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Resistance to rocuronium of rat diaphragm as compared with limb muscles

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ABSTRACT

Background: Skeletal muscles are composed of different muscle fiber types. We investigated the different potency to rocuronium among diaphragm (DIA), extensor digitorum longus (EDL), and soleus (SOL) *in vitro* as well as to investigate the differences of acetylcholine receptors (AChRs) among these three typical kinds of muscles.

Materials and methods: The isolated left hemidiaphragm nerve-muscle preparations, the EDL sciatic nerve-muscle preparations, and the SOL sciatic nerve-muscle preparations were established to evaluate the potency to rocuronium. Concentration-response curves were constructed and the values of IC_{50} were obtained. The density of AChRs at the end plate and the number of AChRs per unit fiber cross fiber area (CSA), AChR affinity for muscle relaxants were evaluated.

Results: The concentration-twitch tension curves of rocuronium were significantly different. The curves demonstrated a shift to the right of the DIA compared with the EDL and SOL ($P < 0.01$), whereas no significant difference was observed between EDL and SOL ($P > 0.05$). IC_{50} was significantly largest in DIA, second largest in SOL, and smallest in EDL ($P < 0.05$). The number of AChRs per unit fiber CSA was largest in DIA, second largest in EDL, and smallest in SOL ($P < 0.01$ or $P < 0.05$). The DIA showed the lowest affinity of the AChRs, whereas the SOL showed the highest affinity.

Conclusions: The resistance to rocuronium of DIA compared with EDL and SOL was verified. The DIA was characterized by the largest number of AChRs per unit fiber CSA and the lowest affinity of the AChRs. Although compared with SOL, EDL was proved to have larger number of AChRs per unit fiber CSA and the lower affinity of the AChRs. These findings may be the mechanisms of different potency to rocuronium in DIA, EDL, and SOL. The results of the study could help to explain the relationship between different composition of muscle fibers and the potency to muscle relaxants. Extra caution should be taken in clinical practice when monitoring muscle relaxation in anesthetic management using different muscles.

1. Introduction

Skeletal muscles are composed of different muscle fiber types, which includes type I and type II (comprising type II a, type II b, and type II x). Previous studies have reported that neuromuscular junction (NMJ) morphology varies across muscle fiber types. For example, NMJs at type I and IIa fibers are smaller and less complex compared with NMJs at type IIx

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and/or IIb fibers [1]. NMJ is the junction between a motor neuron and a muscle fiber, which directly modulates muscle activity by conducting nerve impulse to induce muscle contractions [2]. NMJ achieving its physiologic functions depends on its presynaptic and postsynaptic factors. Presynaptic factors include the modulation of acetylcholine (ACh) release from motor nerve terminals, whereas postsynaptic factors include acetylcholine receptors (AChRs) and the rate of ACh hydrolysis by acetylcholinesterase (AChE) [3]. AChRs accept ACh released from the nerves, thus evoking a muscle impulse.

Findings of previous clinical practices have indicated that different monitoring positions for muscle relaxation in anesthetic management represented different extent of neuromuscular blockade. Some clinical reports showed that the orbicularis oculi muscle was more resistant to non-depolarizing muscle relaxants (NDMR) than the adductor pollicis muscle [4]. In addition, Saitoh *et al.* [5,6] found that neuromuscular block recovered more quickly in the great toe than in the thumb after the administration of vecuronium in their series of reports. They also revealed that the mechanism underpinning this observation was that the flexor *hallucis brevis* muscle contains more type II fibers than the adductor pollicis muscle [7] and type I muscle fiber was more resistant to NDMR [5,8–10]. Additionally, compared with the adductor pollicis muscle, dose-response curves of the diaphragmatic response demonstrated a shift to the right [11,12]. Furthermore, the recovery of the diaphragm (DIA) -evoked response occurred earlier than at the adductor pollicis muscle [13,14]. Besides the factors that might influence the metabolism and absorption of NDMR such as blood flow, oxygen consumption, and distance from the heart and heart output [15,16], different muscle fiber composition of these muscles was a well-known explanation to these findings. However, there are contradictory opinions about the relationship between the neuromuscular blockade and the composition of muscle fibers in different muscles. As we have mentioned above, type I muscle fiber is more resistant to NDMR [17,18]. So muscles with higher contents of type I muscle fibers are more resistant to NDMR than those with higher type II muscle fibers [19]. Chen *et al.* [3,20] reported that chronic dexamethasone treatment led to desensitization of the rat DIA to rocuronium and that susceptibility to rocuronium associated with decreased muscle fiber type II. Nevertheless, there are other reports demonstrated that muscle type composition cannot explain the difference in muscle relaxation effect [3,21]. Some researchers drew a conclusion that there was no association between the composition of muscle fiber types and sensitivity to muscle relaxants [21,22]. Zhou *et al.* [21] showed that the *orbicularis oris* muscle contained fewer type I fibers than the gastrocnemius muscle but displayed more desensitization to rocuronium. The density of AChRs at the end plate and the number of AChRs per unit fiber cross-section area (CSA), AChRs affinity for muscle relaxant may play the most important role in the sensitivity of skeletal muscles to NDMR [21].

