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Effects of sepsis on the neuromuscular blocking actions of d-tubocurarine on rat adductor and abductor laryngeal muscles

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Abstract
Purpose. We evaluated the effects of sepsis on the neuromuscular blocking actions of d-tubocurarine (dTc) in the lateral cricoarytenoid (LCA) and posterior cricoarytenoid (PCA) muscles, an adductor muscle and an abductor muscle of the vocal cords, respectively, in vitro.
Methods. Sepsis was induced in rats by cecal ligation and puncture (CLP) to elicit panperitonitis. Electromyograms (EMGs) and endplate potentials (EPPs) were recorded from the LCA and PCA muscles of CLP-operated septic rats and sham-operated nonseptic rats, using extracellular and intracellular microelectrodes, respectively.
Results. EMG and EPP (amplitude and quantum content) were depressed by dTc, but the dTc-induced neuromuscular blocking effects were attenuated by sepsis. The suppressive effects of dTc on EMG and EPP (amplitude and quantum content) were less intense in the LCA muscle than in the PCA muscle under both sepsis and nonsepsis conditions.
Conclusion. Our study shows that sepsis has a depressive effect on dTc-induced neuromuscular blocking actions at both the adductor and abductor muscles of vocal cords in the larynx.

Key words Sepsis · Cecal ligation and puncture · Laryngeal muscles · Neuromuscular block · d-Tubocurarine · Neuromuscular transmission · Presynaptic · Transmitter release

Introduction

The systematic maintenance of airway patency and the sphincter activity of the larynx is regulated by the balance in tension of an abductor and some adductor muscles of the vocal cords in the larynx [1]. Laryngeal muscles have different sensitivities to a nondepolarizing neuromuscular blocker. It has been reported that the posterior cricoarytenoid (PCA, an abductor) muscle is more sensitive to d-tubocurarine (dTc), a nondepolarizing neuromuscular blocker, than is the lateral cricoarytenoid (LCA, an adductor) muscle in normal rats [2].

Considering the different sensitivities of laryngeal muscles to neuromuscular blockers, it is possible that airway patency transiently decreases during the induction of anesthesia or airway management in the intensive care unit (ICU) when using a neuromuscular blocker to insert a tracheal tube. In the period of several minutes between the injection of the neuromuscular blocker and following completion of the neuromuscular block, when the blood concentration of the neuromuscular blocker increases and thus these laryngeal muscles are differentially and gradually paralyzed, the less intense sensitivity of the adductor muscle than that of the abductor muscle to the neuromuscular blocker can elicit a temporary tendency to close the vocal cords [2,3]. To avoid the risk of upper airway obstruction during anesthetic/sedative induction, an understanding of neuromuscular blocker-induced differential paralysis of the laryngeal muscles is of great importance.

Of interest, sepsis is also known to decrease the sensitivities of some skeletal muscles to nondepolarizing neuromuscular blockers [4–6]. It is thought that sepsis alters the sensitivity of each laryngeal muscle and, accordingly, the airway patency of the larynx. However, the overlapping actions of these differential sensitivities and the effects of sepsis on the laryngeal muscles have not been investigated.

The hypothesis tested in the present study is that sepsis affects the differential actions of nondepolarizing neuromuscular blockers on adductor and abductor laryngeal muscles in complex ways. The objective of this study was to evaluate the effects of sepsis on the actions of a nondepolarizing neuromuscular blocker, dTc, on laryngeal adductor and abductor muscles. The effects of

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dTc on endplate potentials (EPPs) recorded from in vitro LCA and PCA muscle preparations of the rat larynx were investigated under the conditions of late sepsis and nonsepsis.

