

# Acute Late Sepsis Attenuates Effects of a Nondepolarizing Neuromuscular Blocker, Rocuronium, by Facilitation of Endplate Potential and Enhancement of Membrane Excitability In Vitro

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**Background:** Sepsis attenuates the muscle-relaxing effects of nondepolarizing neuromuscular blockers. The authors investigated the effects of acute late sepsis on neuromuscular transmission and neuromuscular actions of rocuronium to clarify the mechanisms by which sepsis attenuates the effects of nondepolarizing neuromuscular blockers.

**Methods:** Sepsis was induced by cecal ligation and puncture operation. Endplate potentials, acetylcholine potentials, and electrotonic potentials were recorded from the motor endplates of isolated diaphragms from acute late septic and nonseptic rats.

**Results:** (1) Sepsis did not influence the effect of rocuronium to decrease endplate potential amplitude, which was increased by sepsis itself; (2) sepsis facilitated the effect of rocuronium to decrease quantal acetylcholine release, which was increased by sepsis itself; (3) sepsis did not influence the effect of rocuronium to decrease acetylcholine sensitivity, which was decreased by sepsis itself; (4) sepsis decreased critical depolarization, and rocuronium did not influence critical depolarization.

**Conclusions:** These results indicate that acute late sepsis facilitates endplate potentials and enhances excitability of the muscle membrane, indicated by a decrease of critical depolarization. It is thought that these elicit the sepsis-induced attenuation of the muscle-relaxing effects of rocuronium.

NONDEPOLARIZING neuromuscular blockers (NDNBs) have frequently been used to facilitate tracheal intubation during management of anesthesia and intensive care for septic patients. Sepsis clinically and experimentally alters the muscle-relaxing effects of NDNBs<sup>1-6</sup> and also influences the actions of intravenous anesthetics/sedatives on the muscle-relaxing effects of NDNBs.<sup>7,8</sup> Septic patients are initially normotensive, with increased cardiac output, low peripheral resistance, and increased total oxygen consumption, but finally become hypotensive with decreased cardiac output. Therefore, stringent and careful administration of anesthetics/sedatives and NDNBs is required for the perioperative management and intensive care of patients in late septic shock. Elucidation of the pathophysiologic mechanisms by which critical acute sepsis alters the effects of NDNBs would be

useful for determining appropriate doses of NDNBs and for safe use of NDNBs.

The pathophysiologic mechanisms by which sepsis alters the effects of NDNBs have been investigated more in the condition of chronic sepsis than in the condition of acute sepsis. Chronic animal sepsis induced by nonlethal panperitonitis or intraperitoneal *Escherichia coli* endotoxin showed hypersensitivity or hyposensitivity to d-tubocurarine, an NDNB, and resulted in a decrease or no change in acetylcholine receptor (AChR) numbers *in vivo*.<sup>2,6</sup> Therefore, under the condition of chronic sepsis, changes in AChR numbers are manifold and changes in the muscle-relaxing effects of NDNB are inconsistent. In our previous study, we demonstrated that acute sepsis induced by cecal ligation and puncture (CLP) operation,<sup>9</sup> which induces bacterial panperitonitis from intestinal perforation, stage-dependently and differentially attenuated the muscle-relaxing effects of NDNBs *in vitro*.<sup>1</sup> Endotoxin-induced increases in the probability of quantal acetylcholine release<sup>10</sup> and quantal contents of endplate potentials<sup>11</sup> have been suspected as the mechanism by which sepsis attenuates the effects of NDNBs<sup>1</sup>; however, the influences of acute late sepsis on the neuromuscular transmission and on the neuromuscular blocking effects of NDNBs have not been investigated in detail.

The aim of this study was to verify the hypothesis that acute late sepsis attenuates the neuromuscular blocking effects of NDNBs by influencing the mechanisms of neuromuscular transmission. We electrophysiologically investigated acute late sepsis-induced changes in neuromuscular transmission and influences of acute late sepsis on the neuromuscular blocking effects of rocuronium bromide, an NDNB, using hemidiaphragm preparations from panperitonitis-induced acute late septic rats. The reasons why we used rocuronium for this experiment are as follows: (1) Rocuronium produced a concentration-dependent twitch depression in a manner similar to those of other NDNBs,<sup>1</sup> (2) rocuronium-induced twitch depression was attenuated by sepsis,<sup>1</sup> and (3) rocuronium is one of the NDNBs widely used clinically.

## Materials and Methods

The methods used in this study were approved by the Animal Care and Use Committee of Sapporo Medical University (Sapporo, Hokkaido, Japan). Adult

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male Wistar rats (weighing 250–350 g, 8–12 weeks old) were randomly divided into sham and CLP groups ( $n = 63$ , each) and operated on as follows under oxygen–isoflurane anesthesia. In the CLP group, acute sepsis was induced by bacterial peritonitis using a CLP operation; *i.e.*, a midline abdominal incision was made, the cecum was ligated below the ileocecal valve, three perforations were made on the cecum with an 18-gauge needle, the cecum was gently compressed until feces were extruded, and the incision was closed. This CLP model shows acute sepsis, the pathophysiology of which is similar to that of clinical intraabdominal sepsis<sup>9,12</sup> and the mortality rate of which is 100% at 36 h after the CLP operation.<sup>3</sup> In the sham group, sham laparotomy was performed; *i.e.*, a midline abdominal incision was made, the cecum was manipulated but not ligated or punctured, and the incision was closed. The sham model shows no sequential septic changes in systemic organs.<sup>1,3</sup> All rats were resuscitated with saline solution (5 ml/100 g body weight) injected subcutaneously in the back at the time of the operations and were deprived of food but had free access to water after the operations.

