Effect of acute peritonitis on rocuronium-induced intraperitoneal pressure reduction and the uptake function of the sarcoplasmic reticulum

JIAN-YOU ZHANG[1], YUAN GONG[2], MEI-RONG YANG[2], JIN WU[2] and SHI-TONG LI[1]

[1]Department of Anesthesiology, Shanghai General Hospital of Nanjing Medical University; [2]Department of Anesthesiology, Shanghai General Hospital, Shanghai 200080, P.R. China

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Abstract. Previous studies have reported the incomplete relaxation effect of neuromuscular blockers on skeletal muscles in acute peritonitis (AP) and other inflammatory processes; however, the underlying mechanisms responsible for this effect have not yet been satisfactorily identified. The impaired removal of cytosolic Ca^{2+} through sarcoendoplasmic Ca^{2+}-ATPase (SERCA) and defects in sarcoplasmic reticulum (SR) Ca^{2+} uptake are the major contributing factors to diastolic dysfunction. Previous studies on the effects of neuromuscular blockers have primarily focused on neuromuscular transmission. Because of the reduced calcium uptake in the SR itself, even when neuromuscular transmission is fully blocked, the muscle is not able to relax effectively. In the present study, the impact of AP on rocuronium-induced intraperitoneal pressure reduction and rectus abdominal muscle relaxation, and SERCA uptake function was investigated. AP was induced via gastric perforation and changes in the intraperitoneal pressure before and after the administration of rocuronium were recorded. Muscle contractile properties, uptake and release functions and SERCA activity in the rectus abdominal muscles of AP model rats were measured. The half-relaxation time in the AP group was significantly prolonged compared with that in the control group ($P<0.01$). The peak rate of SR Ca^{2+} uptake for whole muscle homogenates was significantly reduced ($P<0.05$) in AP model rats without reduction of the rate of Ca^{2+} release evoked through $AgNO_3$. In conclusion, gastric perforation-induced AP attenuates the intraperitoneal pressure-reducing effect of rocuronium, and AP induces diastolic dysfunction of the rectus abdominal muscle. The SR Ca^{2+}-ATPase uptake rate was also reduced by AP.

Correspondence to: Dr Shi-Tong Li, Department of Anesthesiology, Shanghai General Hospital of Nanjing Medical University, 100 Haining Road, Hongkou, Shanghai 200080, P.R. China
E-mail: lishitongs@hotmail.com

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Introduction

D-tubocurarine has been used as a myorelaxant drug to provide muscle relaxation in anaesthesia since it was identified in 1942 (1). However, rocoronium is now used widely as a general anaesthetic (2). Myorelaxant drugs are used for various surgeries under general anaesthesia. For anaesthesiologists, the benefits of using neuromuscular blocking agents include the amelioration of intubating conditions and the reduction of postoperative laryngeal hoarseness (3). In clinical practice, many surgeons strongly advocate complete intraoperative muscle paralysis to improve surgical conditions (4). In a previous study, 98% of patients undergoing radical retropubic prostatectomy with neuromuscular blockade had acceptable surgical conditions compared with 72% of patients who had not received a neuromuscular blockade (5). However, during emergency surgery for acute peritonitis (AP) and other septic progresses, tetanic contraction of the abdominal wall muscles has been observed (6-8), and in this surgery the incomplete muscle relaxation effect of neuromuscular blockers and increased dosage of myorelaxant drugs is a serious problem that affects the success of the surgery. A number of pharmacokinetic and pharmacodynamic factors, including acetylcholine receptors (AChRs) could potentially contribute to this effect (6-8). Studies on the effects of neuromuscular blockers have primarily focused on neuromuscular transmission in systemic inflammation models (6). The regulation of calcium levels in skeletal muscle cells is important for each contraction and relaxation (9). The increase in inflammation-induced cytokines, free radicals and T-cell infiltration in skeletal muscle tissue induces changes in Ca^{2+} homeostasis and decreases the Ca^{2+} uptake function of the sarcoplasmic reticulum (SR) (10-14). The SR is a special membrane system that controls the cytosolic free calcium concentration, and SERCA is an integral membrane protein that pumps calcium ions from the cytosol to the lumen of the SR and is essential for mediating contraction and relaxation (15).

