

Propofol Enhances a *d*-Tubocurarine-Induced Twitch Depression in Septic Rat Diaphragm

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We estimated the effect of *d*-tubocurarine (dTc) on neuromuscular transmission and the action of propofol on dTc-induced twitch depression by using sham control and septic rat nerve-hemidiaphragm preparations *in vitro*. Isometric twitch tension elicited by indirect (phrenic nerve) or direct (muscle) stimulation at 0.1 Hz was evaluated. Sepsis induced by panperitonitis attenuated the twitch tension elicited by indirect and direct stimulation ($P < 0.01$ in each group) in the absence of significant morphological inflammatory damage to the diaphragm. dTc (1 μM) decreased the twitch tension elicited by indirect stimulation ($P < 0.01$) less intensely in the septic group than in the sham group ($P < 0.01$). Propofol accentuated dTc-induced depressed twitch

more intensely in the septic group ($P < 0.01$ or 0.05). These results demonstrate that sepsis attenuates both muscle contractile force and the effect of a neuromuscular blocker and that propofol more intensely enhances dTc-induced twitch depression during sepsis. **Implications:** Propofol and nondepolarizing muscle relaxants are widely used for various clinical cases, including sepsis. Interactions between nondepolarizing muscle relaxants and propofol during sepsis are interesting from a clinical point of view. We demonstrated that propofol significantly enhances *d*-tubocurarine-induced twitch depression *in vitro* in the septic rat model compared with that in the nonseptic rat model.

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Muscle weakness induced by motor deficiency, which elicits respiratory pump failure, is a remarkable complication that influences the clinical course of septic patients (1). Clinically, sepsis-induced polyneuropathy and skeletal myopathy may be the mechanisms of motor unit deficiency (1,2). In animal sepsis models, respiratory pump failure induced by muscle weakness has been observed *in vivo* (3–5). Impairment of muscle contractility in a dissected diaphragm induced by several septic inflammatory mediators or bacteremia has also been reported (6,7). Sepsis-induced change in neuromuscular transmission has been reported *in vitro* (8). However, the mechanism by which sepsis induces motor unit deficiency has not been investigated.

Propofol is often used in combination with nondepolarizing muscle relaxants (NDMRs) in septic patients. A large concentration of propofol enhances the

neuromuscular action of vecuronium in normal rat diaphragms *in vitro* (9). However, our previous study demonstrated that sepsis attenuates the intensity of the neuromuscular blocking effect of *d*-tubocurarine (dTc) accompanying depression of muscle contractility in the rat diaphragm (10).

On the basis of the above results, it is conceivable that sepsis alters propofol-induced potentiation of NDMRs. Therefore, we investigated the effect of propofol on the neuromuscular blocking actions of dTc *in vitro* in nerve-hemidiaphragm preparations from normal rats and rats with sepsis induced by panperitonitis.

Methods

After approval was given by our animal care and use committee, 48 adult male Wistar rats (10–11 wk old, weighing 250–300 g) were used for this study. The rats were divided into sham and septic groups. In the septic group, sepsis was surgically induced by the cecal ligation and puncture (CLP) method (10,11). The rats were anesthetized with O_2 -isoflurane, a midline abdominal incision was made, the cecum was ligated below the ileocecal valve, and three perforations were made in the cecum with an 18-gauge needle. In the

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sham group, a midline abdominal incision was made, and the cecum was manipulated but not ligated or punctured. Saline (5 mL/100g of body weight) was subcutaneously injected in the back to prevent dehydration. The animals were allowed water but no food after the operative procedures.