The rats were killed with excessively deep oxygen–isoflurane anesthesia and bled out 18 h after the CLP or sham operation, at which time the rats in the CLP group were considered to be in the stage of late sepsis.<sup>13–15</sup> Strips of the left hemidiaphragm (10 mm in width) with attached phrenic nerve including the central tendon and rib cage intact, which had been cut parallel to the muscle fibers, were rapidly dissected from each rat. Each isolated strip was pinned onto the silicon plastic base of a small chamber (5 ml in volume), which was continuously superfused (3 ml/min) with modified Krebs solution (25°C) bubbled with 95% O<sub>2</sub>–5% CO<sub>2</sub>. The composition of the modified Krebs solution was as follows: 118.0 mM NaCl, 3.7 mM KCl, 2.5 mM CaCl<sub>2</sub>, 1.3 mM MgCl<sub>2</sub>, 26.2 mM NaHCO<sub>3</sub>, 1.2 mM Na<sub>2</sub>HPO<sub>4</sub>, and 11 mM glucose; pH was 7.40 ± 0.05 while bubbling.

Endplate potential (EPP; postjunctional potential induced by quantally released acetylcholine from the motor nerve terminal), acetylcholine potential (AChP; postjunctional potential induced by iontophoretically applied acetylcholine), electrotonic potential (EP; sequential variation of muscle membrane potential elicited by direct current), spontaneous miniature endplate potential (MEPP; postjunctional potential induced by spontaneously and quantally released acetylcholine from the motor nerve terminal), and resting membrane potential (RMP) were investigated. EPP, AChP, and EP were elicited using an electric stimulator and an isolator (SEN-3301 and SS-202J, respectively; Nihon Kohden Co., Tokyo, Japan). EPP, AChP, EP, MEPP, and RMP were recorded from endplate regions of the superficial muscle fiber of the strip with

conventional intracellular microelectrodes (12–35 MΩ) filled with KCl (3.0 M) using a current clamp amplifier (AxoClamp-2B; Axon Instruments, Foster City, CA). The endplate regions were found with a microscope in the region around fine intramuscular nerve branches and were considered adequate when the rise time of the MEPP was less than 1 ms. Data obtained from fibers for which RMP had depolarized by more than 10 mV during the recording period were excluded from analysis. Amplitudes of these potentials, rise times of AChP and MEPP, and time constant of EP were analyzed with an on-line computer analyzing system (Maclab/4s; Bio Research Center Co., Nagoya, Japan). EPP, AChP, and EP recorded in normal modified Krebs solution (before rocuronium application) were defined as baselines. After the baseline recording of EPP or AChP, rocuronium (1, 5, 10, 15, or 20 μM in modified Krebs solution) was applied to the preparations extracellularly from the superfusing bathing solution, followed by recording after stabilization of the effect of rocuronium judged by stability in these amplitudes. EP was recorded in normal modified Krebs solution or that containing rocuronium (5 or 15 μM). Rocuronium was obtained from N.V. Organon (Oss, The Netherlands), and all other drugs were purchased from Sigma (St. Louis, MO).

#### *Experiment 1: Measurements of EPPs and MEPPs*

To terminate nerve stimulation-induced generation of muscle action potentials (MAPs) and following muscle contractions, which disturb the recordings of EPPs, the cut fiber preparation to continuously elevate RMP<sup>16,17</sup> was performed on the muscle strips only when EPPs were measured. After muscle contraction had been terminated, trains of EPPs elicited by phrenic nerve stimulation (0.05-ms square pulse, 2 Hz, 50 times and supramaximal) were recorded. A train of 50 EPPs was recorded at intervals longer than 20 min. To standardize EPP amplitudes for a standard RMP, recorded EPP amplitudes were corrected by using the formula  $[EPP_{CR} = EPP_{UR} \times (70 - RVP)/(RMP - RVP - EPP_{UR})]$ ,<sup>18</sup> where  $EPP_{CR}$  and  $EPP_{UR}$  are the corrected and uncorrected EPP amplitudes, respectively, and RVP is the reversal potential of the endplate membrane. To calculate the quantal content ( $m$ ),  $EPP_{CR}$  was further corrected for the nonlinear summation by using the formula  $[EPP_{CM} = EPP_{CR}/(1 - 0.8 \times EPP_{CR})/(RMP - RVP)]$ ,<sup>19</sup> where  $EPP_{CM}$  is the corrected EPP amplitude. The standard RMP and RVP were taken as -70 mV and 0 mV,<sup>16</sup> respectively, in both formulas. The  $m$  of each EPP was estimated by the variance method using the formula  $[m = EPP_{CM} \times EPP_{AV}/(V_{EPP})]$ ,<sup>20</sup> where  $EPP_{AV}$  and  $V_{EPP}$  are the average and variance of the corrected 50 EPP amplitudes in the train. The train of 50 EPPs at 2 Hz generally displayed an initial run-down (usually in the first 6–10 EPPs in