The change in intracellular calcium level may contribute to the incomplete relaxation effect of non-depolarizing neuromuscular blockers (NDNBs) on abdominal wall muscles; however, little is known about changes in SR function and sarcoendoplasmic Ca^{2+}-ATPase (SERCA). The aim of the present study was, therefore, to investigate the relaxation effect of rocuronium on the abdominis muscle and whether the function of SERCA was changed in a rodent gastric perforation-induced AP model.

Materials and methods

Animal description and care. A total of 40, male Sprague-Dawley rats weighing 225-265 g, aged five weeks, were purchased from Shanghai Animal Research Centre (Shanghai, China). Upon arrival at the animal house, the rats were placed in cages (5 rats/cage) in an environmentally controlled room (21-24˚C and 40-60% relative humidity) with a 12 h light/dark cycle. A 2-week acclimation period was performed, during which standard rat food and water were provided *ad libitum* until the experiment. Rats weighing 245±9.5 g were sacrificed following the collection of the heart blood sample and rectus abdominis muscle by cervical dislocation following the intraperitoneal administration of 50 mg/kg pentobarbital sodium (Sigma-Aldrich; Merck KGaG, Darmstadt, Germany) anaesthetic injected. The experimental protocols were approved and supervised by the Ethics and Animal Care Committee of Shanghai General Hospital (Shanghai, China).

Surgical procedures. Rats were randomly assigned to 3 groups (n=12 each). These were the sham group (Control) and two study groups, 1 h (G1) and 2 h (G2). In G1 and G2 all measurements were made at 1 h and 2 h respectively following acute peritonitis was induced by gastric perforation (GP) (16,17). All rats were weighed and anesthetized via an intraperitoneal injection of pentobarbital sodium (50 mg/kg). The abdomen was subsequently opened via an upper left incision of 6-8 mm under sterile conditions. The stomach was gently mobilized and an anterior longitudinal gastronomy measuring 2 mm was performed at the edge of the stomach to the main stomach located at the greater curvature. The stomach was subsequently replaced in the abdominal cavity, and the abdomen was closed using 4.0 silk running and purse string sutures. Rats in the control group underwent laparotomy and sham GP.

An 18-gauge catheter was introduced under sterile conditions into the abdominal space at a depth of 2 cm and connected to a three-way stop valve. Air was continuously pumped into the abdominal cavity via one pathway of the three-way stop valve, and the intra-abdominal pressure (IAP) was subsequently measured using an electrical pressure transducer connected to the other pathway of the three-way stop valve until a volume of 105 ml was reached; the air volume was increased by 5 ml at a time, and the air and pressure volumes were recorded. A tracheostomy tube was subsequently intubated into the trachea, and a cannula was inserted into the tail vein to allow rocuronium (3.5 mg/kg) infusion manufactured by Organon International (Oss, The Netherlands). Air was injected into the abdominal cavity at a constant pressure of 15 mmHg. Mechanical ventilation was initiated after the administration of rocuronium (3.5 mg/kg) (18) and maintained until the recovery of spontaneous breathing. The IAP was monitored for 15 min.

Sample preparation. The heart blood samples were collected after IAP was measured and left to stand for 1 h at 37˚C and overnight at 4˚C to obtain the serum. The serum was subjected to cytokine analysis for tumor necrosis factor (TNF)-α, interleukin (IL)-6 and IL-13. TNF-α, IL-6, and IL-13 levels were determined via ELISA using commercially available ELISA kits (R&D Systems Inc., Minneapolis, MN, USA). Once animals were anesthetized with pentobarbital sodium, the sheath of rectus abdominis was removed, and the upper part of the rectus abdominis muscle (2 cm) was harvested and homogenized as described below for the SR assays. The left rectus abdominis muscle was removed for the assessment of muscle contractile properties.

