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# EFFECTS OF CHRONIC ADMINISTRATION OF TWO NEWLY SYNTHESIZED NONDEPOLARIZING NEUROMUSCULAR BLOCKING AGENTS

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## ABSTRACT

The potential changes in neuromuscular blockade after chronic (>24 h) administration of sub-paralytic doses of two newly introduced aminosteroidal muscle relaxants were investigated. Org-9426 (rocuronium) and Org-9487 were studied in the *in vivo* guinea pig gastrocnemius muscle-sciatic nerve preparation. The problems seen after prolonged administration of muscle relaxants are thought to arise from up-regulation of AChRs and this phenomenon may be responsible, at least in part, for prolonged muscle paralysis, development of resistance to muscle relaxants and generalized muscle weakness seen with chronic administration. After two weeks of chronic treatment, using implanted osmotic mini-pumps delivering sub-paralytic doses of the test drugs, responsiveness to the muscle relaxants altered significantly so that higher doses were required in the drug pre-treated groups to produce the same twitch block as in matched control animals. A possible explanation for this observation is that, following chronic treatment, up-regulation of AChRs occurs at the neuromuscular junction, thus increasing the required doses in the drug pre-treated animals as compared with the control group.

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**Keywords**: Up-regulation, Neuromuscular junction, Chronic administration, Org-9487, Org-9426.

## INTRODUCTION

Neuromuscular blocking agents are commonly administered to patients in the Intensive Care Unit (ICU). In the presence of an antagonist or other clinical conditions that may decrease the concentration of ACh, there is an up-regulation (increased number) of AChRs and subsequently there will be increased sensitivity to agonists and decreased sensitivity to antagonists. At the neuromuscular junction these effects have been reported following disuse muscle atrophy,[6,9,26] thermal and direct muscle trauma,[3,13,15,19] infection,[24] and chronic treatment (>24 h) with competitive AChR antagonists.[1,2,11,18] The increased sensitivity to agonists, in some patients, may lead to a lethal hyperkalemic response to suxamethonium.[7,9] Down-regulation (decreased number) of the AChRs occurs during continuous agonist stimulation of the receptors due to internal factors, i.e. internalization, decreased synthesis or increased breakdown of AChRs.[14] Typically, down-regulation is associated with decreased sensitivity to agonists such as ACh or suxamethonium and increased sensitivity to antagonists. The terms "up- and down-regulation" generally refer to changes in availability or reactivity of receptors, but these changes usually do not involve or intimate a change in

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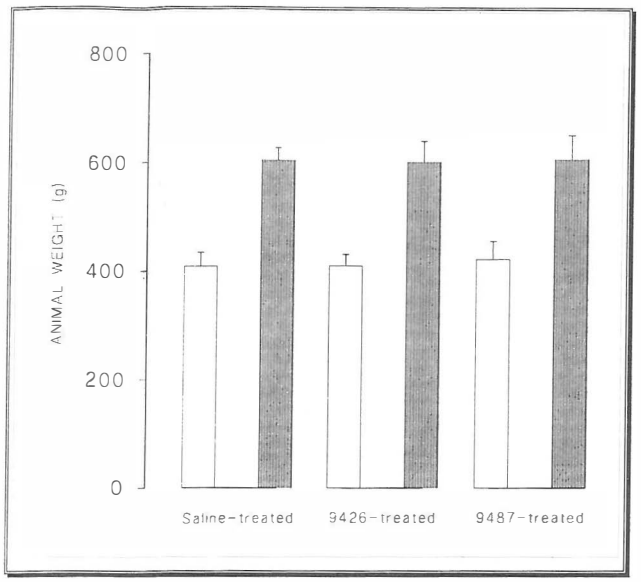


**Fig. 1.** The effect of chronic sub-paralytic doses of neuromuscular blocking drug-treatment on guinea pig body weight. The first group was treated with saline as the control group and the other two groups were treated with drug using osmotic mini-pumps for two weeks. Open columns show animal weight before implantation of mini-pumps and solid columns show animal body weight after two weeks of drug treatment. The points were plotted for at least 8 animal weights in each group and bars indicate S.E.M. values of the mean points. The differences between each group of data were considered to be statistically significant at the level of $p<0.05$.