Materials and methods

This study was approved by the Animal Care and Use Committee of Sapporo Medical University. Adult male Wistar rats (10–12 weeks old, weighing 250–300 g) were used. Sepsis was elicited by the cecal ligation and puncture (CLP) technique described previously [7]. Wistar rats were randomly allocated to one of four groups: (1) a group 10 h after CLP (10-h-CLP group; $n = 5$), (2) a group 10 h after sham operation (10-h-sham group; $n = 5$), (3) a group 16 h after CLP (16-h-CLP group; $n = 5$), and (4) a group 16 h after sham operation (16-h-sham group; $n = 5$).

Each rat was anesthetized with isoflurane, and a midline abdominal incision was made aseptically under spontaneous breathing. The cecum, filled with feces and ligated with a 3-0 silk ligature just below the ileocecal valve without causing bowel obstruction, was punctured in two locations (1 cm apart) using an 18-gauge needle, and feces were extruded. The cecum was then placed back in the peritoneal cavity and the abdomen was closed. Sham-operated rats received the same anesthesia and surgical manipulation without the cecal ligation and puncture. After the surgery, the rats were observed in a recovery cage for 2 h. All of the rats were resuscitated with saline solution (5 ml per 100 g body weight) injected subcutaneously in the back during the operation. The rats were deprived of food but had free access to water after the operative procedure.

At 10 or 16 h after the CLP or sham operation, the rats were killed with isoflurane anesthesia, and the larynx with the attached recurrent laryngeal nerves was dissected free. The preparations were pinned together onto a Sylgard (Dow Corning, Midland, MI, USA) base of a 5-ml tissue bath superfused continuously (5 ml·min^{-1} at 24°C–25°C) with modified Krebs solution bubbled with 95% O_2 and 5% CO_2. The composition of the modified Krebs solution was as follows (in mmol·l^{-1}): NaCl, 135; KCl, 5; $CaCl_2$, 2; $NaHCO_3$, 15; Na_2HPO_4, 1; $MgCl_2$, 1; glucose, 11. The solution was maintained at pH 7.40 ± 0.05 during oxygenation. Contraction of the LCA (adductor) and the PCA (abductor) muscles by supramaximal stimulation of the recurrent laryngeal nerves was confirmed by the electromyographic response and visual inspection [8] before the experiment was started.

The amplitudes of the evoked compound action potentials (CPs) of the EMG were measured with extracellular microelectrodes to assess the action potentials of the LCA and PCA muscles. Thin intramuscular stainless-steel recording electrodes (0.3 mm in diameter; MT Giken, Tokyo, Japan) were inserted into the bellies of these muscles and the recurrent nerve was electrically stimulated (0.1 ms square pulse, 0.1 Hz and supramaximal) with a platinum bipolar stimulating electrode to elicit CPs. Stimulation of the recurrent nerve and the recording of CPs were performed with a Neuromatic 2000 device (Dantec, DISA, Denmark).

EPPs were recorded using intracellular microelectrodes to assess neuromuscular transmission. The preparations were superfused initially with glycerol (400 mmol·l^{-1})-Krebs solution for 90 min to terminate the muscle contraction [9] and then the superfusing solution was changed to normal Krebs solution. Single-barrel borosilicate pipettes (4 to 8 MΩ in resistance) filled with 3.0 mol·l^{-1} KCl were used as microelectrodes. After the complete disappearance of muscle contraction was confirmed microscopically, the electrodes were inserted into the muscle fibers of the LCA or PCA muscles with micromanipulators. Results obtained from muscle fibers with a resting potential of less than –60 mV were used for analysis. Appropriate microelectrode insertion onto the endplate region was judged by the amplitude of miniature endplate potentials (MEPPs) being more than 0.2 mV. To elicit EPPs, the recurrent nerves were stimulated by a supramaximal constant current at 1.0-Hz (duration, 500-μs square pulse), using a stimulator and an isolator (SEN-3201 and SS-202J, respectively; Nihon Kohden Electronic, Tokyo, Japan) and a platinum bipolar stimulating electrode. The signals were amplified with a microelectrode amplifier (AxoClamp 2B; Axon Instruments, Union City, CA, USA) and recorded on a data recorder (RD-101; TEAC, Tokyo, Japan). EPPs with rise times of less than 1.5 ms and amplitudes greater than 2.0 mV recorded in normal Krebs solution were used for analysis. The EPP amplitude was corrected with resting potential (standardized to -80 mV) [10] and nonlinear summation (acetylcholine reverse potential, 0 mV) [11]. The value of the EPP's mean quantum content (m) was calculated using the variance method: m = (SD of the EPP amplitude)$^{-2}$ [12].

