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Comparative fading responses induced by mivacurium, cisatracurium, and d-tubocurarine in the evoked muscular compound action potentials of the cat

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Abstract

Purpose. It has been suggested that the different degrees of fade induced by nondepolarizing neuromuscular blocking agents in repetitive muscular contractions may be due to the varying affinities or binding kinetics of presynaptic nicotinic receptors. We compared the degree of fade induced by mivacurium, cisatracurium, and d-tubocurarine in the cat muscular compound action potential (mCAP).

Methods. In 21 cats, mCAPs of the gastrocnemius muscle were evoked by paired (conditioning and test stimuli) and 2-Hz train-of-four (TOF) sciatic nerve stimulation. The interval between the paired stimuli was changed stepwise from 7 to 1000 ms. The ratios of the amplitude evoked by test stimulus to that evoked by the conditioning stimulus (M2/M1 ratios) and TOF ratios were measured. After baseline variables had been obtained, the cat received either mivacurium ($0.08\,mg\cdot kg^{-1}$, $n = 7$), cisatracurium ($0.05\,mg\cdot kg^{-1}$, $n = 7$), or d-tubocurarine ($0.5\,mg\cdot kg^{-1}$, $n = 7$). A series of M2/M1 ratios and TOF ratios were measured at various levels of partial block during recovery.

Results. At 10% of baseline amplitude, all agents significantly depressed the M2/M1 ratios (i.e., fade) at relatively longer intervals of paired stimuli (mivacurium, $\geq$100 ms; cisatracurium, $\geq$40 ms; and d-tubocurarine, $\geq$20 ms), when compared with baseline. The order of activity to produce fade was mivacurium < cisatracurium < d-tubocurarine. A similar result was obtained in TOF ratios measured at various levels of neuromuscular block.

Conclusion. Our results suggest that mivacurium shows a lesser degree of fade during partial neuromuscular block than cisatracurium and d-tubocurarine.

Key words Nondepolarizing neuromuscular blocking agents · Fade · Electromyography

Introduction

Previous work has suggested that various nondepolarizing neuromuscular blocking agents produce fade of muscle tensions [1–4] and compound action potentials [5–7] or rundown of endplate currents [4,8] in response to repetitive motor nerve stimulation, such as paired [5,6], train-of-four (TOF) [2,3,5,6], and tetanic stimulation [1,4,7,8]. It is generally accepted that both fade and rundown are caused by inhibition of presynaptic acetylcholine receptors that should modulate transmitter release [4,8–12]. Recent work revealed the existence of neuronal nicotinic acetylcholine receptor subunits on mouse phrenic nerve fibers, including the nerve terminals [13].

The differences in degrees of fade between several nicotinic receptor antagonists could be due to the difference in affinities or binding kinetics of presynaptic receptors [9,10]. In particular, d-tubocurarine produces more profound fade and rundown than other clinically used relaxants [2,3,5,6]. However, the relative effect of mivacurium or cisatracurium on fading response, as compared with d-tubocurarine, has not been sufficiently examined. The purpose of this study was to compare the degrees of fade induced by three benzylisoquinolines in the cat evoked muscular compound action potentials.

Materials and methods

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

Twenty-one cats of either sex, weighing 2.5 to 4.0 kg, were anesthetized with 15 to $25\,mg\cdot kg^{-1}$ ketamine intramuscularly followed by intermittent administration of pentobarbital sodium intravenously. Lactated Ringer's solution was infused at a rate of $5\,ml\cdot kg^{-1}\cdot h^{-1}$ via an intravenous cannula placed in the forelimb. The trachea

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was intubated without the use of a neuromuscular blocking drug, and the lungs were artificially ventilated with air using an animal respirator. Ventilation was adjusted to maintain the end-tidal carbon dioxide concentration at 4.0% using an expired gas monitor. Electrocardiograms and arterial blood pressures from a unilateral carotid artery were monitored concurrently, and the esophageal temperature was maintained above 38°C with a heating mattress throughout the experiments.

The cat's lumbar spinous processes and unilateral calcaneous were immobilized in the prone position. The ipsilateral sciatic nerve was severed, and its distal part was attached to a bipolar stimulating platinum electrode after dissection from the surrounding tissue. Another bipolar platinum electrode for measurement of the muscular compound action potential (mCAP) evoked by sciatic nerve stimulation was fixed on the ipsilateral fascia covering the gastrocnemius muscle.