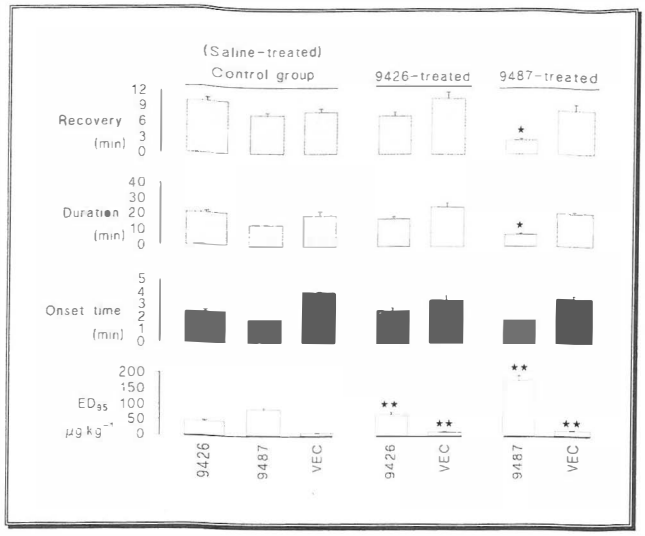


**Fig. 2.** The time course of neuromuscular block of acute treatment with Org-9426 or Org-9487 on the *in vivo* gastrocnemius-sciatic nerve preparation of anesthetized guinea pigs stimulated indirectly (0.1 Hz for 0.2 ms). Each axis break shows different treatments used for each group of animals. Vecuronium was used as control drug in all three groups. The differences between the obtained data for $ED_{95}$, duration, and recovery in each animal experimental group were considered to be statistically significant (Student's paired t-test) at the level of $^{**}p<0.05$ and $^{*}p<0.03$. The complete results of the study are given in Table I.

amino acid composition of the receptor subunits.[17] Whether chronic exposure of central or peripheral AChRs to an antagonist result in proliferation of that receptor is unclear. Org-9426 (rocuronium), a recently introduced intermediate compound, and Org-9487, a novel potentially alternative compound for suxamethonium (succinyldicholine) were tested. In this study the hypothesis that chronic infusion of these two competitive antagonists of acetylcholine receptors (AChR), even in the absence of immobilization or paralysis, may generate up-regulation of the AChR and hence tolerance to the competitive nondepolarizing neuromuscular relaxants, was investigated.

## MATERIALS AND METHODS

The compounds were obtained from Organon Scientific Development Group, Newhouse, Scotland. Male Dunkin-Hartley guinea pigs weighing 400 g were used. Animals were allowed to orientate in the animal unit for at least seven days before commencing the study. The first part of the experiment (insertion of the osmotic mini-pumps) was performed under aseptic conditions. The animals were anesthetized with pentobarbitone sodium (35-45 mg/kg i.p.) and subsequent doses were given as required. After the fur on the back of the neck was shaved and the skin was washed with an isopropyl alcohol solution, an incision was made about 2 cm in length in the interscapular area of the back and a subcutaneous pocket was made by blunt dissection. After insertion of the osmotic pumps, the incision was closed using 2-0 silk, the wound was washed with isopropyl alcohol and the animal was wrapped in bubble-plastic to avoid loss of temperature and left in it until complete recovery before being returned to its cage in the animal unit. Animals received careful postoperative care, including food and drink *ad libitum* and exposure to rhythmic light and dark environmental cycles. In each animal an osmotic mini-pump was filled with either 2.1 mg of Org-9426 (rocuronium), 3.6 mg of Org-9487 or saline (0.9% w/v) and inserted into the subcutaneous pocket. The mini-pumps were filled to deliver a steady rate of drug solution and this amount of infusion was assumed to be constant for two weeks. The control guinea pigs received the same volume of saline. To obtain some data to inform the choice of doses for the infusion of the test drugs, dose-response data ($ED_{50}$) for rocuronium and Org-9487 were established employing guinea pigs without infusion mini-pumps. The $ED_{50}$ values for block of twitch responses of the

**Table I.** The summary results from infusion study. Effective doses and time course of neuromuscular blocking effects of Org-9426 and Org-9487 on the *in vivo* guineapig gastrocnemius-sciatic nerve preparation. The effective concentrations were chosen to produce 80-90% twitch depressions. Results are mean ± S.E.M. and "n" is the number of experiment. Dose is the concentration of drug used in μg/kg to produce 80-90% twitch depressions. Inhibition is percentage of depression produced by effective concentrations. Onset is the time from drug injection to the first maximally depressed twitch (min). Duration is time from injection to 90% twitch recovery (min). Recovery index is time taken to recovery from 75% of the block to 25% of the block (min).

