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Prejunctional Effects of Vecuronium in the Cat

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This study explored the prejunctional actions of vecuronium using the *in vivo* cat soleus nerve–muscle preparation. Vecuronium doses of 1 to 5 μg/kg iv suppressed the repetitive firing of the motor nerve endings, and the obligatory potentiation of the twitch response following high-frequency conditioning at 400 Hz for 10 s without attenuating neuromuscular transmission at 0.4 Hz. It was also found that extremely low doses of vecuronium had excitatory effects at the cat soleus motor nerve endings: doses of 0.5 and 1 μg/kg iv evoked a modest postdrug repetitive firing of the nerve endings and a concomitant potentiation of the muscle responses. These dose-related agonist and antagonist activities suggest that at the motor nerve endings, vecuronium is a weak partial agonist. The major action of vecuronium at the motor nerve endings, however, was suppressive, and this antagonist action contributed to the neuromuscular blocking action of this muscle relaxant. (Key words: Neuromuscular junction: motor nerve terminal; neuromuscular end-plate; prejunctional; postjunctional; posttetanic repetition and potentiation; synapse. Neuromuscular relaxants: vecuronium.)

VECURONIUM IS a nondepolarizing skeletal muscle relaxant of intermediate duration of action that has been recently introduced to clinical practice. From studies of tetanic maintenance in rats,[1] and from studies of quantal transmission parameters in the toad,[2] it has been concluded that vecuronium acts not only at postjunctional receptors but also at prejunctional sites. Other nondepolarizing muscle relaxants, such as *d*-tubocurarine and pancuronium, have also been shown to have prejunctional sites of action in addition to their well-established postjunctional effects.[3–8] This investigation studied the prejunctional effect of vecuronium using the *in vivo* cat soleus nerve–muscle preparation as developed by Riker *et al.* and Standaert.[9,10]

The endings of the cat soleus motor nerves possess the capacity to generate repetitive discharges after high-frequency tetanic conditioning.[9,11] After such conditioning, subsequent single stimuli evoke a repetitive discharge of the nerve terminals, termed posttetanic repetition. Posttetanic repetition causes an obligatory posttetanic potentiation of the muscle contractile responses. This posttetanic potentiation occurs in the absence of neuromuscular blocking agents and is not the same phenomenon as that which occurs when the muscle response is partially blocked by these agents.[12] Posttetanic repetition and posttetanic potentiation are sensitive indices of motor nerve-ending function and are markedly affected by agents with pharmacologic or toxicologic actions on the motor nerve endings.[12,13]

Investigations of drugs affecting the neuromuscular junction are limited by technique. Currently, it is not possible to record directly from motor nerve terminals. Intracellular microelectrodes do directly record postjunctional events, but prejunctional events are inferred in this *in vitro* technique. Recording action potentials from ventral root axons, however, is done *in vivo* and allows the monitoring of prejunctional events in their natural milieu. The *in vivo* experimental technique uses the accessible myelinated motor axons in the ventral roots as direct leads to the motor nerve endings, *i.e.*, the unmyelinated terminal and the distal node of Ranvier. As a result of certain experimental conditioning (high-frequency tetanic stimulation or facilitatory drug administration), an excitability can be provoked in the motor nerve endings through an interaction between the unmyelinated nerve terminal and the most distal node of Ranvier. This interaction initiates repetitive discharges in responses to single nerve stimulation. Monitoring these repetitive discharges discloses this *in vivo* excitability of the motor nerve endings and provides an opportunity to assess the effects of drugs and pathologic factors on this activity.[13] Using this technique, Standaert was among the first to show that *d*-tubocurarine had prejunctional actions.[3]

The present study found that vecuronium had prejunctional effects at the neuromuscular junction using posttetanic repetition and posttetanic potentiation as indicators of motor nerve ending function in a dose range that did not affect neuromuscular transmission.

Methods and Materials

A total of 21 cats of either sex, anesthetized with alphachloralose, 90 mg/kg iv, were used in this study, which employed the *in vivo* soleus nerve–muscle preparation. This method has been previously described.[9,11] A tracheostomy was performed, and ventilation was controlled with a respirator. End-tidal P_{CO_2} ranged from 34–40

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mmHg. The gastrocnemius muscle was excised, and all nerves except that innervating the soleus muscle were ligated and cut. The soleus tendon was connected to a strain gauge for recording the isometric muscle contractile responses. The soleus nerve was continuously stimulated supramaximally with rectangular pulses of 0.1 ms duration at 0.4 Hz except when interrupted for 10 s at a tetanic frequency of 400 Hz. The minimal time between the 400 Hz/10 s tetanic conditioning was 5 min.