Muscle contractile assessment. A strip with a width of ~8 mm and a length of ~3 cm of the left rectus abdominis muscle was immersed in a temperature-controlled organ chamber (37˚C) filled with Krebs solution and continuously perfused with a 95% O_2 and 5% CO_2 mixture. The modified Krebs solution comprised 137 mM NaCl, 4 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM KH_2PO_4, 12 mM $NaHCO_3$, and 6.5 mM glucose; the pH was adjusted to 7.40-7.50 via bubbling. One end of the strip was tied to a rigid support using a 3-0 silk suture and the other end was fastened to an isometric force transducer mounted to a micrometer (Shanghai Alcott Biotech Co., Ltd., Shanghai, China). Each strip was vertically positioned in a tissue chamber containing 40 ml Krebs solution. The muscle was placed between two platinum stimulating electrodes, and 12 μM d-tubocurarine (Sigma-Aldrich; Merck KGaG) was added to the Krebs solution to completely abolish neuromuscular transmission (19).

The strip was equilibrated for 15 min in the organ bath and subsequently placed at the optimal length (Lo), determined when the maximal tension was produced in a fixed electrical stimulation, and the micro-positioner and stimulation voltage were adjusted to obtain the maximum twitch force. The MPA2000m system (Shanghai Alcott Biotech Co., Ltd.) was used to supply and measure all electrical stimuli was supplied and isometric twitch tension for the *in vitro* experiment. The length of the strip was also measured. The following measurements were assessed using the stimulation at Lo.

Twitch characteristics. Two twitches (frequency 0.1 Hz, pulse duration 0.2 msec) were induced at Lo to determine the peak twitch tension (Pt) and time to peak tension (TPT). The half-relaxation time (1/2RT) was measured during the decline phase as the time required for the force to decline to 50% of the maximum. The mean values were used for further analysis.

Maximal tetanic tension. The maximal tetanic tension was recorded as the maximum tension elicited at 120 Hz.

Force-frequency relationship. Each muscle strip was stimulated (0.2 msec pulse duration, 250 msec train duration) at 7 different frequencies from 10-120 Hz in 10-Hz increments at 2-min intervals.

Fatigue protocol. Fatigue properties were determined using 330-msec stimulations (0.2 msec pulse duration, 670 msec train duration) repeated every sec at 25 Hz for 5 min (20). The ratio of the forces obtained after 120 sec was determined, and the first sec was defined as the fatigue index.

After all parameters were assessed, the strips were blotted dry and weighed. All force values were normalized to cross-sectional areas (CSA), calculated by dividing the muscle weight by the muscle length and specific density, as previously described (21).

SR calcium release and reuptake. The functional characteristics of SERCA were assessed using crude muscle homogenates. After weighing, the rectus abdominis muscle was placed in ice-cold homogenization buffer containing 250 mM sucrose, 20 mM N-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), 0.2% sodium azide and 0.2 mM phenylmethylsulfonyl fluoride (pH 7.5). The muscle was subsequently minced and homogenized on ice for three 15-sec bursts at 567 x g (Tissue-Tearor 985370; Biospec Products, Bartlesville, OK, USA) in 10 volumes of fresh buffer. The duration of tissue extraction and homogenization was typically 10 min. The homogenate was centrifuged at 1,600 x g for 15 min at 4˚C. The supernatant was rapidly frozen in liquid N_2 and stored at -80˚C for use in subsequent experiments. The protein concentration was determined using the bicinchoninic acid assay method.