the train) followed by a plateau. The run-down of EPPs was estimated by using ratios of  $EPP_{21-50amp}/EPP_{1amp}$ , where  $EPP_{1amp}$  is amplitude of the first EPP ( $EPP_1$ ) and  $EPP_{21-50amp}$  is amplitude of averaged EPP from the 21st to the 50th EPPs ( $EPP_{21-50}$ ). MEPPs were recorded from the endplate region of muscle strips without the cut fiber preparation for 60 s, and MEPP frequencies (times/s) and MEPP amplitudes were measured.

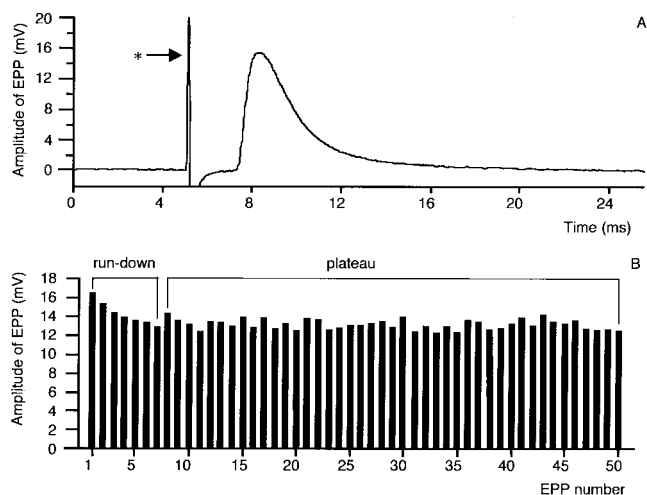
### Experiment 2: Measurement of AChPs

Acetylcholine potentials elicited by iontophoretically applied acetylcholine, which was released from an extracellular microelectrode (7–10 M $\Omega$ ) filled with acetylcholine chloride (3.0 M) by an outward current (400 nA, 50–150 ms, every 60 s, retaining current of 100 nA), were recorded. The extracellular microelectrode was manipulated near the endplate region where the intracellular recording microelectrode had been inserted. The position of the extracellular microelectrode was considered appropriate when the rise time of AChPs was less than 300 ms. Acetylcholine sensitivity was determined as the ratio of the amplitude of AChP to the electric charge of iontophoretically applied acetylcholine (mV/ $\mu$ C).

### Experiment 3: Measurement of EPs

Electrotonic potentials were recorded by stepwise depolarizing muscle membrane potential by approximately 1 mV until an MAP was generated using the direct current (15–80 nA, 25 ms, every 0.5–2.0 s), which was injected through the inserted recording electrodes. Threshold potential (TP) and critical depolarization (CD) were regarded as the membrane potential and membrane depolarization at the step just before generation of an MAP, respectively. Membrane resistance was estimated as the quotient between the membrane depolarization and applied current, and membrane capacitance was estimated as the quotient between the time constant of EP and membrane resistance.

Differences between the sham and CLP groups in the effects of rocuronium on  $EPP_{1amp}$ ,  $m$  of  $EPP_1$  ( $m_1$ ), and acetylcholine sensitivity were evaluated by competition analysis data (50% inhibitory concentration [IC<sub>50</sub>] and slope at IC<sub>50</sub> [Slope<sub>IC50</sub>]), which were determined from a four-variable logistic sigmoidal dose-response model fitted to the rocuronium concentration-response curves with the computer program Prism 4 (GraphPad Software, Inc., San Diego, CA). All results are expressed as mean  $\pm$  SD, except for IC<sub>50</sub> values, which are expressed as mean (95% confidence interval). Statistical significance for IC<sub>50</sub> values was calculated from log IC<sub>50</sub>. The Student unpaired *t* test, Mann-Whitney U test, and one- and two-way factorial analysis of variance with Bonferroni/

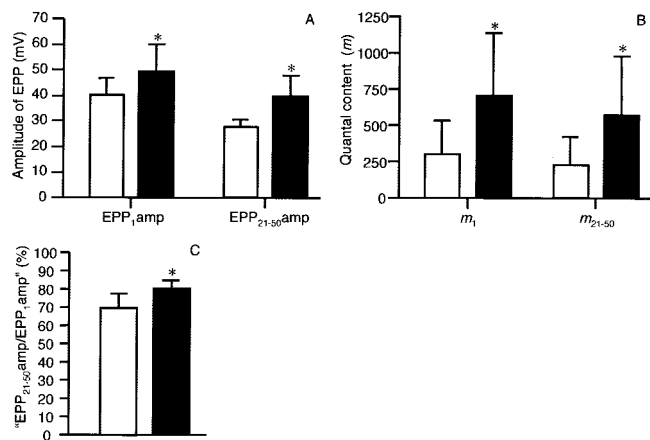


**Fig. 1.** An example of endplate potential (EPP) elicited by phrenic nerve stimulation (A) and a train of 50 EPPs at 2 Hz (B) from isolated rat diaphragm. To terminate nerve stimulation-induced muscle action potentials and following contractions, the cut fiber preparation was performed on the isolated diaphragm when EPPs were recorded. Amplitudes of recorded EPPs were approximately 10–20 mV. The train of EPPs displayed an initial run-down (in the first seven EPPs in the train) followed by a plateau. \* Stimulus artifact.