The CP and EPP amplitudes, which were averaged in groups of ten, were analyzed. After the amplitude of the CP or EPP had stabilized in normal Krebs solution, the control CP or EPP was recorded. Then several concentrations of dTc (Sigma Chemical, St. Louis, MO, USA) were applied through the superfusing modified Krebs solution. After stabilization of the effect of dTc was confirmed, the CP or EPP was again recorded.

Data are expressed as means ± SD. The CP amplitude, EPP amplitude, and m are expressed as percentages of the control values (defined as CP amplitudes without exposure to dTc), mV, and number, respec-

tively. Statistical analysis was performed on a Macintosh computer (Apple Computer, Cupertino, CA, USA) using the software package StatView 4.02 (Abacus Concepts, Berkeley, CA, USA). Intergroup comparisons at the same time after CLP or the sham procedure in the same LCA or PCA muscle were performed using analysis of variance (ANOVA) with repeated measures and the Bonfferoni/Dunn post-hoc test. Intragroup comparisons were made by two-factor ANOVA and the Bonfferoni/Dunn post-hoc test. $P < 0.05$ was accepted as statistically significant.

Results

Supramaximal stimulation of the laryngeal recurrent nerve elicited CPs of the LCA and PCA muscles in the 16-h-sham group (50 ± 10 μV and 46 ± 11 μV, respectively) and in the 16-h-CLP group (47 ± 12 μV and 55 ± 10 μV, respectively; Fig. 1A). In the 10-h- and 16-h-sham groups, dTc at concentrations of 0.25, 0.5, and 0.75 μmol·l^{-1} significantly reduced the CP amplitudes (Fig. 1A) of both the LCA and PCA muscles ($P < 0.01$ each; Fig. 2). There were no significant differences in the dTc-induced reduction of CP amplitudes between the LCA and PCA muscles in the 10-h-sham and 16-h-sham groups. The dTc-induced CP depression was completely reversible by washout with modified Krebs solution without dTc. In the 10-h-CLP group, the CP amplitudes of the LCA and PCA muscles were significantly decreased by dTc, with the magnitude of depression being significantly less intense in the LCA muscle than in the PCA muscle ($P < 0.05$; Fig. 2A).

The dTc-induced reductions in CP amplitudes of the LCA and PCA muscles at dTc concentrations of 0.25–0.75 and 0.25–0.50 μmol·l^{-1} in the 10-h-CLP group were significantly less than those in the 10-h-sham group ($P < 0.01$ each; Fig. 2A). In the 16-h-CLP group, 0.25, 0.5, and 0.75 μmol·l^{-1} dTc did not significantly reduce the CP amplitudes of the LCA and PCA muscles. There were significant differences in the changes in CP amplitude between the 16-h-sham and the 16-h-CLP groups in