Stimulation patterns and variables

A supramaximal square-wave pulse with a duration of 0.2 ms was applied serially to the sciatic nerve at a frequency of 0.1 Hz during the experiments. Following nerve stimulation, the gastrocnemius mCAPs were observed and recorded using a module type EMG. After the mCAPs had been stable for at least 30 min, two patterns of stimulation, as described below, were applied for recording controls.

Paired stimuli

Pairs of pulses at intervals of 7, 8, 9, 10, 20, 40, 60, 80, 100, 500, and 1000 ms were applied to the sciatic nerve in turn, with a 10-s pause (Fig. 1). The first stimulus of the pair was termed the "conditioning stimulus (S1)" and the second the "test stimulus (S2)." The ratio (M2/M1) of the amplitude of the mCAPs evoked by S2 (M2) to that of S1 (M1) was determined.

Train-of-four stimulation

TOF stimulation at a frequency of 2 Hz was applied to determine the TOF ratio.

Administration of nondepolarizing neuromuscular blocking agents

After baseline variables had been obtained, mivacurium (0.08 mg·kg^{-1}), cisatracurium (0.05 mg·kg^{-1}), or d-tubocurarine (0.5 mg·kg^{-1}) was administered intravenously. If complete block was not obtained, cumulative doses consisting of half of the first dose were applied until the mCAP amplitude was abolished. The development of and recovery from each level of block was characterized by serial depression of the amplitude of

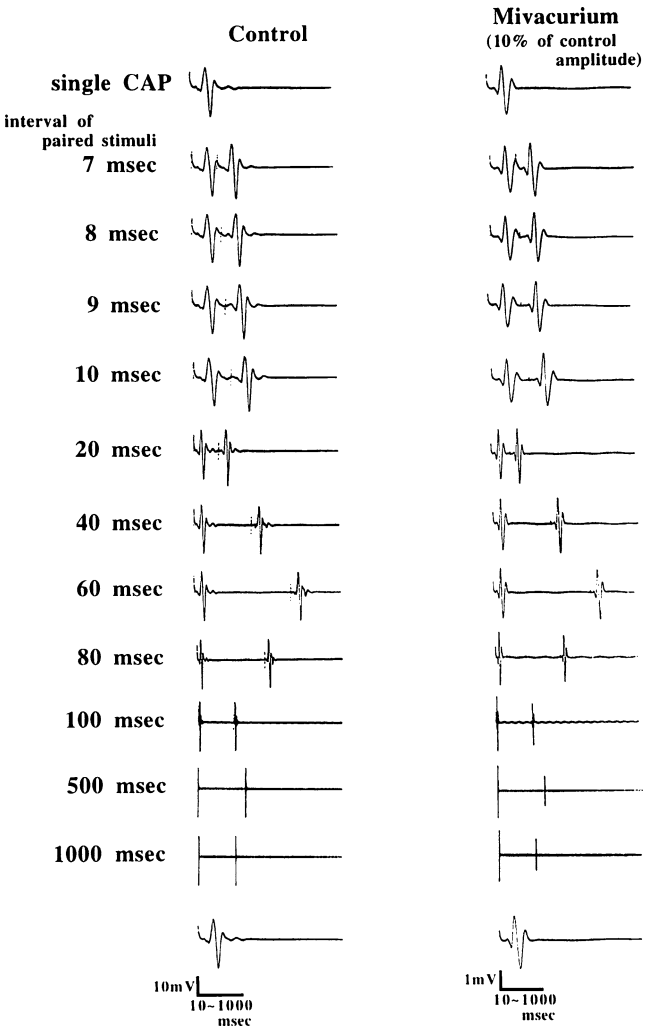

Fig. 1. An example of actual paired muscular compound action potentials (*mCAPs*) with various time intervals ranging from 7 to 1000 ms recorded in order. The **left** tracing was recorded as a baseline, and the **right**, in which fade is demonstrated, was obtained at 10% of baseline amplitude during recovery from mivacurium-induced block

single mCAPs evoked by sciatic nerve stimulation at a frequency of 0.1 Hz throughout the experiments.