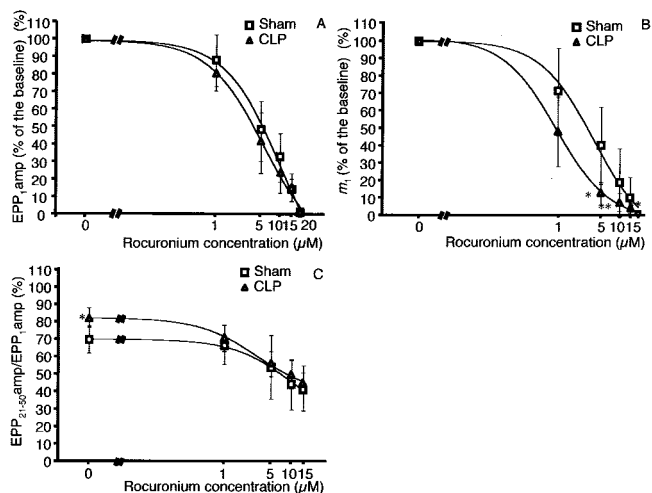
Dunn analysis were used for statistical comparison, and  $P < 0.05$  was considered as significant.

## Results

Rats in the CLP group demonstrated low spontaneous activities, a decrease in escaping movements, and napped hair before dissection. In gross inspection on dissection, CLP rats showed severe inflammatory changes in systemic organs (inflammatory ascites, and



**Fig. 2.** Influences of acute late sepsis on endplate potential (EPP) at baseline. Amplitudes of the first EPP and averaged EPP from the 21st to the 50th EPPs ( $EPP_{1amp}$  and  $EPP_{21-50amp}$ , respectively; A), quantal content of  $EPP_1$  and  $EPP_{21-50}$  ( $m_1$  and  $m_{21-50}$ , respectively; B), and an indication of EPP run-down ( $EPP_{21-50amp}/EPP_{1amp}$ ; C) were significantly ( $P < 0.01$ , each) increased in the cecal ligation and puncture group (filled columns) compared with those in the sham group (open columns). \*  $P < 0.01$  versus sham;  $n = 30$ , each.



**Fig. 3.** Influences of acute late sepsis on the effects of rocuronium on the first endplate potential (EPP) amplitude (EPP<sub>1</sub>amp; *A*), quantal content of the first EPP ( $m_1$ ; *B*), and EPP<sub>21-50</sub>amp/EPP<sub>1</sub>amp (the quotient between averaged amplitude from the 21st to the 50th EPPs; EPP<sub>21-50</sub>amp, and EPP<sub>1</sub>amp), which indicates EPP run-down (*C*). Rocuronium dose-dependently decreased EPP<sub>1</sub>amp (*A*),  $m_1$  (*B*), and EPP<sub>21-50</sub>amp/EPP<sub>1</sub>amp (*C*) in both the cecal ligation and puncture (CLP) group and the sham group. There was no significant difference in the rocuronium concentration–EPP<sub>1</sub>amp curves (% of baseline) between the sham and CLP groups (*A*), but the rocuronium concentration– $m_1$  curve (% of baseline) and rocuronium concentration–EPP<sub>21-50</sub>amp/EPP<sub>1</sub>amp curve in the CLP group significantly ( $P < 0.01$ , each) shifted leftward and upward, respectively, compared with those in the sham group (*B* and *C*). \*  $P < 0.01$  versus sham; \*\*  $P < 0.05$  versus sham;  $n = 30$  at  $0 \mu\text{M}$  rocuronium (baseline) and  $n = 6$  at  $1, 5, 10, 15,$  and  $20 \mu\text{M}$  rocuronium, each.

edema of intestines, liver, kidneys, lungs, and so forth), but few in diaphragms. Rats in the sham group did not show such changes.

### Experiment 1

Endplate potentials (approximately 10–20 mV in amplitude at baseline; fig. 1A) were recorded from the endplate regions of muscle strips, of which RMP was depolarized by the cut fiber preparation without significant difference between sham and CLP groups ( $-42.5 \pm 5.6$  and  $-40.6 \pm 11.0$  mV, respectively). The train of 50 EPPs at 2 Hz displayed an initial

run-down (in the first 7 EPPs in the train) followed by a plateau (fig. 1B). At baseline ( $n = 30$ , each), EPP<sub>1</sub>amp and EPP<sub>21-50</sub>amp in the CLP group were significantly ( $P < 0.01$ , each) larger than those in the sham group (fig. 2A). EPP<sub>21-50</sub>amp/EPP<sub>1</sub>amp and  $m$  of EPP<sub>1</sub> or EPP<sub>21-50</sub> ( $m_1$  or  $m_{21-50}$ , respectively) in the CLP group were significantly ( $P < 0.01$ , each) larger than those in the sham group at baseline (figs. 2B and C).