The rate of Ca^{2+} uptake was kinetically obtained from the muscle fraction after measuring the fluorescence signal of the Ca^{2+}-binding dye fura-2 (pentapotassium salt; Sigma-Aldrich; Merck KGaA) using a fluorescence microplate reader (Varioskan Flash; Thermo Fisher Scientific, Inc., Waltham, MA, USA) as previously described (22-24). This method has been validated against the gold standard of SR vesicle preparations (16). The incubation buffer comprised 100 mM KCl, 20 mM HEPES, 7.5 mM pyrophosphate and 0.5 mM Mg^{2+} (pH 7.0, 37˚C). In addition, 2 μM free Ca^{2+} and 1 μM fura-2 pentapotassium salt were added as a Ca^{2+} indicator. Fura-2 was excited at 340 and 380 nm, and fluorescence emission was detected at 500 nm. A total of 10 μl homogenate protein and 190 μl incubation buffer (100 mM KCl, 20 mM HEPES, 7.5 mM pyrophosphate and 0.5 mM Mg^{2+}, supplied by Sigma-Aldrich; Merck KGaG) were loaded onto a plastic plate. Uptake was initiated after adding 10 μl of 20 mM Na_2-ATP. At 4 min after Na_2-ATP was added, Ca^{2+} release was initiated after the addition of 10 μl $AgNO_3$ (500 μM). Both Na_2-ATP and $AgNO_3$ were injected at a speed of 420 μl/sec into either well with the instrument dispensers. After dispensing, the plate was shaken. Between measurements, the dispensers were soaked in 70% alcohol for 30 min for 2 wash cycles and rinsed in distilled water 10 times. The data for the fluorescence signal were continuously recorded, and the experiment was performed at 20-22˚C. The ratio of the fluorescence at two excitation wave lengths, 340 and 380 nm, with background subtraction, was sampled using a SkanIt computer software, version 2.4.5 (Varioskan Flash; Thermo Fisher Scientific, Inc., Waltham, MA, USA) and converted into free (Ca^{2+}) based on the ratiometric method of Grynkiewicz *et al* (25). The free Ca^{2+} concentration (Ca^{2+})f was calculated using the following equation: $(Ca^{2+})f=Kd^{*}(Sf2/Sb2)^{*}(R-Rmin)/(Rmax-R)$. Kd represents the equilibrium constant for the interaction between Ca^{2+} and Fura-2. R represents the fluorescence ratio recorded at two excitation wavelengths (F340 and F380), and Rmin and Rmax are the fluorescence ratios under Ca^{2+}-free and Ca^{2+}-saturating conditions, determined at the completion of the assay after the addition of 3.5 mM ethylene glycol-bis (β-aminoethyl ether)-N, N,N',N'-tetraacetic acid and 5.0 mM $CaCl_2$, respectively (26). Sf2 and Sb2 are the fluorescence values under Ca^{2+}-free and Ca^{2+}-saturating conditions. Rmin, Rmax and Sf2/Sb2 were determined after each run.

The rates of SR Ca^{2+} uptake and release were determined as the steepest negative (uptake) and positive (release) slopes of the free Ca^{2+} vs. time curve, normalized to the total supernatant protein concentration. Uptake and release assays were performed in triplicate for each group.

Statistical analysis. All data are expressed as the mean ± standard error of the mean. The data were analyzed using an unpaired t-test and two-way analysis of variance. Significant differences between means were calculated using the Bonferroni post hoc test. Statistical analyses were conducted using SPSS 20.0 (IBM SPSS, Armonk, NY, USA). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Body weights and muscle preparation measurements. Similar weights were observed for rats in the control, G1 and G2 groups. The weight, length and cross-sectional areas of the rectus abdominis muscle preparations were not significantly different among the three groups, as shown in Table I.

Measurement of cytokines in the evolution of GP-induced AP. The systemic response in rats was characterized after detecting systemic levels of TNF-α, IL-6 and IL-13 in abdominal aorta blood at different time points (1 and 2 h) following the induction of GP. Serum IL-13 concentrations increased markedly during the first hour, and a mild increase was observed during the second hour. However, this difference was not statistically significant compared with the control group. GP rats were found to have elevated levels of serum IL-6 and TNF-α at 1 and 2 h, respectively, although the differences between the G1 group and the control group were not statistically significant. Notably, the serum levels of IL-6 and TNF-α in the G2 group were significantly increased ($P<0.01$ vs. control; Fig. 1).

IAP change following GP-induced AP and relaxation effect of rocuronium on the abdominal muscles. The intra-abdominal pressure and volume were measured following the induction of GP and sham surgery. With the development of GP-induced AP, IAP was significantly elevated. The volume-pressure relationship curves were fitted in different groups, and the curves in the G1 and G2 groups were significantly shifted leftward ($P<0.05$; Fig. 2). Additionally, the IAP gradually decreased following the administration of rocuronium (Fig. 3). However, the reduction in the IAP observed in the control group was significantly higher compared with that in the two GP groups ($P<0.05$).

Muscle contractile properties

Twitch characteristics. Table II shows the twitch characteristics of the abdominis rectus muscles obtained from sham and GP rats.

Table I. Rat body weights and rectus abdominis muscle strip dimensions.