A dorsal laminectomy exposed the spinal cord from L-4 to S-1, and the ventral roots L-7 and S-1 were cut proximal to their exit from the cord. The ventral root was teased into small filaments that contained one to four axons. Each filament was placed on a recording electrode after: (1) stimulation of the filament evoked a contractile response from the soleus muscle; and (2) stimulation of the peripheral soleus nerve determined the number of axons in the filament. The surgical wounds in the back and leg were covered with paraffin oil maintained at 37° C by radiant heat.

In cats that have received no other drugs except alpha-chloralose (the anesthetic agent), each single stimulus-evoked action potential invading the soleus motor nerve endings triggers the generation of a repetitive discharge for a short period of time following the 400 Hz/10 s tetanic conditioning.[9] The repetitive discharges are conducted antidromically to the ventral roots from which they are recorded. The number of action potentials in a repetitive discharge usually varies from one to five, in addition to the stimulus-evoked potential.

Before vecuronium administration, 10–15 ventral root filaments, each containing one to four functional soleus axons, were identified. Using two recording electrodes, the number of axons from which repetitive action potentials were recorded was determined. The number of axons tested in each cat ranged from 31 to 41 axons. Following the administration of vecuronium, the number of axons exhibiting repetition was determined over the time of peak drug action. Repetition was defined as a minimum of one repetitive action potential following the evoked response. Once repetitive activity was recorded or if no repetitive activity was recorded following 12 single stimulations of the nerve, the filament on the recording electrode was replaced with an untreated one. In this manner, the incidence of axons exhibiting posttetanic repetition was determined before and after the administration of vecuronium.

The repetitive discharge generated by the soleus muscle evoked an obligatory potentiation of the muscle contractile responses, which is known as posttetanic potentiation. Within certain limits, posttetanic potentiation serves as an indirect readout of the repetitive discharge capacity of the entire population of soleus motor nerve endings and is easily quantitated.

A preliminary study in one cat determined that the maximum suppressive effect of vecuronium on neural repetitive firing and muscle potentiation was achieved 5–10 min after injection. Thus, the 400 Hz/10 s tetanic conditioning was delivered 5 min after vecuronium administration and was repeated every 5 min thereafter until full recovery occurred. In determining the incidence of repetitive firing, nine cats were used, with each dose (1.0, 2.5, and 5.0 μg/kg) being given to three animals. Because the incidence of repetitive firing was determined only at peak drug effect, doses were repeated until all sampled axons were tested, waiting for full recovery between doses.

The experiments quantitating vecuronium effects on posttetanic potentiation of the muscle response due to the repetitive discharges of the motor nerve endings and the blockade of neuromuscular transmission were performed in another series of five cats. The vecuronium effect on the peak tension of the posttetanic potentiation was first determined, with intravenous doses being given randomly and waiting for the full recovery of muscle potentiation between doses. Because vecuronium suppressed both the peak and duration of the muscle potentiation, the area of posttetanic potentiation before and after vecuronium administration was measured to determine the time course of drug action using a Zweiss MOP 30® digital image analyzer.

In quantitating neuromuscular blockade, no high-frequency conditioning was given, and recovery of neuromuscular transmission was accomplished spontaneously.

In the experiments studying the neural repetition induced by vecuronium, the incidence of axons exhibiting vecuronium-induced repetition was determined in three cats at a dose of 1.0 μg/kg iv. In another three cats, vecuronium doses of 0.5 and 5.0 μg/kg iv were administered. In all animals, doses were repeated until all sampled axons were tested, waiting 1–2 h between injections.

The range of vecuronium doses employed in the entire study varied from 0.5 to 40 μg/kg and were administered intravenously in a random order.

Posttetanic neural repetition data were analyzed using the Chi square test. Duncan's test was used to analyze the suppression of peak posttetanic potentiation, and the areas of posttetanic potentiation were analyzed using the analysis of variance. Dose–response regressions were determined using the least-square method. The slopes of the regressions were compared using the Student's t test. Statistical significance was $P \leq 0.05$.