Rocuronium significantly ( $P < 0.01$ , each) decreased EPP<sub>1</sub>amp,  $m_1$ , and EPP<sub>21-50</sub>amp/EPP<sub>1</sub>amp in a dose-dependent manner in the sham and CLP groups ( $n = 30$  at baseline and  $n = 6$  at  $1, 5, 10, 15,$  and  $20 \mu\text{M}$  rocuronium, each). There was no significant difference in the rocuronium concentration–EPP<sub>1</sub>amp curve (% of baseline) and IC<sub>50</sub> and Slope<sub>IC50</sub> of the curve between the sham and CLP groups (fig. 3A and table 1). However, the rocuronium concentration– $m_1$  curve (% of baseline) in the CLP group significantly ( $P < 0.01$ ) shifted leftward compared with that in the sham group (fig. 3B). In the rocuronium concentration– $m_1$  curve, IC<sub>50</sub> in the CLP group was significantly ( $P < 0.01$ ) lower than that in the sham group, but there was no significant difference in Slope<sub>IC50</sub> between the sham and CLP groups (table 1). The rocuronium concentration–EPP<sub>21-50</sub>amp/EPP<sub>1</sub>amp curve in the CLP group significantly ( $P < 0.01$ ) shifted upward compared with that in the sham group (fig. 3C). EPP<sub>21-50</sub>amp/EPP<sub>1</sub>amp at baseline in the CLP group was significantly ( $P < 0.01$ ) larger than that in the sham group, but there was no significant difference in EPP<sub>21-50</sub>amp/EPP<sub>1</sub>amp at  $1, 5, 10,$  and  $15 \mu\text{M}$  rocuronium between the sham and CLP groups. There was no significant difference in the MEPP amplitude between the sham and CLP groups ( $0.34 \pm 0.07$  and  $0.39 \pm 0.12$  mV, respectively;  $n = 12$ , each), but the MEPP frequency in the CLP group ( $1.80 \pm 0.58$  times/s) was significantly ( $P < 0.01$ ) larger than that in the sham group ( $1.00 \pm 0.31$  times/s).

### Experiment 2

Acetylcholine potentials (approximately 1.0–2.5 mV in amplitude at baseline) were recorded from the endplate

**Table 1.** Influences of Acute Late Sepsis on the Competition Analysis Parameters of Rocuronium Concentration–EPP<sub>1</sub>amp,  $-m_1$ , and –Acetylcholine Sensitivity Curves

	EPP <sub>1</sub> amp		$m_1$		Acetylcholine Sensitivity	
	Sham	CLP	Sham	CLP	Sham	CLP
LogIC <sub>50</sub>	$-5.30 \pm 0.05$	$-5.43 \pm 0.04$	$-5.71 \pm 0.08$	$-6.06 \pm 0.01^*$	$-5.88 \pm 0.09$	$-5.79 \pm 0.06$
IC <sub>50</sub>	4.98 (3.67–6.75)	3.73 (2.93–4.75)	1.96 (1.10–3.50)	0.87 (0.80–0.96)	1.31 (0.72–2.37)	1.63 (1.09–2.41)
Slope <sub>IC50</sub>	$1.69 \pm 0.28$	$1.55 \pm 0.16$	$1.18 \pm 0.22$	$1.45 \pm 0.08$	$0.90 \pm 0.15$	$1.14 \pm 0.14$

Results are expressed as mean  $\pm$  SD, except for IC<sub>50</sub> values, which are expressed as mean (95% confidence interval).

\*  $P < 0.01$  from the value in the sham group.

CLP = cecal ligation and puncture; EPP<sub>1</sub>amp = the first endplate potential amplitude; IC<sub>50</sub> = 50% inhibitory concentration of the rocuronium concentration–EPP<sub>1</sub>amp,  $-m_1$ , or –acetylcholine sensitivity curve;  $m_1$  = quantal content of the first endplate potential; Slope<sub>IC50</sub> = slope at IC<sub>50</sub>.

