lead to a progressive decrease in the amount of ACh released per stimulus during TOF stimulation, accounting for the TOF fade phenomenon.^{8,17,18} Based on this mechanism, differences among drugs in the production of the TOF fade can be accounted for by different affinities for prejunctional receptors, *i.e.*, the greater the affinity, the greater the TOF fade.^{5,10,12–14} If drug-dependent differences in TOF fade are determined pharmacodynamically, as with affinity for prejunctional receptors, the experiments studying this relationship should be performed under pseudo-steady-state conditions, such as in an organ bath. An organ bath allows the drug concentration to equilibrate between the bath and the neuromuscular junction and between the prejunctional and postjunctional sites, and fails to account for differences in the rate of drug diffusion and elimination. The lack of differences in TOF fade *in vitro* between pancuronium, vecuronium, or rocuronium suggests that there are no differences in the affinity for prejunctional receptors between these three NBAs.

Two *in vivo* studies have examined the differences in the TOF fade between pancuronium and vecuronium,^{4,19} and both investigations concluded that pancuronium caused a greater TOF fade than vecuronium during recovery from neuromuscular block. Additionally, Shiraishi *et al.*¹⁹ demonstrated that the TOF fade produced by rocuronium is less than that produced by pancuronium. These results support our findings.

Few studies have addressed TOF fade produced by NBAs under steady-state conditions.^{15,20} When TOF fade at a given degree of neuromuscular block is measured at steady-state, its value is determined by the relative difference between the pre and postjunctional effects. It is possible that the TOF fade at steady-state is different for different NBAs *in vivo*, since the rate of drug distribution and elimination may influence the time-course of T₄ relative to T₁, and the pharmacokinetic differences among the three NBAs may cause the differences in TOF fade. It has been reported that plasma concentrations of vecuronium and rocuronium decrease more rapidly than the concentration of pan-

curonium^{21,22} which is supported by our experiments. Because the infusion rate necessary to maintain a 95% block *in vivo* was approximately 10 and 25 times higher with vecuronium and rocuronium than with pancuronium, although the potencies of vecuronium and rocuronium are one half and one-fifth of pancuronium's *in vitro*.

In humans, the block/fade relationship following a single bolus of a NBA differs depending on the phase of neuromuscular block, *i.e.*, all NBAs used clinically show less TOF fade during onset than during recovery.^{3,10,12,23} Bartkowski *et al.*²⁴ have explained this difference based on pharmacokinetic differences between muscle compartments. Generally a muscle is composed of several compartments with regard to the rate of drug distribution and elimination.^{15,25} These authors concluded that a rapid change (not only an increase but also a decrease) in the plasma NBA concentration diminish the TOF fade. We believe that the rapid changes in the plasma concentrations associated with vecuronium and rocuronium distribution affected their TOF fade. Consequently, even though the prejunctional effect of the NBAs used in this study was the same, as shown by the similarity of the TOF fade at pseudo-steady-state *in vitro*, the effect of these agents *in vivo* is different because the pharmacokinetics affect the degree of prejunctional block. This proposed mechanism of action may account for the difference in TOF fade observed during *in vivo* and *in vitro* conditions, but fade *in vitro* would be expected to be closer in the case of drugs whose concentration change little *in vivo*, compared with drugs which undergo big changes in concentration. In fact, opposite happens, it has limitation.

We used the sciatic nerve-tibialis anterior muscles *in vivo* and the phrenic nerve-diaphragms *in vitro* experiments. We used different muscles because it is difficult to measure twitch tension of diaphragm *in vivo* (the twitch is influenced by the movement of thorax) and because it is impossible for oxygen to diffuse in the tibialis anterior muscle in an organ bath as the muscle is not thin. The possibility that the difference in the block/fade relationship depends on differences between the limb and respiratory muscle can not be denied. Also, we realize that this study has been restricted to three steroidal NBAs of similar chemical structure. Thus, it is desirable that this work is extended to include non-steroidal NBAs of different chemical structure and metabolism, and with a short and long duration of action.

In conclusion, the prejunctional effects of pancuronium, vecuronium, and rocuronium are similar because the block/fade relationships are the same under pseudo-steady-state conditions. In contrast,

under non-steady-state conditions, such as during recovery, the intrinsic TOF fade for each individual NBA was affected by a factor, possibly the rate of clearance in local drug concentrations, which exists *in vivo*, but not *in vitro*. We postulate that, within a series of structurally related compounds, longer recovery rates are associated with greater TOF fade. Non-depolarizing NBAs for shorter recovery rates are required to have less TOF fade to confirm the recovery.

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