A series of paired stimuli was applied when the amplitude of the mCAP recovered to 10%, 25%, 50%, and 75% of baseline mCAP amplitude from complete block. Thereafter, a TOF stimulation was applied when the mCAP recovered to 12.5%, 37.5%, 62.5%, and 87.5% of baseline.

Statistical analyses

All data were expressed as means ± SEM. Statistical analyses were performed using the Wilcoxon signed-ranks test or the Kruskal–Wallis test. A P value of <0.05 was considered to indicate a statistically signifi-

cant difference. If a significant P value was obtained in multiple comparisons, further group comparisons were made by Scheffé's F-test.

Results

M2/M1 Ratios of Paired mCAPs

Figure 1 shows an example of the serial traces of single and paired mCAPs evoked at various stimulation intervals. The left series are controls and the right series are mCAPs recorded when the single mCAP amplitude had recovered to 10% of baseline from mivacurium-induced neuromuscular block. The mean M2/M1 ratios measured as baselines and those measured when the amplitude of the single mCAP had recovered to 10% of baseline from mivacurium-, cicatracurium-, or d-tubocurarine-induced block were plotted against the intervals of paired stimuli (Fig. 2A–C). The baseline M2/M1 ratios observed at short intervals (≤20 ms) of paired stimuli were slightly above 100%, and those observed at relatively longer intervals (≥40 ms) were almost equal to 100%, as were those observed in previous studies [4,8]. In the partially curarized state, mivacurium caused significant potentiation of the M2/M1 ratios at shorter intervals of paired stimuli from 7 to 20 ms (Fig. 2A); however, such a marked potentiation was not observed in the cisatracurium and d-tubocurarine group (Fig. 2B and C). All tested muscle relaxants significantly depressed the M2/M1 ratios when paired stimuli were applied at relatively longer intervals (mivacurium, ≥100 ms [Fig. 2A]; cisatracurium, ≥40 ms [Fig. 2B]; and d-tubocurarine, ≥20 ms [Fig. 2C]). The greatest depression of the ratios was observed when paired stimuli were applied at 500-ms intervals. Figure 3 shows significant differences in the M2/M1 ratios measured at 10% of baseline mCAP amplitude among tested muscle relaxants. The order of activity to produce fade at stimulus intervals of 20 ms and longer was mivacurium < cisatracurium < d-tubocurarine. Figure 4 shows a comparison of the M2/M1 ratios (stimulation interval of paired stimuli, 500 ms) obtained when the single mCAP amplitude had recovered to 10%, 25%, 50%, and 75% of baseline during spontaneous recovery from mivacurium-, cisatracurium-, and d-tubocurarine-induced block. The degree of fade was gradually reduced with greater recovery from each neuromuscular block.

TOF ratios

Figure 5 shows the mean TOF ratios observed during spontaneous recovery from neuromuscular block. The ratios observed at the same levels of single mCAP amplitude curarized by mivacurium, cisatracurium, and d-tubocurarine differed significantly. The order of activity to produce TOF fade was same as that of the M2/M1 ratios (mivacurium < cisatracurium < d-tubocurarine), and significant differences were observed in the TOF ratios at any level of neuromuscular block among all muscle relaxants.