Sixteen hours after the sham or CLP operation, twitch tension was blindly estimated. The left hemidiaphragms, together with the phrenic nerve, central tendon, and rib cage, were rapidly removed from the operated rats under O₂-isoflurane-anesthesia. Immediately before removal, the rats were killed with deep O₂-isoflurane-anesthesia and bleeding. Strips of diaphragm (10 mm in width) with the phrenic nerve attached were then dissected, by a modification of the method described by Bülbring (12). The section plane of the diaphragm was parallel to the muscle fibers. Each isolated strip was mounted vertically in a 25°C temperature-controlled tissue chamber (25 mL in volume) that was filled with modified Krebs solution and oxygenated with 95% O₂-5% CO₂ gas. The strip was suspended by its central tendon from a force displacement transducer (Grass FT-03; Astro-Med, Inc., West Warwick, RI) by using a 3.0 silk suture, and the rib cage was fixed inferiorly. The composition of the modified Krebs solution was as follows (in mM): NaCl (118.0), KCl (3.7), CaCl₂ (2.5), MgCl₂ (1.3), NaHCO₃ (26.2), Na₂HPO₄ (1.2), and glucose (11), and pH was 7.40 ± 0.05 while oxygenated. The dissected preparation was initially rinsed several times with modified Krebs solution. Each strip was stretched to optimal length by applying a preload of 3–5 g, and the peak isometric twitch tension was measured during indirect (phrenic nerve) or direct (muscle) stimulation at 0.1 Hz. Stimulation was performed with a supramaximal constant current (duration: 0.06 ms for indirect and 0.2 ms for direct) using a Grass S 48 stimulator and constant current unit. The phrenic nerve was positioned on wire bipolar platinum stimulating electrodes when indirect twitch tension was elicited. A pair of bipolar platinum stimulating plate electrodes (25 mm × 12 mm square) was used when direct stimulation was performed. When direct stimulation was done, the diaphragm was pretreated with 15 μM dTc to extinguish the effect of nerve stimulation by completely blocking neuromuscular transmission. The twitch tension was recorded, via a force transducer, on a thermal chart recorder.

Stability of twitch tension for at least 30 min was confirmed before any study commenced. dTc and propofol were applied to the preparation extracellularly from the bathing solution. After determination of control (drug free) twitch tension elicited by indirect stimulation, 1 μM dTc was applied, and the effect was determined. After the peak effect of 1 μM dTc was attained and maintained for at least 30 min, propofol (100, 200, 400, and 800 μM) was added stepwise to dTc

(1 μM), and the effects of propofol on dTc were evaluated. Moreover, the effects of these four concentrations of propofol and the corresponding concentrations of intralipid, used as a solvent for propofol, on indirectly and directly elicited twitch tension were also determined. After exposure to the drugs, the diaphragm was rinsed with modified Krebs solution to verify whether twitch tension would return to 95%–105% of initial values in each study. Each experimental group consisted of different rats (*n* = 6). All drugs other than propofol and intralipid were purchased from Sigma (St. Louis, MO). Propofol (formulated in Intralipid[®] as Diprivan[®]; Zeneca, London, UK) and Intralipid[®] (Pharmacia AB, Stockholm, Sweden) were used. Data of twitch tension were expressed as means ± SE of each group of six preparations and as grams or % of control. Statistical comparisons between values were performed by paired or unpaired two-tailed Student's *t*-tests, and those within or between whole curves were performed by one- or two-way repeated-measures analysis of variance, respectively. *P* < 0.05 was considered significant.

Histopathological changes in the diaphragm, lung, and liver were also examined to confirm the systemic influences of pancreatitis on the induction of sepsis. Formalin-fixed, paraffin-embedded tissues that were sectioned to 10 μm in thickness were stained by hematoxylin and eosin for microscopic observation at 100-times magnification (*n* = 12).

Results

The weight, length, and cross-sectional area of diaphragm preparations were similar in the two groups. Twitch tension elicited by indirect or direct stimulation was determined in the sham and septic groups (*n* = 6 in each group, Figure 1). Despite the fact that the muscle strips used in these experiments were mostly the same size (10 mm in width), twitch tensions elicited by indirect and direct stimulation were significantly (*P* < 0.01) less in the septic group than in the sham group (38.7% and 35.8% of the sham group, respectively).

