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Fading responses in the evoked EMG after rocuronium in cats

This study was performed to evaluate the inhibitory effect on motor nerve terminals by rocuronium using recovery curves of muscle compound action potentials (CAPs) and train-of-four ratios (TOFRs) in anaesthetized cats, and to compare the results with other relaxants reported previously. Recovery curves were derived from the amplitude of the CAP induced in the gastrocnemius muscle by the second of a paired stimulus (test response) to the sciatic nerve and compared with results evoked by the first component (conditioning response). The interval between the paired stimuli was increased stepwise from 7 to 1,000 msec, and the differences in amplitude of the test and conditioning responses were plotted on a graph by relating the changes in paired intervals. The recovery curve after rocuronium was less inhibited than after pancuronium, ($100.4 \pm 5.9\%$, $82.3 \pm 6.7\%$ and $68.5 \pm 6.7\%$ at 60, 100 and 500 msec intervals, compared with $70.3 \pm 3.3\%$, $59.0 \pm 4.7\%$ and $46.7 \pm 4.3\%$ after pancuronium ($P < 0.05$). The recovery curves with d-tubocurarine were more depressed than with pancuronium; however, the RC with vecuronium was similar to that of rocuronium. The degree of fade in TOF by rocuronium was also less than those seen with d-tubocurarine and pancuronium. The results obtained suggest that rocuronium has less inhibitory effect on motor nerve terminals than do d-tubocurarine and pancuronium, and has a similar effect to that of vecuronium.

Cette étude évalue l'effet inhibiteur du rocuronium sur les terminaisons nerveuses motrices en utilisant des courbes de récupération de potentiels d'action composés (CAP) et de

rapports de train-de-quatre (TOF) chez des chats anesthésiés et compare ses résultats avec ceux d'autres relaxants déjà connus. Les courbes de récupération sont dérivées de l'amplitude de CAP provoqués dans le muscle gastrocnémien par le deuxième d'une paire de stimuli (test de réactivité) appliqués au nerf sciatique et comparé avec le résultat obtenu après la première excitation (réponse de conditionnement). L'intervalle entre le couple de stimuli a été augmenté par paliers de 7 à 1000 msec et la différence d'amplitude du test de réactivité et de conditionnement est portée sur un graphique en relation avec les changements enregistrés par couple d'intervalles. La courbe de récupération du rocuronium est moins inhibée que celle du pancuronium ($100,4 \pm 5,9\%$, $82,3 \pm 6,7$ et $68,5 \pm 6,7\%$ à des intervalles de 60, 100, et 500 msec comparativement à $70,3 \pm 3,3\%$, $59,0 \pm 4,7\%$ et $46,7 \pm 4,3\%$ après pancuronium : $P < 0,05$). Les courbes de récupération sont encore plus abaissées avec la d-tubocurarine qu'avec le pancuronium, alors que celles du vecuronium sont identiques à celles du rocuronium. Le degré d'affaiblissement de la réponse du TOF par le rocuronium est aussi moindre que celui observé avec la d-tubocurarine et le pancuronium. Ces résultats suggèrent que le rocuronium a un effet inhibiteur moindre sur les terminaisons du neurone moteur que la d-tubocurarine et le pancuronium et un effet identique à celui du vecuronium.

Key words

MEASUREMENT TECHNIQUES: electromyography;
NEUROMUSCULAR RELAXANTS: d-tubocurarine,
pancuronium, rocuronium, vecuronium;
NEUROMUSCULAR TRANSMISSION.

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Non-depolarizing or competitive neuromuscular block is characterized by a waning or fading response of the affected muscle to indirect repetitive stimulation. The second response of paired repeated stimuli show different responses such as potentiation or wane depending on the interval between the two stimuli.¹⁻³ Paired stimuli have been applied to peripheral nerves in humans and in cats, and the amplitude of the compound action potentials (CAPs) evoked in the muscle by the second member of the stimulus pair (test response) has been compared with those evoked by the first (conditioning response). If the interval between the two components (the pair interval) is increased stepwise from 7 to 1,000 msec, the differences in amplitude of the test and conditioning responses resulting from the changes in pair intervals produces a recovery curve (RC).¹¹ It has been demonstrated that the recovery curve during partial block with nondepolarizing relaxants shows a characteristic pattern of slight potentiation at very

short intervals of less than 20 msec of stimuli, followed by considerable depression at longer intervals. The degree of the potentiation and depression differed with the different neuromuscular blocking drugs under similar levels of block.¹⁻³

The present study was undertaken to compare the RC during partial paralysis by rocuronium, a new steroidal relaxant, with results using other neuromuscular blockers in anaesthetized cats.