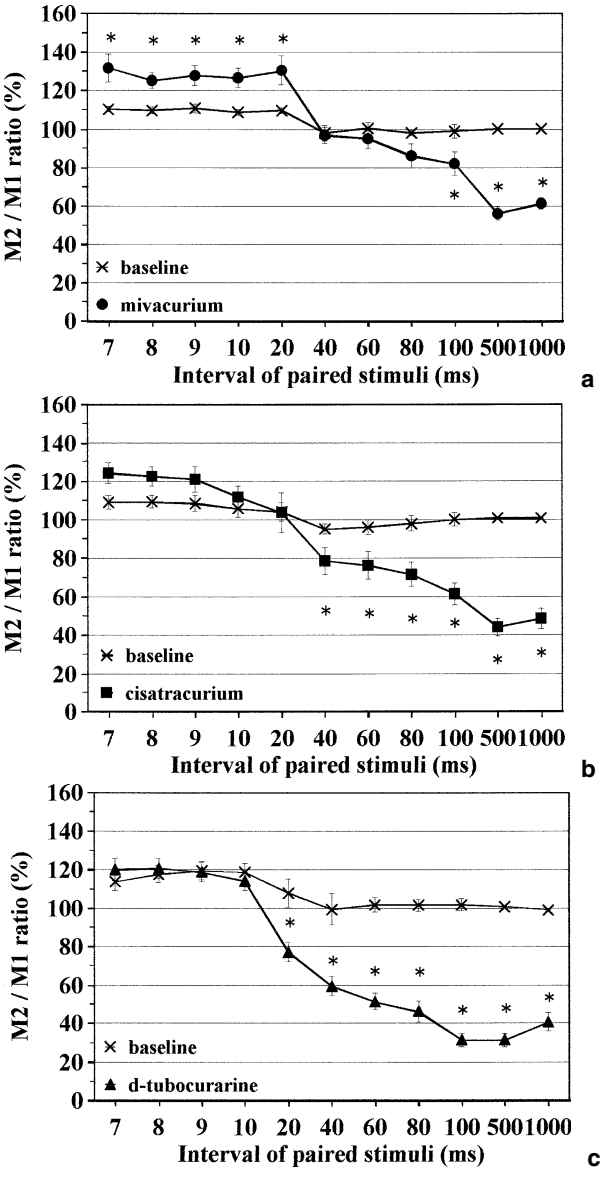

Fig. 2. M2/M1 ratios in response to paired stimuli observed at 10% of baseline amplitude inhibited by (**a**) mivacurium, (**b**) cisatracurium, and (**c**) d-tubocurarine. The ordinate and abscissa show M2/M1 ratios (mean ± SEM) and intervals of paired stimuli, respectively. *$P < 0.05$ compared with baseline

Discussion

It is well known that d-tubocurarine has a potent waning effect on muscle contractions [2,3], mCAPs [5,6], and endplate currents [8] in the neuromuscular junction

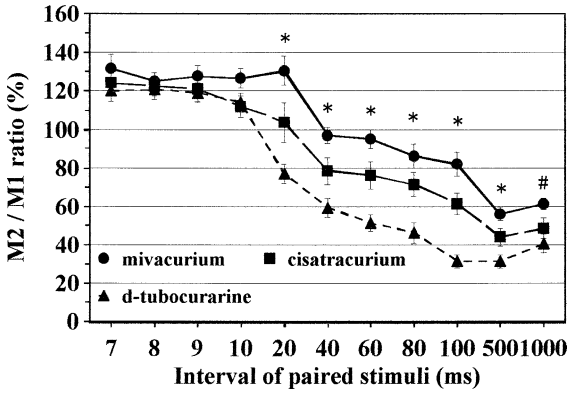

Fig. 3. Comparative M2/M1 ratios depressed by mivacurium, cisatracurium, and d-tubocurarine in response to paired stimuli observed at 10% of baseline amplitude. The ordinate and abscissa show M2/M1 ratios (mean ± SEM) and intervals of paired stimuli, respectively. $^{*}P < 0.05$ among all drugs. $^{\#}P < 0.05$ between mivacurium and cisatracurium, or d-tubocurarine

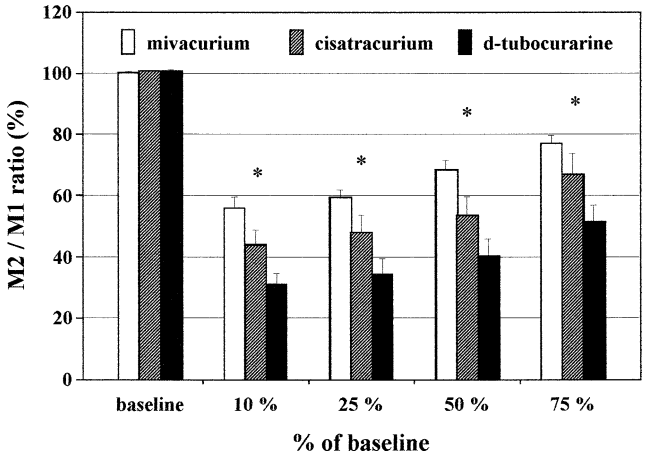

Fig. 4. Comparative M2/M1 ratios (stimulation interval of paired stimuli: 500 ms) obtained during spontaneous recovery from mivacurium-, cisatracurium-, and d-tubocurarine-induced block. The ordinate and abscissa show M2/M1 ratios (mean ± SEM) and levels of inhibited amplitude, respectively. $^{*}P < 0.05$ among all drugs