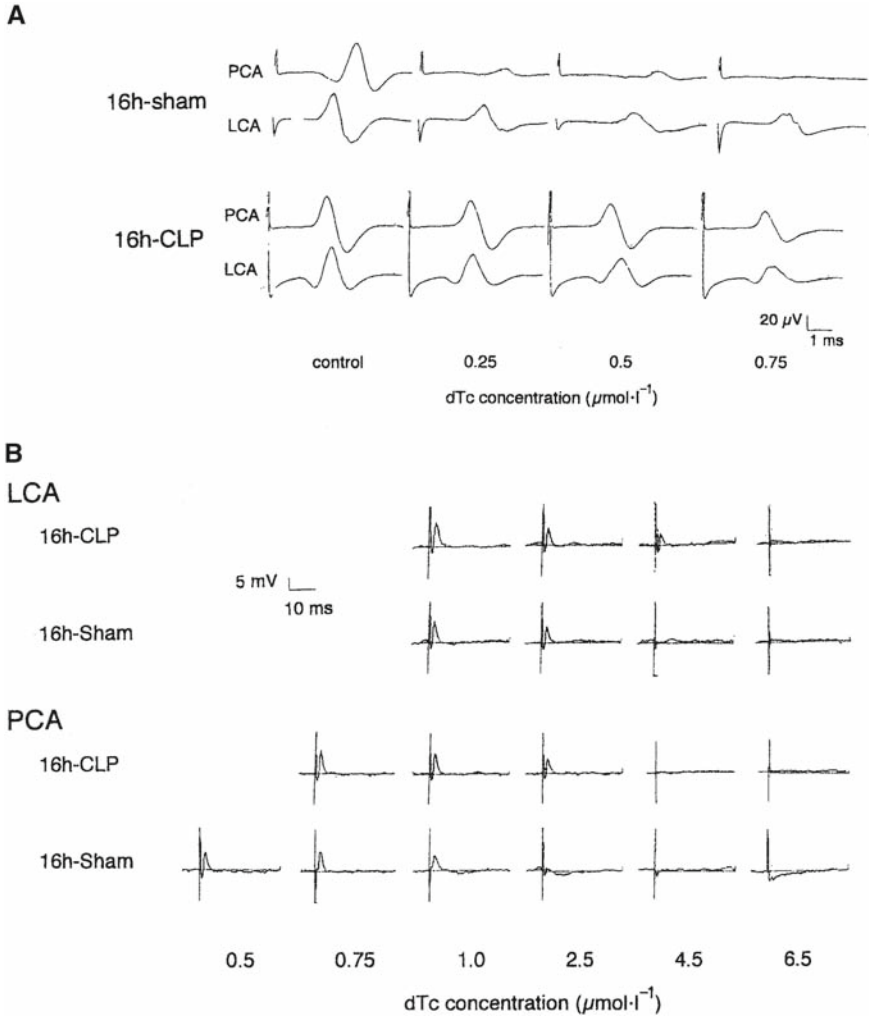

Fig. 1A,B. Compound action potentials (**A**) and endplate potentials (**B**) on the posterior cricoarytenoid (*PCA*) and lateral cricoarytenoid (*LCA*) muscles in the 16-h-sham and 16-h-cecal ligation and puncture (*CLP*) groups following administration of d-tubocurarine (*dTc*)