| $ED_{95}$ μg/kg | $ED_{50}$ μg/kg | Recovery (min) | Duration (min) | Onset (min) | Inhibition % | Dose μg/kg | Drug | |
|---|---|---|---|---|---|---|---|---|
| 45.4±3.0 | 34.7±5.2 | 9.95±0.6 | 21±1.1 | 2.45±0.2 | 87.6±1.03 | 42±2.7 | Org-9426 n=4 | Saline-pre-treated group |
| 7.85±0.8 | 4.5±0.35 | 8.1±0.7 | 20±2.8 | 4.1±0.1 | 81.5±0.6 | 5.3±0.3 | Vec n=3 | |
| 84.0±4.4 | 59.9±13.5 | 7.4±0.5 | 13.7±0.5 | 1.8±0.11 | 89.5±1.1 | 81.8±5.8 | Org-9487 n=5 | |
| 11.95±1.74 | 8.3±1.2 | 10.8±1.2 | 25.4±3.0 | 3.5±0.4 | 86.3±2.6 | 10.6±0.9 | Vec n=5 | ORG-9426 pre-treated group |
| 68.99±7.42 | 49.5±3.5 | 7.4±0.8 | 17.8±1.5 | 2.7±0.2 | 88.8±2.5 | 65.4±8.2 | Org-9426 n=7 | |
| 15.6±1.65 | 10.7±1.3 | 8.4±1.2 | 21.2±1.1 | 3.6±0.2 | 83.2±3.7 | 12.7±1.3 | Vec n=5 | ORG-9487 pre-treated group |
| 178.1±16.2 | 107.2±13.0 | 2.7±0.25 | 7.8±0.6 | 1.9±0.03 | 84.4±3.9 | 174.8±9.1 | Org-9487 n=7 | |

gastrocnemius muscle for Org-9426 and Org-9487 in non-implanted guinea pigs were 35 μg/kg and 60 μg/kg respectively. The $ED_{50}$ values for each drug were used to calculate the exact delivery rate for each drug. It was previously showed[11] that an effect of vecuronium and tubocurarine can be detected when it is delivered at a constant rate of 2.75% of its $ED_{50}$ per hour. Therefore, as the test drugs have one-seventh the potency of vecuronium, the infusion rate of 1.65 μg/kg/h and 0.96 μg/kg/h was used for Org-9487 and Org-9426 respectively.

The animals were reanesthetized two weeks after mini-pump implantation in the same manner. Animals, once fully anesthetized, were artificially ventilated via a tracheal cannula with room air (100 mL air for 100 g animal body weight). Further details of surgical procedures to gain access to the jugular vein, blood and heart rate monitoring, drug administration and stimulus parameter were performed according to the method of Hogue et al.[11] The twitch responses of gastrocnemius muscle were allowed to stabilize for at least 10 minutes prior to injecting any drugs. Each drug-treated animal received the same drug as they were treated with via the mini-pumps. In addition, vecuronium bromide was used as the control drug in all three experimental groups. After stabilization of the twitch, a desired dose of drug solution was administered through the jugular vein to achieve around 20% and 80% of twitch depression of the gastrocnemius muscle. The ability of the drug to reduce the twitch response before administration of the test compound was expressed as percentage of control twitch force as follows:

$$\%\text{Block} = 100 \times \frac{T_C - T_B}{T_C}$$

where $T_C$ is the control twitch force and $T_B$ is twitch force after injection of the test compounds into each animal.

The effective doses of each drug for suppression of twitch height (tension) to 50% and 95% of baseline values ($ED_{50}$ and $ED_{95}$) were then calculated and averaged for all three groups of the experimental animals. At the end of the experiments the animals were sacrificed with an overdose injection of anesthetic followed by air. The differences between the data were considered to be statistically significant at the level of $p < 0.05$ using one-way analysis of variance (ANOVA).