Methods

The study was approved by the Ethical Committee for Animal Investigation at Nihon University School of Medicine, and Surugadai Nihon University Hospital.

Materials and preparation

Ten cats of both sexes were anaesthetized with 15 to 25 mg · kg⁻¹ ketamine *im* followed by intermittent administration of 5 to 10 mg · kg⁻¹ of pentobarbital sodium *iv*. The lungs were ventilated with air using an animal respirator keeping the end-tidal carbon dioxide concentrations at 3.5% with an expired gas monitor. Cardiovascular variables were monitored by ECG and direct measurement of BP from the carotid artery and recorded (Recti-Horiz-8K21-L6®). The oesophageal temperature was maintained at 38° C with a heating mattress. The cat's lumbar spinal processes and unilateral calcaneus were immobilized. The ipsilateral sciatic nerve was severed, and its peripheral part was isolated from the surrounding tissue and attached to two stimulating electrodes. Another two electrodes to measure CAPs were fixed on the fascia covering the gastrocnemius muscle. These electrodes and exposed tissues were maintained in a paraffin pool at 38° C.

A rectangular pulse using supramaximal strength and a duration of 0.2 msec was applied every ten seconds (0.1 Hz). Compound action potentials were induced and recorded using a module type EMG instrument (Medelec MS6®) with the time constant during 0.16 to 16 kHz. The amplitude of CAPs prior to administration of rocuronium (control) showed 13.4 ± 1.4 mV (mean $\pm$ SD), and the CAPs during partial block were amplified to nearly control levels.

Stimulation patterns

Single shock stimulation at 0.1 Hz was applied serially during the experiments, and the amplitude of CAP to the stimulation before rocuronium was used as the control. Paired pulses at each interval followed by train-of-four (TOF) pulses were applied with a pause of ten seconds after the last stimulation in the train to obtain control patterns of RC and TOFR. After the control values were obtained, neuromuscular block by rocuronium was verified

by a single pulse at 0.1 Hz. When the amplitude of CAP by single stimuli recovered from complete block to 10%, 25%, 75% and 100%, the same stimulation patterns to obtain RC and TOFR were performed.

Variables

AMPLITUDE OF CAPS

The amplitudes of the CAPs from single shock stimulation at 0.1 Hz were compared before and after administration of the test agent and were expressed as percentage of control.

Recovery curve (RC) of CAPs

The original method of obtaining RC has been reported previously.¹ A pair of pulses was applied to the sciatic nerve with a ten-second pause. The first stimulus of the pair was termed the "conditioning stimulus" and the second the "test stimulus." The ratio of the amplitude of the muscle CAP (M2) evoked by the test stimulus to that (M1) of the conditioning stimulus was obtained. The interval between paired stimuli ranged from 7 to 1,000 msec. Recordings of the paired responses were made every millisecond and between 7 and 10 msec, every 20 msec between 10 and 100 msec, and every 500 msec between 100 and 1,000 msec, respectively. When each M2/M1 ratio was plotted against the interval between the paired stimuli, a curve, i.e., a recovery curve (RC), for the muscle CAP could be obtained. The recording at each paired interval was accomplished with one measurement, because it had been shown previously that a constant ratio was obtained by preliminary measurements repeated in the same paired interval.¹⁻³ We then measured the responses to a series of paired stimuli to obtain a RC of approximately 120 sec.

Train-of-four ratio (TOFR) of CAPs

We then applied a train-of-four stimulation at 2 Hz to obtain the ratio (TOFR) of the amplitude of the fourth response (M4) to the amplitude of the first response (M1) expressed as a percentage.

Administration of rocuronium

After control variables had been obtained, 0.35 mg · kg⁻¹ of rocuronium was administered *iv*. If complete neuromuscular block was not obtained, cumulative doses from 0.15 to 0.35 mg · kg⁻¹ were applied until the twitch response was abolished. Twitch responses from single stimuli with a frequency of 0.1 Hz were obtained during the experiments to estimate the level of neuromuscular blockade. Recovery curves and TOFRs of CAPs were recorded as described above.