Therefore, the aim of the present study was to investigate the different responses to rocuronium among DIA (represents mixed muscles), extensor digitorum longus (EDL) (with predominantly fast-twitch fibers), and soleus (SOL) (with predominantly slow-twitch fibers) *in vitro* as well as to investigate the differences of AChRs among these three typical kinds of muscles. We compared the density of AChRs at the end plate and the number of AChRs per unit fiber CSA, AChRs affinity for muscle relaxants. Therefore, the purpose of the present study was to draw inferences about the relationship between the functional results and morphologic changes.

2. Materials and methods

2.1. Animals

The study was approved by the Animal Care Committee in Shanghai Jiaotong University (Shanghai, China). Twenty male Sprague–Dawley rats (Experimental Animal Center of the School of Medicine, Shanghai Jiaotong University, Shanghai, China), weighing 200–240 g, were housed in groups of three. They were fasted but allowed to have free access to water and food before the experiments.

2.2. Muscle preparations

12 rats were killed with 60 mg/kg pentobarbital intraperitoneally. The isolated left hemidiaphragm nerve-muscle preparations, the EDL sciatic nerve-muscle preparations, and the SOL sciatic nerve-muscle preparations were established for indirectly electrical stimulation as described previously [23–25]. Body temperature was maintained at 37°C using a heating blanket and radiant heat.

The left hemidiaphragm with attached phrenic nerve, central tendon, and rib cage intact was rapidly removed from each of the rats to investigate rocuronium potency. The right hemidiaphragm were rapidly removed for morphologic analysis. Either the EDL or the SOL was exposed in one leg. After measurements were completed for one muscle, the other was then exposed in the other leg. The SOL was exposed by sectioning the tendons connecting the plantaris and gastrocnemius muscles to the heel and reflecting the muscles back. Silk thread was attached to the distal tendon of the SOL and the tendon was sectioned. The muscle was then carefully freed of surrounding tissues, ensuring the blood supply remained intact, and the sciatic nerve was sectioned. The EDL was prepared in the similar manner after first exposing the muscle by reflection of the anterior tibialis muscle. The silk sutures were tied to the proximal and distal tendons of the EDL and/or SOL muscles and the muscles were removed, tendon to tendon. The EDL or the SOL muscles on the other leg were removed simultaneously for morphologic analysis.

The isolated nerve-muscle preparations were dipped immediately into Plexiglass chambers (ALC-M System for Isolated Tissue-Organ Research; Shanghai Alcott Biotech, Shanghai, China; 40 mL in volume) filled with Krebs solution, maintained at 37°C, and bubbled with 95% oxygen and/or 5% CO_2. The composition of the Krebs solution was as follows: 137 mM NaCl, 4 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM KH_2PO_3, 12 mM $NaHCO_3$, and 6.5 mM glucose, with a pH 7.40 ± 0.05 during bubbling.

EDL, SOL, and DIA strips of the other side were dissected for ATPase staining. The strips for ATPase staining were embedded separately in plastic holders and rapidly frozen in