**Table 2. Influences of Acute Late Sepsis or Rocuronium on Properties of the Muscle Membrane**

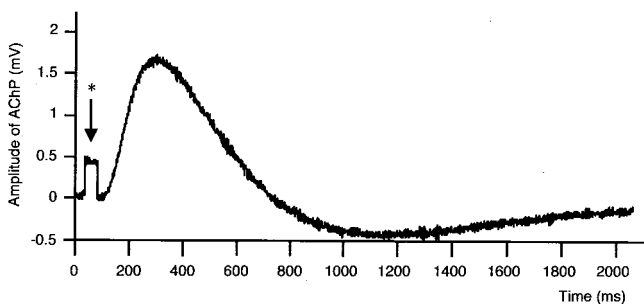
Rocuronium, $\mu\text{M}$	Resting membrane potential, mV		Threshold potential, mV		Critical depolarization, mV		Time constant, ms	
	Sham	CLP	Sham	CLP	Sham	CLP	Sham	CLP
0 (Baseline)	71.15 $\pm$ 3.28	69.71 $\pm$ 3.40	62.52 $\pm$ 3.51	62.25 $\pm$ 4.68	8.63 $\pm$ 1.81	7.46 $\pm$ 1.95†	5.60 $\pm$ 1.51	5.12 $\pm$ 1.58
5	71.35 $\pm$ 3.71	70.79 $\pm$ 2.97	62.19 $\pm$ 3.64	62.60 $\pm$ 3.55	9.16 $\pm$ 1.70	8.19 $\pm$ 1.70†	5.50 $\pm$ 1.17	5.60 $\pm$ 1.61
15	72.24 $\pm$ 4.78	71.91 $\pm$ 4.34	62.61 $\pm$ 4.41	63.93 $\pm$ 4.46	9.63 $\pm$ 1.79	7.98 $\pm$ 2.15*	5.33 $\pm$ 1.03	4.95 $\pm$ 1.49

regions (fig. 4). There was no significant difference in the rise time of AChP between the sham and CLP groups at baseline ( $222.2 \pm 50.7$  and  $235.3 \pm 45.8$  ms, respectively;  $n = 30$ , each). Acetylcholine sensitivity in the CLP group ( $24.0 \pm 12.7$  mV/ $\mu\text{C}$ ) was significantly ( $P < 0.01$ ) smaller than that in the sham group ( $43.0 \pm 23.0$  mV/ $\mu\text{C}$ ) at baseline (fig. 5A).

Rocuronium significantly ( $P < 0.01$ ) decreased acetylcholine sensitivities in a dose-dependent manner in the sham and CLP groups ( $n = 30$  at baseline and  $n = 6$  at 1, 5, 10, 15, and 20  $\mu\text{M}$  rocuronium, each). There was no significant difference in the rocuronium concentration–acetylcholine sensitivity curve (% of baseline) and  $\text{IC}_{50}$  and  $\text{Slope}_{\text{IC}_{50}}$  of the curve between the sham and CLP groups (fig. 5B and table 1).

### Experiment 3

At 0 (baseline), 5 and 15  $\mu\text{M}$  rocuronium ( $n = 25$ , each), CD, and membrane resistance in the CLP group were significantly ( $P < 0.05$  or  $P < 0.01$ ) smaller than those in the sham group (fig. 6 and table 2), but there was no significant difference in RMP, TP, and the time constant of EP between the sham and CLP groups (table 2). At baseline, there was no significant difference in membrane capacitance between the sham and CLP groups. In both groups, membrane resistance and membrane capacitance at 15  $\mu\text{M}$  rocuronium were significantly ( $P < 0.01$ , each) different (smaller and larger, respectively) than those at baseline, but there was no significant difference in RMP, TP, CD, and the time constant of EP between 5 or 15  $\mu\text{M}$  rocuronium and baseline (table 2).



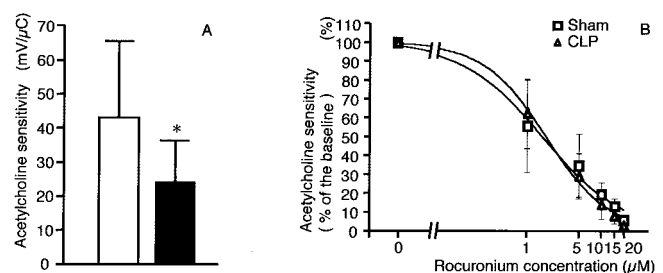
**Fig. 4.** An example of acetylcholine potential (AChP) elicited by iontophoretically applied acetylcholine from isolated rat diaphragm. Amplitudes of recorded AChPs were approximately 1.0–2.5 mV. \* Stimulus artifact.

## Discussion

The results of the current study demonstrate that acute late sepsis electrophysiologically attenuates the neuromuscular blocking effect of rocuronium modulating the functions of neuromuscular transmission by facilitations of EPPs and excitability of the muscle membrane.

Cecal ligation and puncture animals generally represent “hyperdynamic and hypermetabolic” early sepsis and “hypodynamic and hypometabolic” late sepsis within 10 and 12 h after the CLP operation, respectively.<sup>13–15,21</sup> In our previous studies, a CLP operation, the procedure of which is the same as that performed in this study, succeeded in producing acute late sepsis at 18 h after the operation.<sup>1,8</sup> From these findings in the previous studies and also in the current study, the CLP rats were regarded to be in a state of acute late sepsis at the time of dissection.