## RESULTS

Daily evaluation of the activity of the animals showed no obvious differences between the groups of control

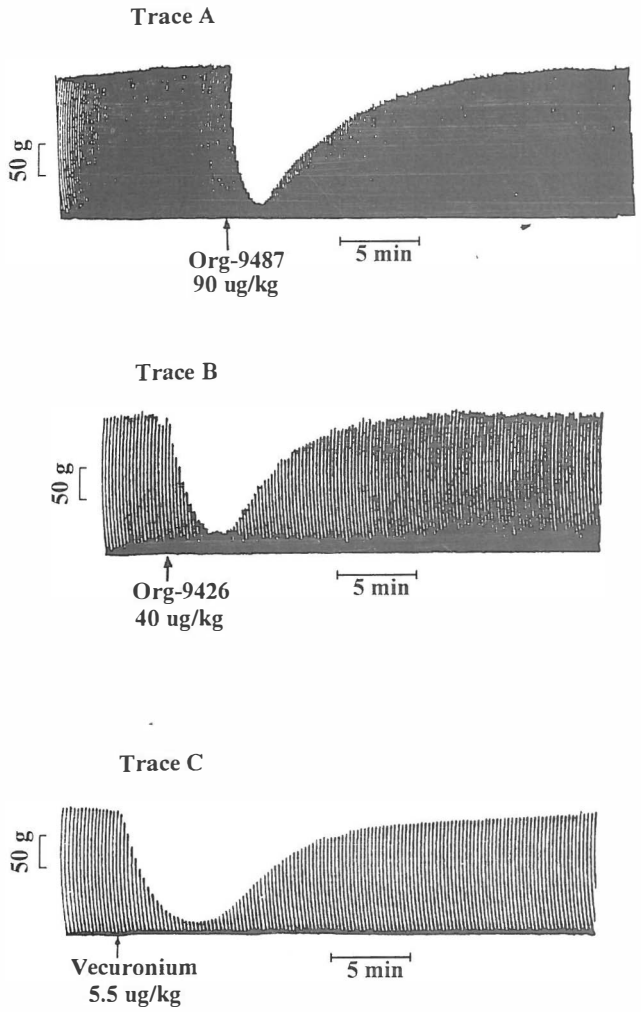


**Fig. 3.** Representative traces for saline pre-treated guinea pigs. Trace A is Org-9487 (90 μg/kg), trace B is Org-9426 (40 μg/kg) and trace C is vecuronium (5.5 μg/kg) used as control drug in this experiment.

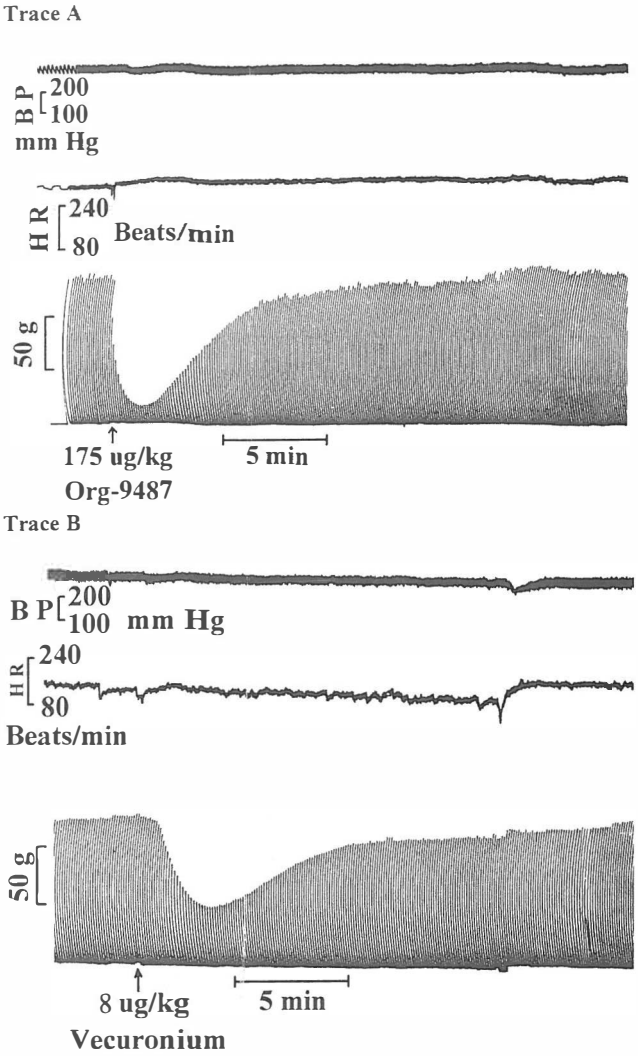


**Fig. 4.** Representative traces for Org-9487 pre-treated guinea pigs. Trace A is Org-9487 (175 μg/kg) and trace B is vecuronium (8 μg/kg) used as control drug in this experiment.