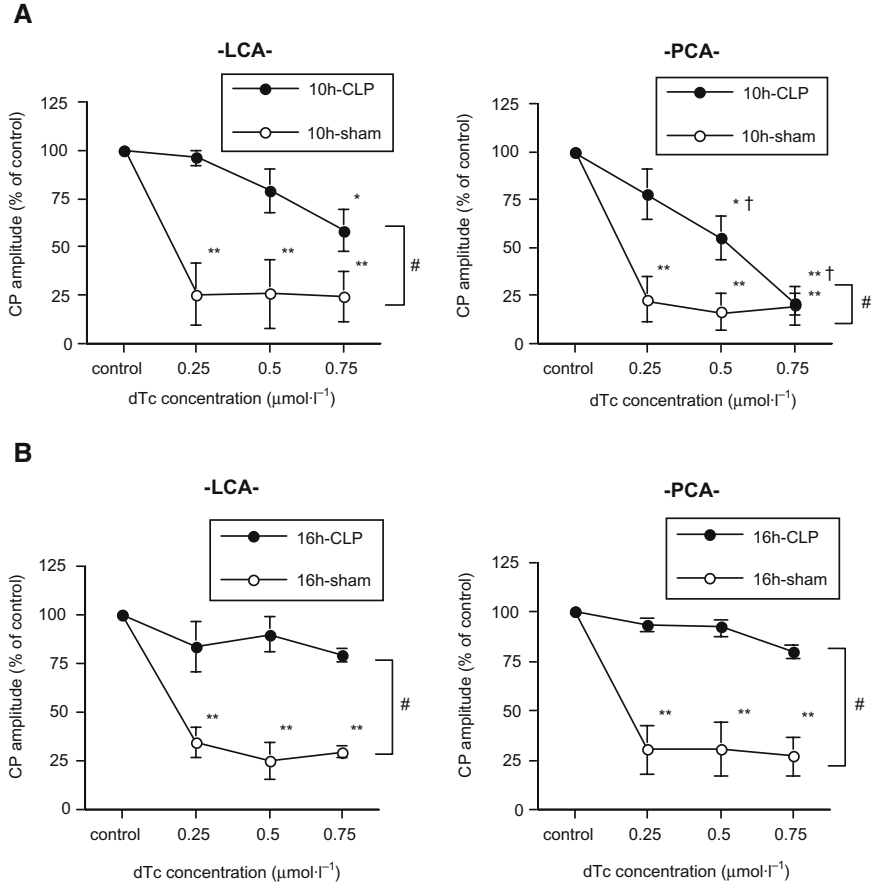

Fig. 2A,B. Effects of d-tubocurarine (*dTc*) on compound action potential (*CP*) amplitudes on the posterior cricoarytenoid (*PCA*) and lateral cricoarytenoid (*LCA*) muscles in the10-h-sham and 10-h-CLP groups (**A**) and in the 16-h-sham and 16-h-CLP groups (**B**). Values are expressed as means ± SD. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs control value; $^{\dagger}P < 0.05$ vs the LCA muscle in the same group at the same dTc concentration; $^{\#}P < 0.01$ between the CLP and sham groups by analysis of variance (ANOVA)

both the LCA and PCA muscles ($P < 0.01$ each; Fig. 2B). The CP amplitudes of the LCA muscle at dTc concentrations of 0.75 μmol·l^{-1} and of the PCA muscle at dTc concentrations of 0.5 and 0.75 μmol·l^{-1} were larger in the 16-h-CLP group than in the 10-h-CLP group ($P < 0.05$).

Supramaximal stimulation of the laryngeal recurrent nerve elicited EPPs of the LCA and PCA muscles in the 16-h-sham group (30 ± 3 mV and 30 ± 2 mV, respectively) and in the 16-h-CLP group (32 ± 2 mV and 32 ± 2 mV, respectively; Fig. 1B). These laryngeal muscles in the 16-h-CLP and 16-h-sham groups were exposed to several concentrations of dTc (0.25, 0.5, 0.75, and 1.0 μmol·l^{-1}). The minimal concentrations of dTc at which EPPs could be detected by counteracting the action potentials were 1.0 μmol·l^{-1} in LCA muscles in both the 16-h-sham and 16 h-CLP groups, and 0.5 and 0.75 μmol·l^{-1} in PCA muscles in the 16-h-sham and 16-h-CLP groups, respectively (Fig. 1B). In both the 16-h-sham and 16-h-CLP groups, dTc decreased the EPP amplitudes of the LCA and PCA muscles in a concentration-dependent manner ($P < 0.01$ each). There were significant differences between the 16-h-sham and 16-h-CLP groups in dTc-induced reduction of EPP amplitudes in both the LCA and PCA muscles ($P < 0.01$ each). In both the 16-h-sham and 16-h-CLP groups, the dTc-induced reduction of EPP amplitudes was greater in the PCA muscle than in the LCA muscle ($P < 0.01$ each; Fig. 3A). In both the 16-h-sham and 16-h-CLP groups, dTc decreased *m* of EPPs in the LCA and PCA muscles in a concentration-dependent manner ($P < 0.01$ each). In the LCA and PCA muscles, the dTc-induced reduction of *m* was less in the 16-h-CLP group than in the 16-h-sham group ($P < 0.01$ each). In both the 16-h-sham and 16-h-CLP groups, the dTc-induced reduction of *m* in the LCA muscle was less than that in the PCA muscle ($P < 0.01$ each; Fig 3B).