The effect of propofol on dTc-induced twitch depression during indirect stimulation was evaluated in the sham and septic groups (*n* = 6 in each group, Figure 2). Application of dTc (1 μM) significantly decreased the twitch tension to approximately 23.5% ± 0.06% of the control in the sham group and 47.6% ± 0.08% of the control in the septic group (*P* < 0.01 each), and then the decreased twitch tension remained stable. The dTc (1 μM)-induced twitch depression in the sham group was significantly more intense than that in the septic group (*P* < 0.01). In the septic group, the dTc (1 μM)-depressed twitch tension was significantly further depressed in a dose-dependent manner

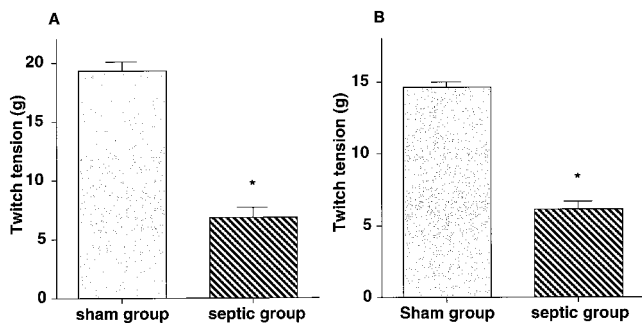


Figure 1. The effect of sepsis on twitch tension in the diaphragm. Sepsis significantly decreased the contraction force of the diaphragm by both indirect (A) and direct (B) stimulation in the septic group. Data are expressed as mean + SE, $n = 6$ in each histogram. * $P < 0.01$ versus the septic group by unpaired t -test.

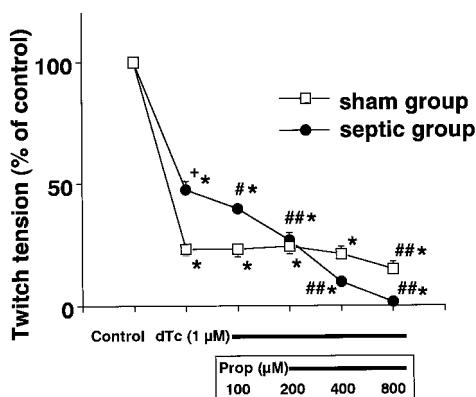


Figure 2. The effect of propofol (Prop) on *d*-tubocurarine (dTc)-depressed twitch tension elicited by indirect (nerve) stimulation in the phrenic nerve-diaphragm preparation. dTc 1 μ M depressed the twitch tension elicited by indirect stimulation to about 25% and 45% of control in the sham group and in the septic group, respectively. This difference was statistically significant. The potentiating effect of propofol on the action of dTc was found in the septic diaphragm. In contrast, propofol potentiated the dTc-induced twitch depression only at the largest concentration in the sham group. * $P < 0.01$ versus control. ** $P < 0.01$ and # $P < 0.05$ versus dTc 1 μ M by paired t -test. + $P < 0.01$ versus the sham group dTc 1 μ M by unpaired t -test.

by 100, 200, 400, and 800 μ M propofol to 39.9% \pm 0.03% ($P < 0.05$), 26.8% \pm 0.07% ($P < 0.01$), 9.8% \pm 0.03% ($P < 0.01$), and 1.6% \pm 0.01% ($P < 0.01$) of the control, respectively. Similar dose-dependent depression with propofol was observed in the sham group, though significant depression was observed only with the highest concentration tested (14.9% \pm 0.08% of the control at 800 μ M, $P < 0.05$).