Sepsis did not influence the effect of rocuronium to decrease EPP amplitude, which was increased by sepsis itself. EPP<sub>1</sub>, which is elicited under the condition of sufficiently long intervals, represents under the fundamental condition of mobilization of synaptic vesicles and the probability of quantal acetylcholine release. Sepsis increased EPP<sub>1amp</sub> at baseline but did not influence the rocuronium-induced decrease in EPP<sub>1amp</sub>, indicating that EPP amplitudes are larger under the condition of sepsis both at baseline and in the pres-



**Fig. 5.** Influences of acute late sepsis on acetylcholine sensitivity at baseline (A) and on the effect of rocuronium on acetylcholine sensitivity (B). Acetylcholine sensitivity in the cecal ligation and puncture (CLP) group (filled column) was significantly ( $P < 0.01$ ) lower than that in the sham group (open column) at baseline (A). Rocuronium dose-dependently decreased acetylcholine sensitivity, but there was no significant difference in the rocuronium concentration–acetylcholine sensitivity curves (% of baseline) between the sham and CLP groups (B). \*  $P < 0.01$  versus sham;  $n = 30$  at 0  $\mu\text{M}$  rocuronium (baseline) and  $n = 6$  at 1, 5, 10, 15, and 20  $\mu\text{M}$  rocuronium, each.

Table 2. (continued)

Rocuronium, $\mu\text{M}$	Membrane resistance, $\text{M}\Omega$		Membrane capacitance, nF	
	Sham	CLP	Sham	CLP
0 (Baseline)	0.90 $\pm$ 0.25	0.73 $\pm$ 0.24†	6.65 $\pm$ 1.92	7.29 $\pm$ 2.27
5	0.82 $\pm$ 0.22	0.66 $\pm$ 0.18*	7.09 $\pm$ 1.87	8.81 $\pm$ 2.58*
15	0.66 $\pm$ 0.17‡§	0.52 $\pm$ 0.21‡	8.47 $\pm$ 2.28‡	11.00 $\pm$ 5.37†‡

Results are expressed as mean  $\pm$  SD.

\*  $P < 0.01$  from the value in the sham group. †  $P < 0.05$  from the value in the sham group. ‡  $P < 0.01$  from the value at 0  $\mu\text{M}$  rocuronium (baseline). §  $P < 0.01$  from the value at 5  $\mu\text{M}$  rocuronium. ||  $P < 0.05$  from the value at 5  $\mu\text{M}$  rocuronium.

CLP = cecal ligation and puncture.

ence of rocuronium. These results can explain the sepsis-induced attenuation of the muscle-relaxing effect of rocuronium observed in our previous study.<sup>1,3</sup>

Prejunctionally, sepsis facilitated the effect of rocuronium. At baseline, sepsis increased  $m_1$  involving increased MEPP frequency. These results indicate that sepsis increases quantal acetylcholine release by increasing the probability of release, which is reflected by elevated MEPP frequency. Sepsis caused a leftward shift of the rocuronium concentration- $m_1$  curve (% of baseline), indicating that sepsis enhances the rocuronium-induced decrease in quantal acetylcholine release, which is increased by sepsis itself. Because sepsis enhanced the effect of rocuronium on  $m_1$  without altering  $\text{Slope}_{\text{IC}_{50}}$ , it is thought that sepsis facilitates the rocuronium-induced decrease in quantal acetylcholine release by a presynaptic mechanism that does not involve binding between rocuronium and prejunctional AChRs. Sepsis increased  $\text{EPP}_{21-50\text{amp}}/\text{EPP}_{1\text{amp}}$  at baseline, indicating that sepsis suppressed EPP run-down. However, the increased probability of acetylcholine release should theoretically enhance EPP run-down because a higher probability of release can decrease releasable synaptic vesicles for the next

release. A possible explanation for the increased releasing probability and suppressed EPP run-down is sepsis-induced facilitation of mobilization of synaptic vesicles in the nerve terminal. It is thought that the sepsis-induced increase in intracellular  $\text{Ca}^{2+}$  concentration<sup>22,23</sup> contributes to the increases in the probability and facilitation of the mobilization, both of which are intracellular  $\text{Ca}^{2+}$  dependent.<sup>24</sup>

Postjunctionally, sepsis decreased acetylcholine sensitivity at baseline but did not influence the rocuronium-induced decrease in acetylcholine sensitivity, indicating that acetylcholine sensitivity is smaller under the condition of sepsis both at baseline and in the presence of rocuronium. These results cannot explain the sepsis-induced resistance to the muscle-relaxing effects of NDNBs.<sup>1,3</sup> Because acetylcholine sensitivity was estimated using iontophoretically applied acetylcholine, both the function of AChRs and the activity of acetylcholinesterase should be considered as mechanisms inducing the decrease in acetylcholine sensitivity. Sepsis-induced decrease in postjunctional nicotinic AChRs<sup>6</sup> is thought to be one of the mechanisms by which acetylcholine sensitivity is decreased. The spread of acetylcholine receptors along the muscle membrane induced by sepsis,<sup>25</sup> which theoretically increases acetylcholine sensitivity, was not confirmed in the current study because no AChP was recorded in the extrajunctional muscle membrane and sepsis did not increase acetylcholine sensitivity. On the other hand, inhibition of acetylcholinesterase activity induced by infectious inflammation, which theoretically increases acetylcholine sensitivity, has been reported.<sup>26,27</sup> It is thought that the influence of decreased AChRs overcomes that of inhibited acetylcholinesterase activity under the condition of sepsis. Sepsis did not influence the rocuronium concentration-acetylcholine sensitivity curve and its  $\text{Slope}_{\text{IC}_{50}}$ , indicating that sepsis does not influence the competitive blocking action of rocuronium on postjunctional AChRs. Sepsis may decrease acetylcholine sensitivity by affecting some postsynaptic sites, which does not concern acetylcholine binding sites on AChRs.