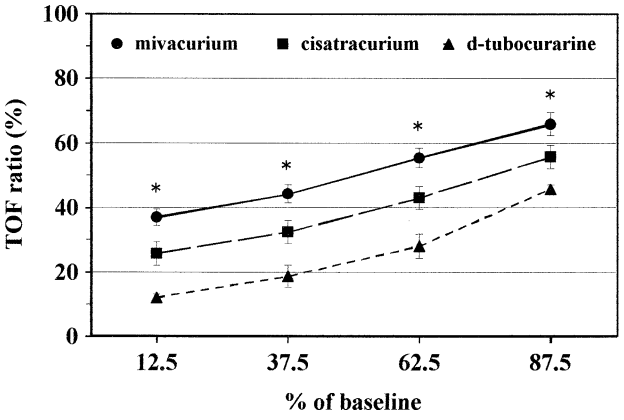

Fig. 5. Comparative train-of-four (*TOF*) ratios obtained during spontaneous recovery from mivacurium-, cisatracurium-, and d-tubocurarine-induced block. The ordinate and abscissa show TOF ratios (mean ± SEM) and levels of inhibited amplitude, respectively. $^{*}P < 0.05$ among all drugs

evoked by repetitive nerve stimulation, when compared with other clinically used relaxants such as pancuronium [2,6], vecuronium [2,3,6], rocuronium [5], and atracurium [2,3]. The present study showed that mivacurium causes a lesser degree of fade in a series of mCAPs evoked by paired and TOF stimuli than cisatracurium and d-tubocurarine. It is generally accepted that fade is due to a depression in transmitter output relative to the initial release in the presence of nicotinic antagonists [4,8–12]. Therefore, mivacurium appears to have a weaker inhibitory effect on acetylcholine release from the motor nerve terminal than cisatracurium and d-tubocurarine.

It is assumed that the amplitude of the mCAP evoked by the test stimulus is influenced by relative changes in the amount of released transmitter in response to the conditioning stimulus and is dependent on the interval between the paired stimuli during partial curarization [5,6]. Electrophysiological study using rat hemidiaphragm-phrenic nerve preparation [14] could give further support to our idea. The study confirmed that there was an increase in the quantal content (i.e., mobilization) in response to the test stimulus after the conditioning stimulus of paired stimuli. Potentiation of the endplate potential following the test stimulus was detected at intervals up to 100 ms after the conditioning stimulus and was not followed by any depression of potentials in control measurement. In the curarized preparation with d-tubocurarine, however, potentiation of transmitter release could only be observed at intervals of paired stimuli as long as 10–15 ms and was followed by a long period of depression (1–10 s). The degree of inhibition of transmitter release from the nerve terminals by nondepolarizing block is dependent on the interval between the paired stimuli. The result shows the same transition with our findings in the M2/M1 ratios and supports our idea that the ratios indirectly represent transmitter release from the motor nerve terminals. Our previous studies [5,6] showed that the pattern of M2/M1 ratios produced by α-bungarotoxin, which irreversibly binds to postsynaptic acetylcholine receptors and does not affect the presynaptic site, was characterized by profound potentiation of the response to test stimuli at short intervals between paired stimuli. The finding also suggests a close relationship between the change in M2/M1 ratios and that in the amount of presynaptic acetylcholine release. In this study, mivacurium significantly augmented the ratios

above 100% at short intervals of paired stimuli up to 20 ms (Fig. 2A), when compared with the baseline value, although cisatracurium (Fig. 2B) and d-tubocurarine (Fig. 2C) could not cause similar potentiation. Mivacurium may have a weaker inhibitory effect on acetylcholine release from the motor nerve endings than cisatracurium and d-tubocurarine, and therefore the increased concentration of acetylcholine in the neuromuscular junction after a test stimulus may be sufficient to temporarily antagonize mivacurium at postsynaptic acetylcholine receptors. In addition, mivacurium showed the least degree of fade when paired stimuli with longer intervals and the TOF stimuli were applied. The result also indicates the differences in the degree of presynaptic inhibitory effect among drugs.