Discussion

Our results showed that sepsis attenuated dTc-induced neuromuscular block by inhibiting the dTc-induced reduction of transmitter release in the laryngeal adductor (LCA) and abductor (PCA) muscles, with the adductor muscle showing more resistance to dTc.

We used a CLP septic model, an experimental model of intra-abdominal sepsis, in the present study. CLP-induced sepsis, showing a hyperdynamic state in the early phase (10 h after the CLP procedure) followed by

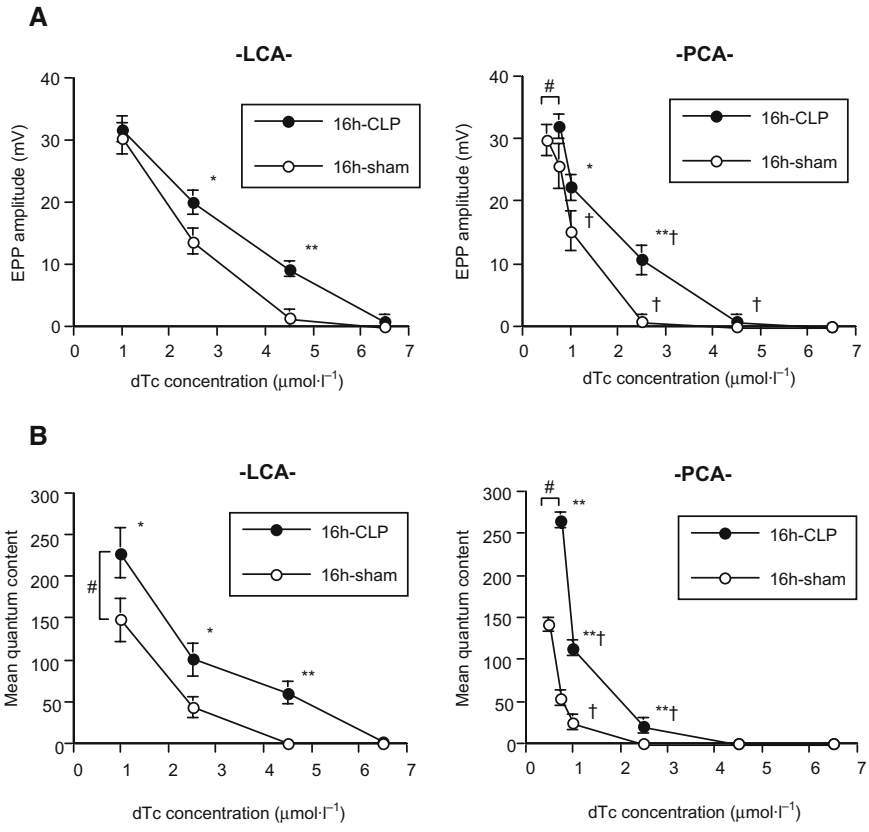

Fig. 3A, B. Changes in endplate potential (*EPP*) amplitudes (**A**) and mean quantum content (**B**) in the lateral cricoarytenoid (*LCA*) and posterior cricoarytenoid (*PCA*) muscles following administration of d-tubocurarine (*dTc*) in the 16-h-sham and 16-h-CLP groups. Values are expressed as means ± SD. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs the 16-h-sham group at the same dTc concentration; $^{\dagger}P < 0.05$ vs the LCA muscle in the same group at the same dTc concentration; $^{\#}P < 0.01$ between the CLP and sham groups by ANOVA

a hypodynamic state in the late phase (16–24 h after the CLP procedure) [13], has been regarded as being more relevant to clinical sepsis than endotoxin-induced animal sepsis [7,14].