Propofol facilitated the directly elicited twitch tension in both the sham ($P < 0.01$) and septic ($P < 0.05$) groups in a dose-dependent manner to 140.1% \pm 3.3% and 160.6% \pm 17.8% of the control at 800 μ M, respectively, although there was no significant difference between the two groups (Figure 3A). Similarly, propofol facilitated the indirectly elicited twitch tension in the sham group ($P < 0.01$ or 0.05) in a dose-dependent manner (113.1% \pm 15.3% of the control at 800 μ M, $P < 0.05$). In the septic group, however, although propofol

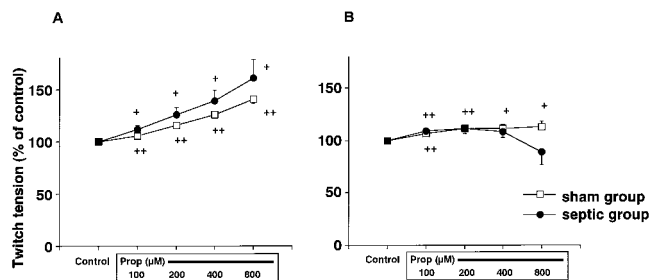


Figure 3. The effect of propofol (Prop) on the twitch tension elicited by direct (muscle) and indirect (nerve) stimulation in the diaphragm. Propofol enhanced the twitch tension of the diaphragm elicited by direct stimulation in both groups in a dose-dependent manner (A). Similarly, propofol facilitated the indirectly elicited twitch tension in the sham group in a dose-dependent manner. In the septic group, however, although propofol facilitated the indirectly elicited twitch tension at 100 μ M, propofol conversely depressed the twitch tension at 800 μ M, although there was no significant difference between the two groups (B). ++ $P < 0.01$ and + $P < 0.05$ versus control by paired t -test.

facilitated the indirectly elicited twitch tension at 100 μ M (109.2% \pm 1.4% of the control, $P < 0.01$), propofol conversely depressed the twitch tension to 88.7% \pm 11.9% of the control at 800 μ M, although there was no significant difference between the two groups (Figure 3B). Intralipid, administered at concentrations corresponding to those of propofol (100, 200, 400, and 800 μ M) did not affect the directly elicited twitch tension in either group (data not shown). After the diaphragm was rinsed with modified Krebs solution after exposure to the drugs, twitch tension returned to 95%–105% of initial values in every study.

Postmortem examination revealed severe septic changes in the lung, liver, and diaphragm in the septic group but not in the sham group. On gross inspection, pulmonary edema and liver swelling were observed. Histological features of the lung were atelectasis, hyaline membrane formation, and acute inflammatory cell infiltrations, and those of the liver were fatty changes and an increase in sinusoidal cells ($n = 12$). However, few obvious changes were found in the diaphragm specimens. Gross examination of the septic diaphragms showed no notable changes. Histologically, the septic diaphragm showed a normal appearance of myofibrils in cross- and parallel-sectioned areas. Slight and limited inflammatory infiltration was found only in the perivascular area and in the lateral surface of the serosa in the septic group ($n = 12$).

Discussion

We demonstrated that propofol potentiates dTc-induced twitch depression, especially in the septic rat diaphragm, which exhibits abnormalities in neuromuscular transmission and muscle contractility as we reported previously (10), i.e., sepsis attenuated the neuromuscular blocking action of dTc and the muscle contractility of the

rat diaphragm in the absence of morphological inflammatory damage to the diaphragm.

The CLP method (11) has been widely used to promote a Gram-negative bacterial septic animal model induced by panperitonitis (13-15). Our pathological findings showed that CLP produced a systemic septic condition in rats [for detailed descriptions, see Reference (10)]. It has been reported that, in this rat model, sepsis reaches the late hypodynamic phase 18 hours after the CLP operation (14), practically when the rats were dissected in this study.