Results

Vecuronium suppressed posttetanic repetition in a dose-related manner. The threshold dose was 1.0 μg/kg, and doses greater than 5 μg/kg completely suppressed posttetanic repetition. These doses did not block neuro-

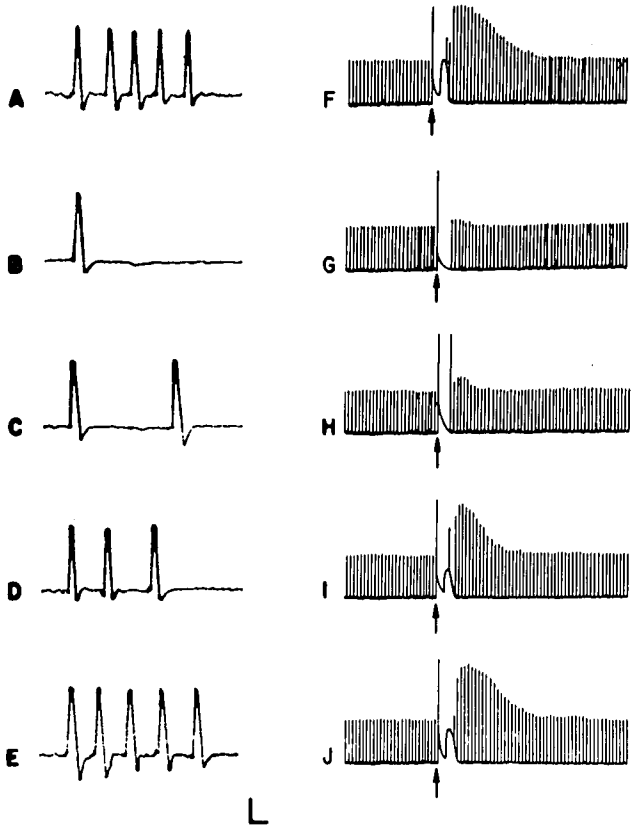

FIG. 1. Time course of suppressant effect of vecuronium (5 μg/kg) dose on the posttetanic repetition (*A–E*) generated by cat soleus motor nerve endings and the obligatory posttetanic potentiation (*F–J*) of the muscle response: *A* and *F*, untreated control; *B* and *G*, 5 min after vecuronium; *C* and *H*, 15 min after vecuronium; *D* and *I*, 30 min after vecuronium; *E* and *J*, 40 min after vecuronium. The soleus nerve was supramaximally stimulated at 0.4 Hz, except when interrupted for 10 s at 400 Hz (*arrows*). Calibration: vertical, 300 g for muscle tension; horizontal, 10 s for muscle responses and 2 ms for posttetanic repetition.

muscular transmission. The typical suppressive effects of a 5 μg/kg dose of vecuronium on posttetanic repetition and posttetanic potentiation are illustrated in figure 1. Partial recovery of posttetanic repetition and posttetanic potentiation was evident at 15 and 30 min, with full recovery occurring at 45 min. Vecuronium reduced the incidence of the posttetanic repetition in a dose-related manner from the control value of 84% (table 1). With the 2.5 and 5 μg/kg doses, the maximal suppressive effect was reached 5 min after vecuronium administration, while the time to maximal effect with the 1 μg/kg dose was reached in 10 min after administration. Table 2 lists the mean times to full recovery of posttetanic potentiation for the 1, 2.5, and 5 μg/kg doses. Larger doses had proportionally longer durations of action.

TABLE 1. Incidence* of Posttetanic Repetition (PTR) Generated at Soleus Motor Nerve Endings in Vecuronium-treated Cats†

Dose (μg/kg) iv	Axons Demonstrating PTR	Per Cent
0	101	84
1	84	70
2.5	35	29
5	5	4

* n = 3 cats for each dose; total axons tested at each dose = 120; axons tested per cat ≥ 39.

† Treated cats *vs.* untreated control, $P < 0.001$.

TABLE 2. Recovery Times* of Posttetanic Potentiation Following Vecuronium Administration†

Doses (μg/kg)	Time (min)
1	23 ± 2.6
2.5	41 ± 1.89
5	55 ± 3.5

* Mean ± SEM.

† n = 5 cats randomly receiving all 3 doses.