(saline-treated) and drug pre-treated animals. The results obtained from animal body weight in all groups were treated by one way analysis of variance and they showed no statistical significance between each animal-pairing group at the level of $p<0.05$ (Figure 1). However, for before or after treatment weights, in all groups, there was a statistically significant increase in weight over the two-week experimental period (Fig. 1, $p<0.05$).

As Table I indicates, the $ED_{95}$ for vecuronium, Org-9426 (rocuronium), and Org-9487 in the saline-treated group was 7.85±0.76, 45.4±2.96 and 84±4.4 μg/kg, respectively. However, following chronic infusion with the test compounds, it can be clearly seen from Table I that effective doses to produce the same neuromuscular blocking effect had to be increased up to two-fold. To produce the same twitch depression as obtained in the saline-treated group the required concentrations were increased in the rocuronium-pretreated group to 12.0±1.74 and 69.0±7.4 μg/kg for vecuronium and rocuronium, respectively and in the Org-9487-pretreated group to 15.6±1.7 and 180±16 μg/kg for vecuronium and Org-9487, respectively. The results are summarized in Table I and the time course of neuromuscular block produced by the test compounds is shown in Fig. 2. Using one-way analysis of variance it was found that the difference between the required concentrations to produce neuromuscular inhibition ($ED_{95}$, μg/kg) to the test muscle relaxants in each group was statistically meaningful (Table I, $p<0.05$). Representative traces for control (saline-treated) and drug-treated guinea pigs are given in Figures 3-5.

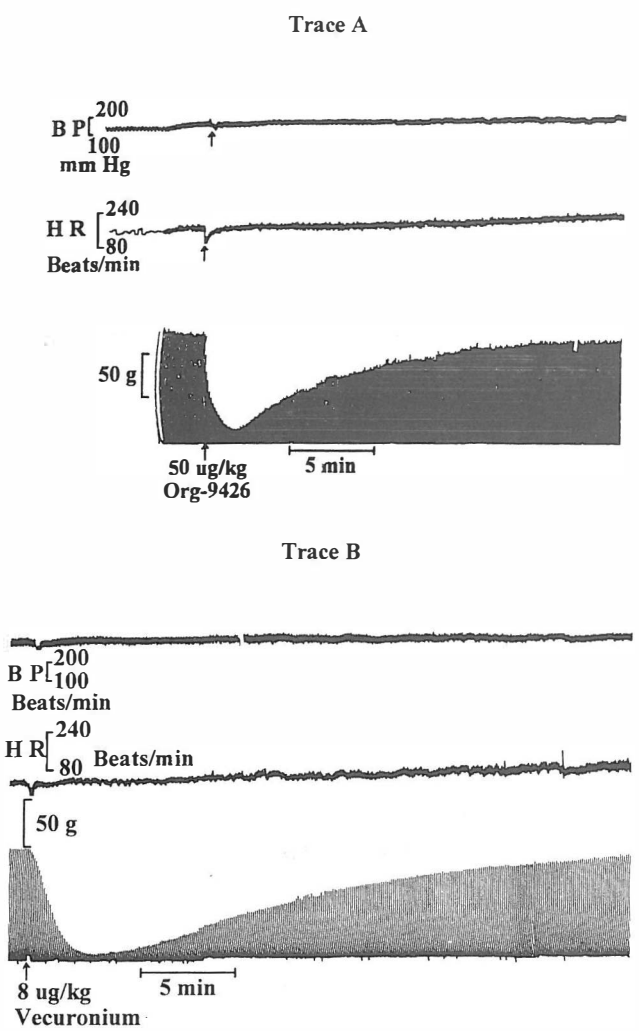


**Fig. 5.** Representative traces for Org-9426 (rocuronium) pre-treated guinea pigs. Trace A is Org-9426 (rocuronium; 50 μg/kg) and trace B is vecuronium (8 μg/kg) used as control drug in this experiment.