FIGURE 1 An example of the serial tracing of single and paired CAPs at various stimulation intervals. The left series are controls in which a CAP from a single pulse stimulation is demonstrated by action potentials from several muscle fibres. It has a first positive peak followed by a large negative peak, and followed by two small positive potentials. The largest positive to negative peak is estimated as the amplitude of the CAP. The second CAPs (test responses) in each paired response show slightly larger amplitudes than those seen with the first CAP (conditioning response) in 100 msec and shorter intervals of stimulation. The right series are CAPs which were depressed to almost 10% of the control amplitudes by rocuronium. The first positive peak in the Figure of CAP of control is split into two small positive peaks, but the second positive reflection is estimated as the measurable positive peak.

Evaluation of results

The results at each time during recovery period from neuromuscular block were compared with control values. Comparisons were also made with results obtained from similar previous studies with other neuromuscular blockers.³ All data were expressed as mean $\pm$ SEM.

FIGURE 2 Recovery curves of muscle CAPs at control and at three levels of neuromuscular block by rocuronium.

Comparisons within experimental protocols were made using a repeated measurement analysis of variance (ANOVA). Multiple comparisons between individual means were performed using Newmann-Keuls method. Differences with $P < 0.05$ were considered significant.

Results

Recovery curves of CAPs

An example of single and paired CAPs at various stimulation intervals is given in Figure 1. It shows a control series (left) and a series obtained during partial block with rocuronium (right) in a cat. The amplitudes of M2 in the control were very slightly potentiated compared to the amplitude of M1 at intervals shorter than 20 msec, and they were almost equal to that of M1 at longer intervals. When the amplitude of the single CAP was inhibited to almost 10% of control levels as shown in the right series of Figure 1, slight potentiation of the amplitudes of M2 compared to those of M1 was found at intervals shorter than 60 msec, and subsequent depression of the amplitudes of M2 compared to those of M1 were observed at longer intervals of paired stimuli.

Similar changes in the amplitudes of M2 in response to a range of intervals of paired stimuli were noted in each cat. Data for recovery curves from the mean ratios of M2/M1 at various time intervals of paired stimuli are presented in Figure 2, showing three RCs at different

FIGURE 3 Recovery curve obtained from the mean ratios ($\pm$ SEM) of M2/M1 when the amplitude of the CAP was inhibited to nearly 10% of control levels by rocuronium is compared with RCs obtained at similar levels of inhibition by the other relaxants evaluated (Reprinted by permission from Katsumata N *et al.*)³

levels of neuromuscular block with rocuronium demonstrating similar patterns and equal levels of changes in every RC.

In Figure 3, a RC obtained from the mean ratios of M2/M1 when the amplitude of the CAP was inhibited to almost 10% of control by rocuronium is presented in comparison with RCs which were obtained during similar levels of inhibition by d-tubocurarine, pancuronium, vecuronium, and α -bungarotoxin.³

The pattern of the RCs resembled that for other non-depolarizing relaxants. Higher levels of RC were found after rocuronium at 20 msec and at longer intervals of the paired stimuli than with those of other non-depolarizing relaxants. For example, the M2/M1 ratios of $100.4 \pm 5.9\%$, $82.3 \pm 6.7\%$ and $68.5 \pm 6.7\%$ at 60, 100 and 500 msec intervals after rocuronium differed from $70.3 \pm 3.3\%$, $59.0 \pm 4.7\%$ and $46.7 \pm 4.3\%$ at the equivalent intervals after pancuronium ($P < 0.05$). The recovery curves with d-tubocurarine were more depressed than with pancuronium and the RC with vecuronium was similar to that of rocuronium. The pattern of RC with rocuronium was different from that of α -bungarotoxin (α -BuTx).

Train-of-four ratios

Mean train-of-four ratios at several inhibited amplitudes of CAPs by several neuromuscular blocking agents³ are

FIGURE 4 Mean train-of-four ratios at four levels of neuromuscular block by rocuronium compared with those by other relaxants (Reprinted by permission from Katsumata N *et al.*)³
*Different from pancuronium and d-tubocurarine ($P < 0.01$).

shown in Figure 4. The degree of fade in the train-of-four (TOF) with rocuronium was less than with d-tubocurarine and pancuronium ($P < 0.01$) at similar levels of block. The fade was not significantly different from that for vecuronium. There was no fade produced by α -BuTx.