Doses of vecuronium greater than 10 μg/kg not only suppressed posttetanic potentiation, but also affected the single impulse transmission, depressing the twitch tension in a dose-related manner (fig. 2). Doses greater than 30 μg/kg completely blocked transmission in all instances. Figure 2 also shows the dose–response regression for the suppression of posttetanic potentiation as well as the blockade of neuromuscular transmission. The slopes of the regressions were found not to be different when tested for parallelism. The ED_{50} for the suppression of posttetanic muscle potentiation was 1.95 μg/kg and the ED_{50} for the suppression of single-impulse transmission was 18.0 μg/kg. Thus, a dose difference greater than nine-fold existed between the actions of vecuronium suppressing posttetanic potentiation and blocking neuromuscular transmission.

The spontaneous recovery of neuromuscular transmission from blocking doses of vecuronium was followed by potentiation of the muscle contractile tension. Although this potentiation was not great, it was consistent. In figure 3*A*, it is 12% and lasted for 80 min in this experiment. The duration of the postdrug potentiation observed in all experiments was 15–90 min.

This vecuronium-induced potentiation was also regularly produced in cats first exposed to extremely small doses of vecuronium (0.5 and 1.0 μg/kg iv). In figure 3*B*, the postdrug potentiation induced by a 1.0 μg/kg dose of vecuronium is biphasic. The potentiation (13%) occurs immediately following the injection, falls back to control, and then occurs again to 37%. In this experiment, the vecuronium-induced muscle potentiation lasted 48 min. At 5 μg/kg, no postdrug potentiation was observed immediately following the vecuronium injection. It should be noted that this dose of vecuronium also suppressed posttetanic repetition.

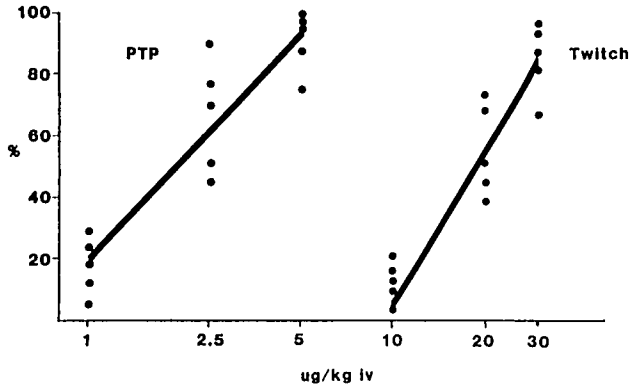

FIG. 2. The dose–response regressions for the suppression of posttetanic potentiation and neuromuscular transmission (twitch tension) at 0.4 Hz. Abscissa: intravenous vecuronium dose, μg/kg, log scale. Ordinate: per cent suppression (100 × [response after vecuronium/response before vecuronium]).

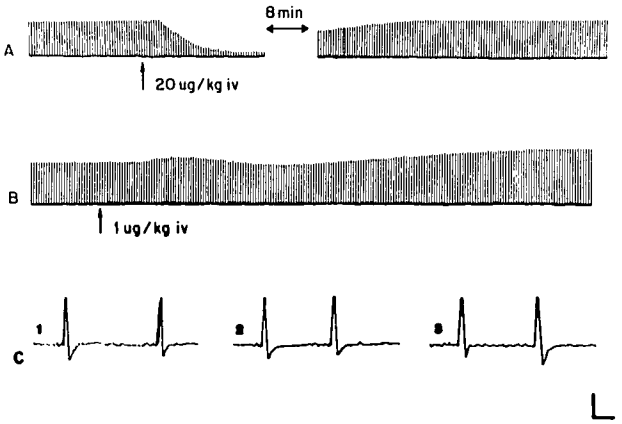

FIG. 3. Vecuronium-induced postdrug repetition (PDR) and postdrug potentiation (PDP). *A.* PDP seen after spontaneous recovery from neuromuscular blockade (first 8 min of recovery not shown) produced by a 20 μg/kg dose of vecuronium given at *arrow. B.* PDP evoked by a 1 μg/kg dose of vecuronium given at *arrows,* which did not block neuromuscular transmission. *C.* Typical PDR (tracings 1–3) evoked by a 1 μg/kg dose of vecuronium and recorded during the PDP of the muscle responses. Calibration: vertical, 300 g of muscle tension; horizontal, 10 s for muscle responses and 2 ms for PDR.