		Muscle preparations		
Group	Body weight (g)	Weight (g)	Lo (cm)	CSA (cm^2)
Control	237.0±9.901	0.338±0.043	3.04±0.191	0.1055±0.012
G1	239.9±8.410	0.345±0.061	2.99±0.192	0.1089±0.016
G2	241.6±7.521	0.349±0.031	2.95±0.210	0.1123±0.122

All data are presented as the mean ± standard error of the mean. n=7. Lo, optimal length; CSA, cross-sectional area.

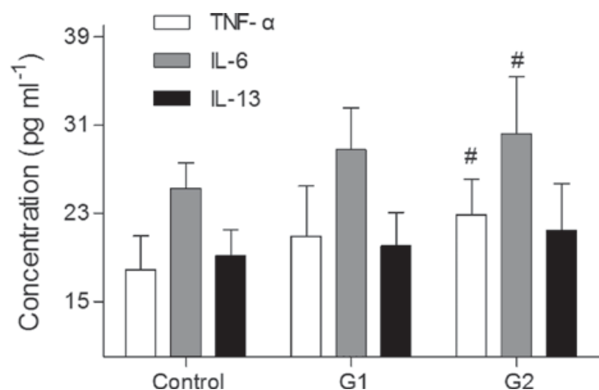

Figure 1. Systemic cytokine levels in the three groups. #P<0.01 vs. control group. TNF, tumor necrosis factor; IL, interleukin. n=12.

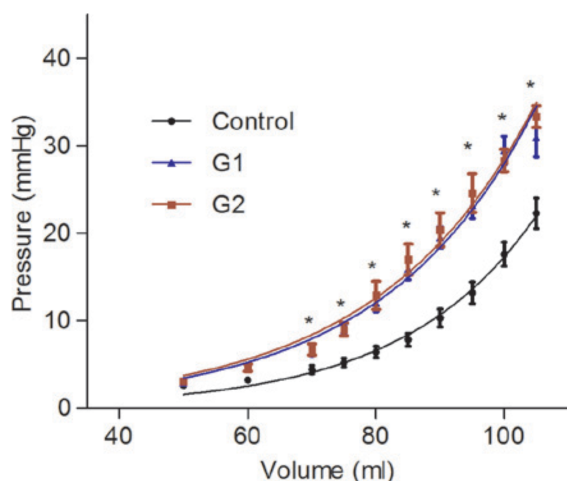

Figure 2. Fitted volume-pressure relationship curves in the three groups. Curves for G1 and G2 were shifted leftward significantly. *P<0.05 vs. control. n=12.

The Pt in the G2 group was significantly higher than that in the control group (P<0.05). In addition, the 1/2RT in the G2 group was significantly increased compared with that in the control group (P<0.01). TPT increased slightly in GP rats; however, this difference was not statistically significant.

Maximal tetanic tension. The peak tetanic tension in the G2 group was significantly higher than that in the control group (P<0.01). No significant difference was observed between the sham and G1 groups (Fig. 4A).

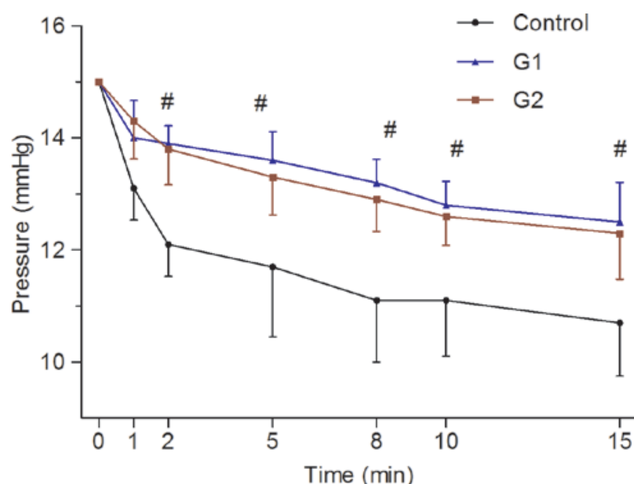

Figure 3. Change of IAP after the administration of rocuronium. #P<0.01 vs. control group. IAP, intra-abdominal pressure. n=12.