The present study demonstrated a decrease in dTc sensitivity of the septic diaphragm, as we showed in our previous study (10). This decrease in dTc-sensitivity may reflect some unrevealed sepsis-induced changes in neuromuscular transmission. Several possible mechanisms underlying this decrease in dTc sensitivity have been proposed. Severe thermal injury is known as another pathophysiological condition that produces similar changes in sensitivity to NDMRs (16-19). In thermal injury, changes in acetylcholine receptor expression have been considered as an explanation of the resistance to NDMRs (20). The thermal injury-induced resistance to NDMRs involves some features concerning its intensity, i.e., the intensity varies with the time course (generally not present within the first week and reaching a maximal level at about five to six weeks after the injury) and with the burn surface area (18,19). These findings suggest that sepsis may contribute to thermal injury-induced resistance to NDMR, because the time course may overlap the probable time period of sepsis in many burn patients, and because sepsis frequently develops in patients with a large burn surface area. Pavlin et al. (21) showed that burned animals with sepsis required a significantly larger dosage of atracurium to obtain the same level of twitch depression compared with those without sepsis. Our results may account for one of the mechanisms of thermal injury-induced resistance to NDMR. Meanwhile, it has been reported that the cyclic adenosine monophosphate level in muscle specimens strongly decreased in a chronic septic mouse model accompanying attenuations in both the indirectly elicited twitch tension and the effect of dTc (8). It is presumed that such changes in second messenger systems may contribute to the mechanism of the decrease in dTc-sensitivity.

Even though the mechanism of the interaction between dTc and propofol is unclear, propofol potentiated the depression of twitch tension by dTc (1 μ M) in the sham group, as was shown in a previous study using vecuronium in nonseptic rats (9), although 800 μ M of propofol was required to elicit a significant depression. This result supports a previously reported finding that propofol (Diprivan[®]) did not demonstrate any significant interactions with NDMRs in nonseptic patients (22) because 800 μ M is obviously higher than the clinical range of propofol concentration. However, our results

demonstrated that propofol (from 100 to 800 μ M) enhanced the directly elicited twitch tension of sham diaphragms, indicating enhancement of muscle contractility, which is in contrast to the results of a previous study using vecuronium (9). The facilitation of muscle contractility is not caused by the intrinsic effect of the solvent for propofol, because concentrations of intralipid (without propofol) corresponding to 100 to 800 μ M of propofol produced no obvious effect on the directly elicited twitch tension. These results indicate that the action of propofol on dTc (1 μ M)-induced twitch depression does not simply reflect enhancement of the dTc-induced neuromuscular blockade.

When indirect stimulation was performed, propofol facilitated twitch tension in the sham group in a dose-dependent manner, similar to the twitch tension elicited by direct stimulation in both the sham and septic groups. In contrast, propofol at the highest concentration depressed the indirectly elicited twitch tension in the septic group. These results indicate the possibility that in a septic condition, propofol by itself facilitates muscle contractility, while it has a blocking effect on neuromuscular transmission and has a much greater effect at a higher concentration. Therefore, it is speculated that this is one of the mechanisms by which propofol potentiates dTc-induced twitch depression in the septic rat diaphragm. It is presumed that propofol may elicit greater enhancement of the dTc-induced neuromuscular blockade than that represented as twitch depression, although the action of dTc on twitch tension may be antagonized by facilitation of muscle contractility induced by propofol.

In the septic group, propofol at a small concentration (from 100 μ M) potentiated the depression of twitch tension decreased by dTc (1 μ M). However, the facilitating effect of propofol on muscle contractility in the septic group was similar to that in the sham group. These results indicate that propofol more strongly potentiates the dTc-induced neuromuscular blockade under the condition of sepsis; nevertheless, dTc resistance is increased by sepsis, as mentioned above. Currently, little is known about neuromuscular transmission under the condition of sepsis or about the interaction between dTc and propofol in neuromuscular transmission. Some reports suggest that increased nitric oxide synthase production may be involved in the diaphragmatic dysfunction after sepsis (23-25), and these appear to be implicated in the altered neuromuscular transmission in sepsis. However, it is still difficult to explain why and how sepsis enhances the potentiating effect of propofol on dTc-induced twitch depression. Meanwhile, our results suggest that a condition of sepsis may cause propofol to potentiate the action of NDMRs. Moreover, in the clinical situation, it is possible that septic patients receiving both NDMRs and propofol will show a greater neuromuscular blocking effect than that in patients receiving only NDMRs.

In summary, this study showed that sepsis in the acute phase attenuates both muscle contractility and sensitivity to a neuromuscular blocker in the diaphragm, and that propofol potentiates dTc-induced twitch depression, especially in the septic rat diaphragm.

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