The postdrug potentiation induced by these vecuronium doses was causally related to a vecuronium-induced repetitive firing of the motor nerve endings, illustrated in figure 3*C*. The postdrug repetition was characterized by one repetitive action potential, in addition to the stimulus-evoked potential, and was evoked in individually sampled axons once out of every two to five nerve stimulations. Table 3 relates vecuronium dose and the incidence of postdrug repetition.

Discussion

The major effect of vecuronium at motor nerve endings is suppressive. Vecuronium doses that suppress posttetanic repetition also suppress postdrug repetition. This suppressive prejunctional action accounts for the suppression of the obligatory posttetanic potentiation. The slopes of the dose–response curves for the suppression of the posttetanic potentiation, a prejunctional effect, and the blockade of the twitch response, a postjunctional effect, are similar. This suggests that the mechanism of action at the two different sites is the same. Thus, the prejunctional action of vecuronium may be due to the occupation of receptors located on the motor nerve endings.

The ability of extremely small doses of vecuronium to evoke postdrug repetition and of higher doses to suppress posttetanic repetition suggests that vecuronium has some partial agonistic activity at the motor nerve endings. These biphasic actions explain the vecuronium effects that are seen in figure 3*B*. Immediately following the injection and as the vecuronium first arrives at the prejunctional site of action, the drug-evoked neural repetition begins and, as a consequence, the muscle potentiation occurs. As the concentration of the prejunctional site is further increased with the arrival of more vecuronium, the ceiling of the vecuronium repetitive activity is reached. As the vecuronium concentration in the cleft is further increased, the repetitive activity becomes suppressed. The result is the reduction of the muscle potentiation. Within a short period of time, the concentration at the prejunctional site falls below the ceiling of the vecuronium-induced neural repetitive activity. Neural repetition is again evoked, and muscle potentiation is observed for a second time. The low incidence of repetition and the fact that only one repetitive action potential was generated when repetition did occur demonstrate the weak agonist property of vecuronium.

Hubbard and Wilson determined that the time course of the prejunctional effects of *d*-tubocurarine had a longer onset and duration of action than that of the postjunctional effects.[6] In the cat, Bowman and Webb found that pancuronium and *d*-tubocurarine had different degrees of depression of peak tetanic tension: a postjunctional effect; and tetanic fade, a prejunctional effect.[14] The muscle relaxants, however, needed to be administered intravenously to produce tetanic fade. These investigators postulated that prejunctional cholinoceptors have less affinity than postjunctional cholinoceptors for nondepolarizing

TABLE 3. Incidence* of Postdrug Repetition (PDR) Generated by Vecuronium at Cat Soleus Motor Nerve Endings

Dose† (μg/kg)	Axons Demonstrating PDR	Per Cent
0.5	11	11
1.0	16	16
5.0	0	0

* Total axons tested for each dose = 100; axons tested per cat ≥31.
† 1.0 μg administered to 3 cats; 0.5 and 5.0 μg to another 3 cats.

muscle relaxants. The intravenous injection of the muscle relaxants assures sufficient time and concentration at the prejunctional sites. In humans, Stanec and Baker found that intravenously administered pancuronium and *d*-tubocurarine have the same order of depression of peak tetanic tension and tetanic maintenance as the cat.[15] The present study found that the prejunctional suppression of posttetanic repetition and posttetanic potentiation had a similar onset as that of the neuromuscular blockade.[16] The duration of the prejunctional suppression, however, is longer. Doses of vecuronium that do not produce neuromuscular block suppress posttetanic repetition and potentiation from 23 to 55 min. Thus, with doses of vecuronium that block neuromuscular transmission, the prejunctional suppression of posttetanic repetition and potentiation would continue for a similar period of time after transmission has spontaneously recovered. The longer prejunctional duration of action explains the residual tetanic fade that is present after spontaneous recovery from vecuronium neuromuscular blockade.

The prejunctional suppressive effect of nondepolarizing muscle relaxants may explain the priming principle.[17] This class of muscle relaxants interferes with the mechanisms of transmitter release.[2,4–9] This interference can occur at concentrations that do not affect postjunctional sites.[7] In applying the priming principle, a small fractional dose of the intubating dose of a nondepolarizing relaxant is first given.[18,19] Ideally, this priming dose does not affect transmission. The present study shows that the range of this dose would certainly have prejunctional effects. The key to the successful use of a priming dose is apparently time: the prejunctional sites of action must be exposed for a sufficient time to assure adequate binding.