Force-frequency curve and fatigue properties. The effects of GP on the force-frequency curves of rectus abdominis muscles are shown in Fig. 4B. The force of the rectus abdominis muscle strip in response to each stimulus frequency is expressed in absolute values. The force-frequency curve for the sham group was significantly lower than those for the two GP groups (P<0.01). The forces at all frequencies aside from 10 Hz, were lower in the sham group than those in the G2 group (P<0.01). However, the differences between the G1 and sham groups were not significant. The fatigue indices for the G1 and G2 groups were significantly lower than those for the control group (P<0.05 and P<0.01, respectively; Table II).

SR Ca^{2+} uptake, release and activity. A schematic of the process is shown in Fig. 5A. The initial fast phase and slower second phase of Ca^{2+} release were measured in the present study. In rat rectus abdominis muscle, the peak rates of SR Ca^{2+} uptake for whole muscle homogenate were significantly reduced in the G1 and G2 groups by 50.7 and 47.7%, respectively (P<0.05; Fig. 5B). Furthermore, the amount of Ca^{2+} sequestered during loading was markedly reduced compared with the control group (P<0.05; Fig. 5C). The rates and amounts of Ca^{2+} release evoked through $AgNO_3$ in GP rats were also reduced compared those in with control rats. As shown in Fig. 5D, there was no significant difference in SERCA activity among the groups.

Table II. Muscle contractile characteristics and fatigue properties.

Group	Pt (kg/cm^2)	1/2RT (msec)	TPT (msec)	Fatigability index (%)
Control	0.0111±0.005	8.375±0.569	17.85±0.931	100.13±24.661
G1	0.0138±0.005	8.833±0.718	18.25±0.805	79.18±16.513^a
G2	0.0165±0.006^a	9.125±0.742^b	18.28±0.583	60.23±24.822^b

All data are presented as the mean ± standard error of the mean. n=12. ^{a}P<0.05 and ^{b}P<0.01 vs. control group. Pt, peak twitch tension; 1/2RT, half-relaxation time; TPT, time to peak tension.

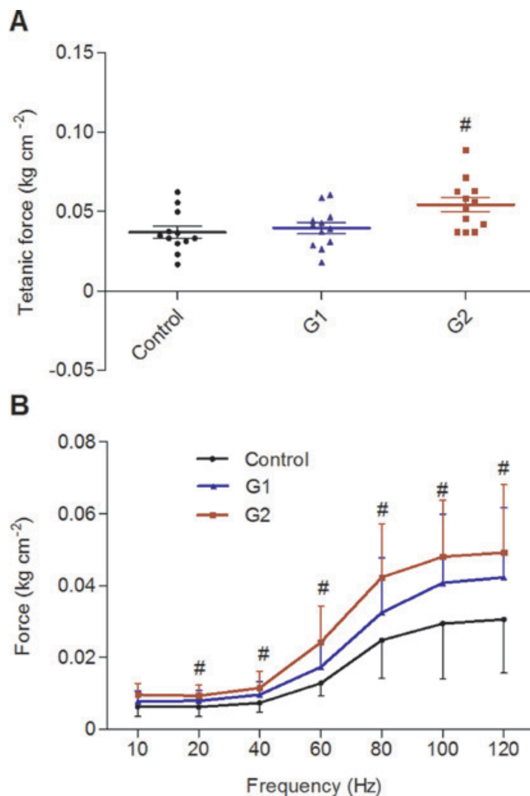

Figure 4. (A) Tetanic forces of the muscle strips in the Control group (closed circles), G1 group (closed triangles) and G2 group (closed squares). Each point represents an individual rat. The horizontal line in the middle represents the mean value of group. (B) Force-frequency relation curves in three groups. #P<0.01 vs. control group. All data are presented as the mean ± standard error of the mean. n=12.

Discussion

The results of the present study demonstrated that rocuronium administration does not reduce the intraperitoneal pressure in this animal model of GP-induced AP. However, AP significantly attenuated the effect of rocuronium on abdominal wall muscle relaxation, and impaired the SR Ca^{2+}-ATPase uptake rate.