## DISCUSSION

Up-regulation of AChRs is accompanied by supersensitivity of the receptor to acetylcholine.[28] Increasing sensitivity of the surface membranes of skeletal muscle to acetylcholine is related to a spread of receptor sites beyond the neuromuscular junction; extrajunctional area and chronic treatment with neuromuscular blocking agents significantly increases this sensitivity.[4] In general, using sub-paralytic regimens of muscle relaxant diminished the responsiveness to and produced tolerance to neuromuscular blocking drugs. However, increased responsiveness can occur when higher chronic doses of these drugs are used.[17,28] The extrajunctional receptor sites have binding properties for neuromuscular nondepolarizing muscle relaxants that are slightly different from those properties of junctional receptors.[4] Mature/innervated AChRs are composed of $\alpha\beta\alpha\delta\varepsilon$ subunits. When there is a clinical neuromuscular dysfunction such as denervation neuronal syndromes, e.g., lower motor neuron injury or direct muscle injury involving a lesion of the sciatic nerve,[11] upper motor neuron denervation such as paraplegia and cerebral hemorrhage[12] and closed head injury,[5,23] immobilization and disuse muscle atrophy [6,9,26] such as seen in ICU patients, the immature or denervated AChR is synthesized with a subunit composition of $\alpha\beta\alpha\gamma\delta$, i.e. the immature type.[8,10,16,20,21] The mature AChRs are metabolically stable, with a half-life of approximately two weeks, whereas denervated or immature AChRs have a metabolic half-life of less than 24 hours.[22,25] What is not clear is if, or how, these new AChRs located in the extrajunctional area can modify neuromuscular transmission. The possibility proposed[17] is that the increasing number of AChRs at the extrajunctional area may act as a sink for accumulating neuromuscular blocking agent molecules. Therefore, in the osmotic mini-pump implanted animals with rocuronium and Org-9487 it is likely that more of the acutely administered muscle relaxant is being bound at extrajunctional areas, hence increasing the dose required to block neuromuscular transmission. Chronic constant infusion of rocuronium or Org-9487 altered the drug concentration-responses so that significantly higher doses (equi-effective doses; $ED_{95}$) were required in the experimental groups (Table II and Fig. 2, $p<0.05$) than in the saline-treated group to produce the same twitch depression. It has been suggested[28] that changes in the number of AChRs, due to acute muscle relaxant-treatment, could be an important factor in the development of drug tolerance in ICU patients.

It has been argued that immobilization alone can result in up-regulation of AChRs[4] and resistance to competitive antagonists.[9] Previously, it has been shown [1,2] that chronic treatment with various neuromuscular blocking agents increases the number and distribution of AChRs in the rat diaphragm. The authors concluded that immobilization was responsible for the receptor up-regulation. It is currently believed that the prolonged paralysis or muscle weakness observed in ICU patients, after chronic administration of neuromuscular blocking agents, is due to patient inactivity and this could lead to up-regulation of AChRs at the neuromuscular junction.[11,17] However, as Figure 1 shows, statistically significant increases in animal weight gain were observed within all groups after the drug-pretreatment period ($p<0.05$). This was evidence for absence of immobilization or animal inactivity due to chronic administration of the test muscle relaxant. All animals were able to move freely about their cages and ran away when attempts were made to remove them from their cages. The absence of any difference in the weight gain among the three groups confirmed what was apparent from daily visual evaluation. Thus any suggested increase in AChR in

the experimental groups was not because of immobilization or animal inactivity and this might prove the hypothesis that up-regulation of AChR can be induced by competitive antagonists even in the presence of preserved muscle activity.

The presently available muscle relaxants were not developed or completely evaluated for prolonged administration in the ICU. Many problems seen in prolonged use, at least in part, are attributed to the use of muscle relaxants or related to the immobilization resulting from the acute use of these agents in ICU patients. However, due to the complexity of clinical conditions in this category of patients and the presence of concomitant medications as well as other factors which directly affect neuromuscular function, such as reduced motor nerve activity, impaired muscle blood flow, sepsis and endotoxemia, it is difficult to single out muscle relaxants as the main cause of the observed complications. As Wierda et al.[27] suggested, long duration administration of the currently available neuromuscular blocking agents should only be applied in exceptional situations, and only when there is no alternative strategy available. In these situations it seems rational to take necessary action concerning monitoring of the neuromuscular blocking effects and titration of the dose to a predetermined end-point. In this study based on the observed results here it can be extrapolated that chronic muscle relaxant treatment in patients might produce proliferation of AChRs. This effect together with the patient's inactivity during the stay in the ICU, i.e. disuse atrophy, which is believed can produce up-regulation of AChRs, may influence the patient's requirement for higher doses of nondepolarizing relaxants to maintain paralysis.

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