Numerous electrophysiological studies at the skeletal muscle neuromuscular junction have suggested that the motor nerve terminal should have some autoreceptors similar to the postsynaptic nicotinic receptors on the endplate [4,8–12]. Such presynaptic nicotinic receptors are thought to modulate transmitter release from the nerve terminal, via a positive [4,8–10], negative [11], or combined feedback theory [4,12], for maintaining normal successive neuromuscular depolarizations. For positive feedback, it is hypothesized that acetylcholine released into the synaptic cleft acts on presynaptic autoreceptors to enhance mobilization of acetylcholine during high-frequency nerve stimulation. On the basis of this theory, nondepolarizing neuromuscular blocking agents bind to presynaptic receptors and suppress transmitter mobilization from reserve vesicles into immediately releasable stores. The depressed transmitter release causes the fading phenomenon during repetitive nerve stimulation. On the other hand, the negative feedback hypothesis proposes that nondepolarizing block of presynaptic receptors inversely accelerates transmitter release at the beginning of repetitive nerve stimulation, but the nerve terminal cannot maintain the elevated level of transmitter output to sustain constant postsynaptic depolarization.

Our results showed that the twitch-TOF ratio relationship differs markedly among three muscle relaxants. In clinical settings, TOF fade in thumb adduction cannot be detected visually or manually when the TOF ratio measured mechanically is over 0.4 [15]. If our results apply to humans, it should be difficult to feel fade at about 12.5%, 62.5%, and 87.5% of baseline response during recovery from mivacurium-, cisatracurium-, and d-tubocurarine-induced block, respectively (Fig. 5). Even in a deep block of the twitches caused by mivacurium, it may become impossible to detect fade. Mivacurium is a short-acting muscle relaxant; however, it should be careful to assess TOF fade and residual block during recovery from mivacurium-induced block.

The previous study by Carroll et al. [16] has shown that TOF fade during the onset of block is greater with cisatracurium, 0.05 mg/kg, than mivacurium, 0.15 mg/kg; however, no significant difference in the degrees of fade was obtained during recovery from neuromuscular block induced by cisatracurium and mivacurium. The difference between the results of our study and the previous one may be caused by several differences in methods. First, there are differences in the muscle and monitoring apparatus used to measure neuromuscular responses. In the human study by Carroll et al. [16], twitch tensions of the adductor pollicis muscle, which is classified as a slow-twitch muscle, were measured by a mechanomyograph (MMG). In contrast, we studied electromyographic (EMG) responses in the fast-twitch gastrocnemius muscle. Day et al. [17] reported that the degree of TOF fade induced by vecuronium was greater in the gastrocnemius (fast) muscle than the soleus (slow) muscle in cats. Furthermore, fade observed in the EMG tends to be greater than that in the MMG during recovery of neuromuscular function from d-tubocurarine-induced block [18,19]. In light of the results, our study might be able to observe a larger degree of fade than the previous study and easily detect the relative differences in fade among three neuromuscular blocking agents. Additionally, we should consider the species difference between the human and the cat.

The different degrees of fading response caused by several nicotinic receptor antagonists could be due to differences in the affinities or binding kinetics of antagonists to presynaptic receptors [9,10]. Furthermore, we should consider that the pharmacokinetics and duration of action-related effects may contribute to the variation in drug-induced fade in vivo. In the present and in previous studies, short (mivacurium)- and intermediate (rocuronium and vecuronium)-acting drugs caused significantly less fade than long-acting drugs (pancuronium and d-tubocurarine) [5–7]. The greater fade observed during the recovery period than during the onset of block [20–22] suggests that the association and dissociation of nondepolarizing neuromuscular blocking agents to presynaptic receptors should be relatively slow. Therefore, it is possible that the concentration of mivacurium and cisatracurium in the presynaptic sites of the neuromuscular junctions may not reach equilibrium sufficiently. The differences in accessibility of drugs to presynaptic receptors are also supposed to correlate with the different degrees of fade [21]. Further investigations are necessary to study this point.

Additionally, there is a notion that the extent of presynaptic effects may be related to the structural distinction of drugs, since benzylisoquinolines tend to show greater fade than steroidal agents [2]. However, the present and previous findings [5,6] cannot support this idea.

In conclusion, the degrees of fade of mCAPs in the cat gastrocnemius muscle evoked by paired and TOF stimuli differ significantly during spontaneous recovery from neuromuscular block induced by three benzylisoquinolines. Mivacurium shows the least fade, and cisatracurium and d-tubocurarine cause greater fade than mivacurium, in that order.

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