Discussion

The present experiments confirmed that rocuronium blocked neuromuscular transmission with a non-depolarizing pattern¹⁻³ in the RC of CAPs induced from the gastrocnemius muscle in cats. However, the depressed RCs during partial block by rocuronium were less than those produced by d-tubocurarine and pancuronium, and were similar to those of vecuronium. The degree of fade in TOF with rocuronium co-responded with the depression of the RCs in a similar fashion to, but less than with, d-tubocurarine and pancuronium. This suggests that rocuronium may have less inhibitory effect on the motor nerve terminals than the other relaxants evaluated.

Using rat diaphragm-phrenic nerve preparations treated with d-tubocurarine or magnesium, Hubbard and Schmidt⁴ and Hubbard⁵ studied the fluctuation of endplate potential (epp) amplitudes by the second impulse (testing volley) and miniature endplate potential (mepp) frequency after a stimulus (conditioning volley) of paired stimuli at various intervals. They concluded that, when measured as epps and mepp for a 100–200 msec period, a single nerve

impulse increased acetylcholine (ACh) release which was followed by a decrease. Wilson^{6,7} demonstrated that this increased release resulted from mobilization of ACh with the residual calcium ion which had been induced by the depolarization process at the nerve terminal by the conditioning impulse, and that the mobilization decreased with time.

According to Waud and Waud^{8,9} and our previous report¹ of partial curariform block, the threshold fibres which have a reduction in available receptors is reflected by the amount of released transmitter. Thus the size of the summated muscle action potential in the partially curarized state is related to the total number of muscle fibres that have reached a potential threshold in the whole muscle.

When paired stimuli are applied to nerves innervating partially curarized muscle in humans and animals, it is assumed that the amplitude of the compound muscle action potential (CAP) by the second stimulus (test response) is influenced by the relative changes of the amount of released transmitter in response to the first stimulus (conditioning response) and is dependent on the interval between the paired stimuli.

Suzuki *et al.*¹ found in anaesthetized patients that the RC of muscle CAPs following gallamine and d-tubocurarine showed an identical time course of mobilization process of transmitter from nerve terminals after the conditioning stimulus in studies with rats by Hubbard *et al.*^{4,5} A similar pattern in the RCs of CAPs had been demonstrated by Katsumata *et al.*³ during partial block with conventional non-depolarizing relaxants in cats. In contrast, the pattern of RCs produced by lignocaine was found to be characterized by marked and serial depression of response to test stimuli at all intervals between paired stimuli,² and the pattern of RCs produced by α -BuTX was characterized by profound potentiation of the responses to test stimuli at shorter intervals between paired stimuli, followed by a gradual recovery.³ They suggested that the RC produced by α -BuTX may demonstrate the physiological time course of the mobilization process of transmitter after a conditioning stimulus, because it is believed that α -BuTX affects only acetylcholine receptors at the endplate, and does not affect prejunctional receptors.¹⁰⁻¹² Further, they³ demonstrated that non-depolarizing relaxants showed marked differences in depression at intervals longer than 40 msec during profound neuromuscular blockade. The depression was most prominent with d-tubocurarine, followed by pancuronium and vecuronium. Also, fade of TOF followed a similar difference with each neuro-muscular relaxant.³ The authors concluded that depression of RCs and fade of TOF by non-depolarizing relaxants may be caused by an inhibitory effect at the motor nerve terminals as many investigators have reported.¹³⁻¹⁷

The results of this study demonstrate that rocuronium has an inhibitory effect on motor nerve terminals, although the effect is less than that produced by d-tubocurarine and pancuronium.

In studies on differences of fade in TOF among non-depolarizing relaxants in clinical cases, several investigators¹⁸⁻²⁰ have found that d-tubocurarine caused a more pronounced fade than did pancuronium or vecuronium. Gibson *et al.*²⁰ noted that pancuronium caused a greater fade than vecuronium which is similar to our results, although Storella *et al.*²¹ reported that such differences among relaxants could not be observed when they were examined at steady-state block in isolated phrenic nerve-diaphragm preparations of the mouse.

Based on clinical studies involving mechanical twitch responses of the thenar muscle to rocuronium, identical levels of TOF fade with vecuronium were found by Mark *et al.*²² and Foldes *et al.*²³ Our results did not demonstrate any difference in TOF fade or RC depression between the two relaxants.

It has not yet been determined whether these effects on the RCs of CAPs and levels of fade in TOF influence the clinical mode of action of the relaxants or not. However, it should be borne in mind that the fade in TOF as a monitor of neuromuscular blockade may be expressed more weakly by rocuronium and vecuronium than by d-tubocurarine and pancuronium at similar levels of blockade.

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