In the present study, the dTc-induced reductions in the CP amplitudes of the LCA and PCA laryngeal muscles were inhibited completely by late-phase sepsis and partially by early-phase sepsis. Because CP reflects a population of action potentials, these results indicate that sepsis stage-dependently inhibits the effect of a nondepolarizing neuromuscular blocker to depress action potentials by blocking neuromuscular transmission. Because the dTc-induced reductions of CP amplitude may reflect a decrease in the number of contracting muscle fibers (in terms of the "all or none" theory of neuromuscular transmission), it is also presumed that sepsis inhibits the neuromuscular blocking effect of dTc to depress the contraction forces of these laryngeal muscles, which are, experimentally, very difficult to measure directly [15].

We found that the dTc-induced reduction of EPP amplitudes was attenuated by late-phase sepsis in these laryngeal muscles, indicating that late-phase sepsis inhibits the effect of a nondepolarizing neuromuscular blocker on neuromuscular transmission. The effects of sepsis on quantal acetylcholine release from motor nerve terminals were investigated from EPPs. Because MEPPs are too small to record under the condition in which postjunctional acetylcholine sensitivity is decreased by dTc, we used the variance method to calculate *m* from the fluctuation of EPP amplitudes [16] in the present study, instead of the direct method [(EPP amplitude)(MEPP amplitude)$^{-1}$] [10]. The values for *m* in the present study were within the range of those reported in previous studies [16,17], being distributed in the range of 100 to 400 quanta per pulse.

In the present study, the dTc-induced reductions of *m* in the laryngeal muscles were less under the condition of late sepsis than under the condition of nonsepsis, indicating that late sepsis inhibits the effect of dTc on quantal acetylcholine release. The sepsis-induced presynaptic effect, i.e., attenuation of the effect of dTc on quantal release, is probably one of the main mechanisms of the effect of dTc on EPP amplitude. However, the mechanisms underlying the influence of sepsis on the effect of dTc are still unclear. It is possible that the effects of sepsis on the peripheral nervous system shown in previous studies; i.e., septic critical illness polyneuropathy and/or septic axonal degeneration [18–20], influence the function of motor nerve terminals to enhance quantal acetylcholine release or result in hyposensitivity to dTc under the condition of upregulation of muscle nicotinic acetylcholine receptors at postsynaptic sites.

The effects of dTc on CP amplitude, EPP amplitude, and *m* in the present study were less intense in the LCA (adductor) muscle than in the PCA (abductor) muscle under both sepsis and nonsepsis conditions. These findings indicate that sepsis does not significantly alter the properties of laryngeal muscles previously reported for normal rats [2], i.e., that the LCA muscle is more resistant to dTc than is the PCA muscle, even when sepsis significantly influences the actions of dTc on these muscles. The higher sensitivity of an abductor laryngeal muscle than that of an adductor muscle to a nondepolarizing neuromuscular blocker suggests that the airway patency of the larynx tends to decrease under the condition of a partial neuromuscular block [3].

In conclusion, the results of the present study indicate that panperitonitis-induced sepsis attenuated the dTc-induced neuromuscular block on both the adductor and abductor muscles of the vocal cords in the larynx. However, sepsis did not significantly influence the differential sensitivities of the LCA and PCA muscles to a nondepolarizing neuromuscular blocker, the LCA muscle being less sensitive than the PCA muscle. The sepsis-induced presynaptic effect, i.e., attenuation of the effect of dTc decreasing quantal transmitter release, had an impact as one of the mechanisms of the suppressive effects of sepsis on dTc-induced neuromuscular block.

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