In clinical practice, NDNBs exert an incomplete neuromuscular block effect on the abdominal wall muscle, despite effective doses, or frequently show rapid recovery from paralysis in the emergency operation of AP. Abdominal muscle and diaphragm contractions are detrimental in open abdominal surgery as they reduce visibility, so many surgeons utilize intraoperative muscle paralysis to improve surgical conditions. Higher doses of NDNBs may be necessary to generate muscle relaxation and improve surgical conditions (4). However, NDNBs have been associated with postoperative residual curarization. Furthermore, NDNBs has been reported to result in histamine release and cardiovascular alteration (27). In a previous study, residual paralysis was observed at 2 h following the administration of nondepolarizing agents with a single intubating dose of ED95X2 (28). Due to these contraindications, minimal use of NDNBs is important.

Patients with gastrointestinal perforation typically develop AP (29). In the present study, AP was induced via GP as a model of clinical gastrointestinal perforation. Although peritonitis induced via perforation is typically limited to a local area and may be self-healed (30), the extent and severity of peritonitis may reflect the continuous dissemination of contaminated gastrointestinal secretion. Surgical intervention is essential to ameliorate the aggravation of AP and prevent overwhelming systemic inflammation, which contributes to severe sepsis or septic shock (31), leading to vital organ dysfunction. NDNBs are often used to facilitate tracheal intubation and relax skeletal muscles in general anesthesia. However, the results of the present study demonstrated that rocuronium administration did not completely relax the abdominal wall muscle or lower the pressure of the abdominal cavity in AP model rats compared with control rats. Additionally, rocuronium did not completely block muscle contraction at the neuromuscular junction. The attenuated effects of neuromuscular blockers in the acute phase of the sepsis and other infectious diseases has previously been reported (6,32,33). The activity of neuromuscular blockers have been shown to be reduced *in vivo* in endotoxin-induced sepsis animal models (34) and *in vitro* in muscle samples obtained from surgical panperitonitis-induced sepsis animal models (19,35-37). The attenuation of neuromuscular blocking in infectious models varies depending on the stage of infection and the type of neuromuscular blocker used (19). Additionally, a previous study showed that neuromuscular blockade produced via d-Tc or vecuronium administration was reduced when the acid concentration of the incubation solution was increased (38). Pharmacodynamic changes include altered AChR physiology or sensitivity, inhibition of serum cholinesterase activity, interactions with plasma constituents, alterations in distribution volume and protein binding. Clearance may also contribute to resistance in several disease stages, as previously described (6-8). However, calcium overload in the cell and the dysregulation of intracellular Ca^{2+} may also induce uncoordinated contraction of myocytes.