Sepsis did not alter MEPP amplitude although sepsis

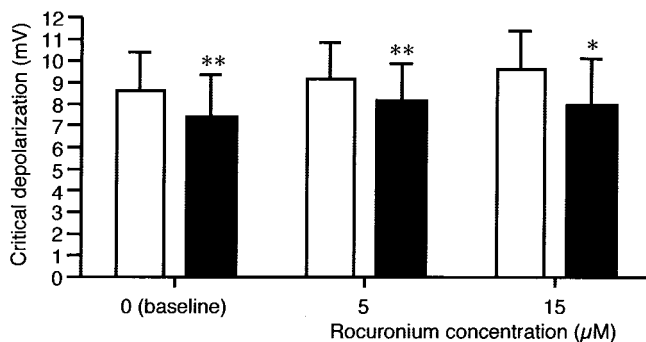


Fig. 6. Influence of acute late sepsis or rocuronium on critical depolarization of muscle membrane. Critical depolarizations in the cecal ligation and puncture (CLP) group (filled columns) were significantly ( $P < 0.05$  or  $P < 0.01$ ) smaller than those in the sham group (open columns) at 0 (baseline), 5, and 15  $\mu\text{M}$  rocuronium, but there was no significant difference between critical depolarization at 0  $\mu\text{M}$  rocuronium (baseline) and that at 5 or 15  $\mu\text{M}$  rocuronium in both the sham and CLP groups. \*  $P < 0.01$  versus sham; \*\*  $P < 0.05$  versus sham;  $n = 25$  at 0 (baseline), 5 and 15  $\mu\text{M}$  rocuronium, each.

decreased acetylcholine sensitivity. Possible explanation for this is sepsis-induced increase in acetylcholine content in each synaptic vesicle mediating sepsis-induced protein kinase A activation.<sup>28,29</sup> The sepsis-induced increase in acetylcholine content in each synaptic vesicle can explain the fact that sepsis did not decrease the effect of rocuronium on EPP amplitude although sepsis facilitated the rocuronium-induced decrease in acetylcholine release but did not alter the rocuronium-induced decrease in acetylcholine sensitivity. For possible explanation for the sepsis-induced increase in EPP<sub>amp</sub> at baseline, it is thought that the influence of sepsis-induced decrease in acetylcholine sensitivity is overcome by that of sepsis-induced increase in acetylcholine release.

Sepsis decreased CD, indicating enhancement of excitability of the muscle membrane, and rocuronium did not influence CD. The influence of sepsis can also explain the attenuation of the muscle-relaxing effects of NDNBs because smaller CD reflects a capability of easier MAP generation with smaller membrane depolarization, which relates to the muscle contraction. Sepsis did not significantly alter TP, which should be decreased by sepsis as CD is. It is thought that it is easier for CD, which is a relative value calculated from TP and RMP, to elicit significant differences than it is for TP or RMP, which is an absolute value and is easily affected by influences of some technical factors such as the insertion of intracellular microelectrodes. At baseline, sepsis decreased membrane resistance, which reflects functions of the voltage-dependent ion channel (generally sodium and potassium channels) on muscle fibers but did not significantly alter membrane capacitance, which is equivalent to the capacitance of lipid bilayers of the muscle membrane. These results indicate that sepsis facilitates the functions of sodium or potassium channels on the muscle membrane but does not influence the lipid bilayers. It has been reported that sepsis up-regulated adenosine triphosphate-sensitive potassium channels in the rat diaphragm.<sup>30</sup> It has also been reported that burn injury in rats, which frequently involves sepsis<sup>4</sup> and shows neuromuscular changes similar to those caused by sepsis (resistance to NDNBs<sup>31</sup> and increased quantal acetylcholine release<sup>32</sup>), increases transcripts of the mature isoform of the sodium channel.<sup>33</sup> It is suspected that the sepsis-induced enhancement of excitability of the muscle membrane originates from the sepsis-induced facilitation of functions of the sodium channels, which reduces the threshold depolarization required to initiate MAP.<sup>34</sup>

In our previous study, we presumed that the endotoxin-induced increase in the probability of release, which theoretically increases acetylcholine release, may be a part of the mechanisms of the sepsis-induced resistance to NDNBs.<sup>1</sup> The results of the current study suggest that the sepsis-induced increase in acetylcholine release, in-

duced by the elevated releasing probability, contributes to the resistance to an NDNB by facilitating EPPs, as we have expected, and that the sepsis-induced increase in excitability of the muscle membrane also contributes to the resistance.

In conclusion, sepsis induces an increase in EPP amplitude, which is advantageous for generating an MAP, and a decrease in CD, which reflects a condition to elicit MAP with smaller EPPs. It is thought that these mechanisms originate the sepsis-induced attenuation of the muscle-relaxing effects of rocuronium and probably other NDNBs.

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