Because both the evocation of postdrug repetition and the suppression of posttetanic repetition occur with doses that do not block the single impulse transmission, it follows that whenever neuromuscular transmission is blocked by vecuronium, the doses necessary to achieve this effect are far greater then those which act selectively at the motor nerve endings. Thus, doses that cause neuromuscular blockade affect both prejunctional and postjunctional sites. When transmission has spontaneously recovered, the vecuronium concentration in the junctional cleft can still be sufficient to affect the prejunctional sites of action. Because the major action of vecuronium at the motor nerve endings is suppressive, this prejunctional activity contributes to the neuromuscular blocking action of vecuronium at the neuromuscular junction.

In summary, while this investigation found that vecuronium had biphasic effects at motor nerve endings, the major effect of vecuronium at the prejunctional site of action was suppressive. The prejunctional suppressive effect contributes to the neuromuscular blocking action of this agent.

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References

1. Bowman WC, Gibb AJ, Marshall IG: Prejunctional and postjunctional effects of vecuronium, Clinical Experiences with Norcuron, Current Clinical Practice Series II. Edited by Agoston S, Bowman WC, Miller RD, Viby-Mogensen J. Amsterdam, Excerpta Medica, 1983, pp 26–32
2. Torda TA, Kiloh N: Myoneural actions of Org NC 45. Br J Anaesth 54:1217–1221, 1982
3. Standaert FG: The action of *d*-tubocurarine on the motor nerve terminal. J Pharmacol Exp Ther 143:181–186, 1964
4. Galindo A: Prejunctional effect of curare: Its relative importance. J Neurophysiol 34:289–301, 1971
5. Galindo A: Curare and pancuronium compared: Effects on previously undepressed mammalian myoneural junction. Science 178:753–755, 1972
6. Hubbard JI, Wilson DF: Neuromuscular transmission in a mammalian preparation in the absence of blocking drugs and the effect of *d*-tubocurarine. J Physiol (Lond) 228:307–325, 1973
7. Blaber LC: The prejunctional actions of some nondepolarizing neuromuscular blocking drugs. Br J Pharmacol 47:109–116, 1973
8. Glavinovic MI: Presynaptic action of curare. J Physiol (Lond) 290: 499–506, 1979
9. Standaert FG: Post-tetanic repetitive activity in the cat soleus nerve. Its origin, course, and mechanism of generation. J Gen Physiol 47:53–70, 1963
10. Riker WF Jr, Roberts J, Standaert FG, Fujimori H: The motor nerve terminal as the primary focus of drug-induced facilitation of neuromuscular transmission. J Pharmacol Exp Ther 121: 286–312, 1957
11. Standaert FG: The mechanisms of post-tetanic potentiation in cat soleus and gastrocnemius muscles. J Gen Physiol 47:987–1001, 1964
12. Riker WF Jr: Prejunctional effects of neuromuscular blocking and facilitatory drugs. Muscle Relaxants. Edited by Katz RL. New York, North-Holland Publishing, 1975, pp 59–102
13. Riker WF, Baker T, Sastre A: Electrophysiological and clinical aspects of glucocorticoids on certain neural systems, Current Topics in Neuroendocrinology, vol. 2, Adrenal Actions on the Brain. Edited by Gaten D, Phaff DW. New York, Springer-Verlag, 1982, pp 69–105
14. Bowman WC, Webb SN: Tetanic fade during partial transmission failure produced by non-depolarizing neuromuscular blocking drugs in the cat. Clin Exp Pharmacol Physiol 3:545–555, 1976
15. Stanec A, Baker T: Prejunctional and postjunctional effects of tubocurarine and pancuronium in man. Br J Anaesth 56:607–611, 1984
16. Miller RD, Rupp SM, Fisher DM, Cronnelly R, Fahey MR, Sohn YJ: Clinical pharmacology of vecuronium and atracurium. ANESTHESIOLOGY 61:444–453, 1984
17. Miller RD: The priming principle. ANESTHESIOLOGY 62:381–382, 1985
18. Mehta MP, Choi WW, Gergis SD, Sokol MD, Adolphson AF: Facilitation of rapid endotracheal intubations with divided doses of nondepolarizing neuromuscular blocking drugs. ANESTHESIOLOGY 62:392–395, 1985
19. Schwarz S, Ilias W, Lackner F, Mayrhofer O, Foldes FF: Rapid tracheal intubation with vecuronium: The priming principle. ANESTHESIOLOGY 62:388–391, 1985