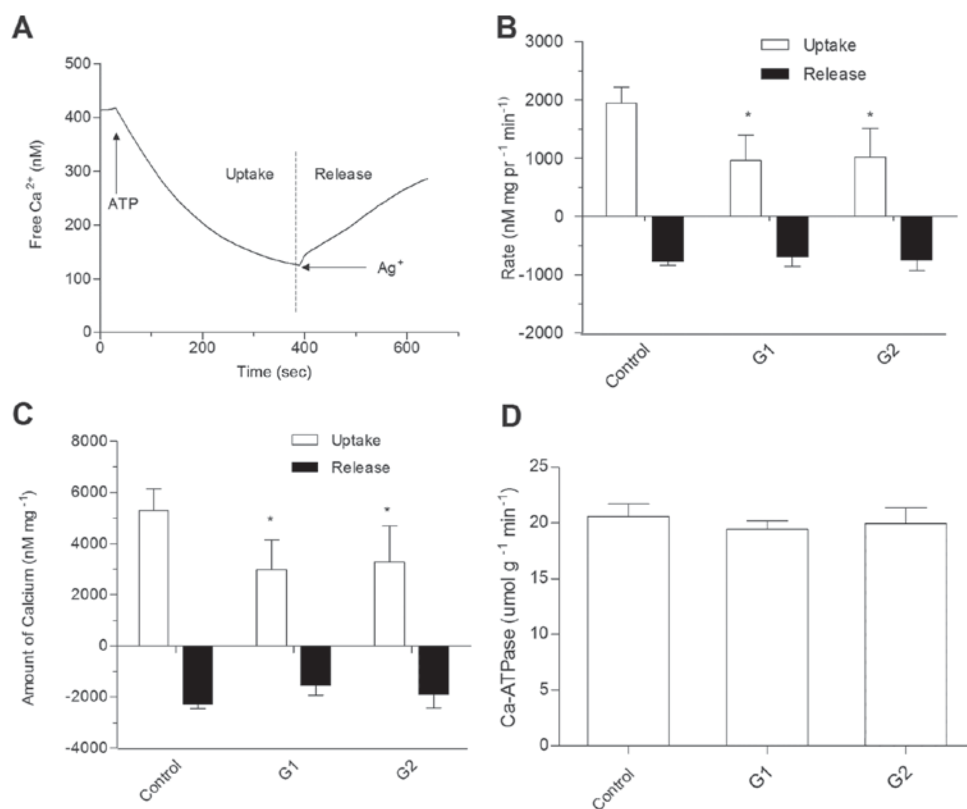

Figure 5. Calcium release and uptake from muscle homogenates. (A) Typical graph showing Ca^{2+} flux over time in SR measured with Fura-2. The maximal rates of Ca^{2+} uptake and release from the SR were calculated from this graph. (B) Peak rates of SR Ca^{2+} uptake as well as $AgNO_3$ stimulated release in SR of rectus abdominis muscle. (C) Maximal amounts of SR Ca^{2+} uptake as well as $AgNO_3$-stimulated release. (D) Activity of SERCA. All data are expressed as the mean ± standard error of the mean. *P<0.05 vs. control. SR, sarcoplasmic reticulum; SERCA, sarcoendoplasmic Ca^{2+}-ATPase. n=12.

Acid-base changes may alter the rate of muscle relaxation, tetanic tension and intracellular Ca^{2+} (39). A previous study reported that SR Ca^{2+}-ATPase in rabbit skeletal muscle was sensitive to HOCl-induced injury (40) and so it may be that the local stimulatory effect of gastric contents (including gastric acid, digestive enzymes and food) on the abdominal muscles and systemic inflammation may have contributed to the decreased SR uptake observed in the present study. Furthermore, TNF-α and IL-13 have been demonstrated to increase the Ca^{2+} response in airway smooth muscle (41). In the present study, TNF-α, IL-6 and IL-13 levels in AP rats were determined, and TNF-α and IL-6, which are inflammation markers, were elevated following GP. No significant increase was observed in IL-13 levels, which is consistent with recent clinical reports in which IL-13 was not increased in sepsis patients and endotoxemic volunteers (42,43). The inflammation-induced increase in intracellular calcium was mediated by the interference of SERCA expression and function, and Ca^{2+} uptake was impaired.

Calcium is a universal important second messenger for mediating muscle contraction, cell proliferation, and the regulation of gene expression (44). The regulation of intracellular calcium is critical for the contraction of striated and smooth muscles (45). In the present study, the force changes and half relaxation time of the rectus abdominis muscle were investigated. The results demonstrated that both twitch and tetanic tensions significantly increased in AP rats, and the frequency-force response curve was elevated. Furthermore, AP markedly affected the regular relaxation of muscle strips. The impaired removal of cytosolic Ca^{2+} and defects in SR Ca^{2+} uptake have been reported as major contributing factors in the progression of diastolic heart failure (46,47). In the present study, the rate of intracellular calcium uptake into the SR lumen significantly decreased in rats following the induction of AP. However, the activity of SERCA was not markedly impaired. Ca^{2+} reflux to the SR is primarily controlled by SERCA. Inhibition or low expression of SERCAs elevates intracellular Ca^{2+} and exhausts SR Ca^{2+} stores, and store-operated Ca^{2+} entry is triggered as a result of SR Ca^{2+} depletion (11). In the present study, disruption of SR Ca^{2+} uptake induced an increase in intracellular calcium levels, resulting in higher muscle force and prolonged relaxation time. This suggests that SERCA regulation is a key aspect of intracellular calcium level regulation. A reduction in SR calcium uptake may therefore induce tetanic contraction of the abdominal wall muscles, and GP-induced AP attenuates the effects of the neuromuscular blocker. Changes in SERCA, the regulated Proteins phospholamban and sarcolipin remain unclear (48,49). In the present study, all of these factors may influence SR calcium uptake; however, due to the limited experimental approach, the detailed mechanisms remain elusive. These results potentially indicate a novel pathway for the attenuation of neuromuscular blockers in GP-induced AP, and this requires clarification in future studies.

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