Tulobuterol Patch Maintains Diaphragm Muscle Contractility for Over Twenty-four Hours in a Mouse Model of Sepsis

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Since the development of tulobuterol as a synthetic $\beta_2$-adrenergic receptor agonist (Kubo et al. 1975), it has been used to treat patients with bronchial asthma or chronic obstructive pulmonary disease (COPD) by 24-hour transdermal patch treatment. It is well known that $\beta_2$-adrenergic receptor agonists, such as isoproterenol (Howell and Roussos 1984), fenoterol (Suzuki et al. 1986), terbutaline (Cairns and Dulhunty 1993) and clenbuterol (Heijden et al. 1997), increase diaphragm muscle contractility. It has also been reported that diaphragm weakness occurs in patients with mild to moderate bronchial asthma (Akkoca et al. 1999; Barbarito et al. 2001; Weiner et al. 2002). We have recently reported that transdermal tulobuterol treatment increases the force-frequency curves of normal diaphragm muscles for 24 hours (Shindoh et al. 2007).

On the other hand, endotoxin is known to induce a decrease in diaphragm muscle contractility (i.e., diaphragm muscle weakness) due to its cellular damage by superoxides or free radicals and to induce TNF-$\alpha$ mRNA expression (Shindoh et al. 1995). Concerning the effect of endotoxin on diaphragm muscle contractility, some cytokines of interleukin-10 (IL-10) (Taneda et al. 1998), interleukin-13 (IL-13) (Takahashi et al. 1999), and interleukin-12 (IL-12) (Nakahata et al. 2001) prevent the depressive effects of endotoxin on diaphragm muscle contractility. These cytokines seem to act competitively against endotoxin to maintain muscle contractile forces.

Furthermore, recent studies have revealed that a complex of CD14 and Toll-like receptor 4 (TLR4) functions as a receptor for endotoxin (Ulevitch and Tobias 1995) and that total serum immunoglobulin E (IgE) is reversely related to endotoxin concentration in patients with bronchial asthma associated with single nucleotide polymorphisms (SNPs) of CD14 (Baldini et al. 1999). It is speculated that exposure to house dust with a high endotoxin concentration is strongly related to the incidence of bronchial asthma; however, in patients with SNPs, it is reversely related to the quantity of endotoxins in the environment during childhood and to non-development of bronchial asthma.

Transdermal tulobuterol treatment is clinically used once a day to stabilize the asthma state; however, it has not yet been clarified whether or not tulobuterol transdermal...
treatment may affect diaphragm muscle contractility against the effects of endotoxin administration. We therefore hypothesized that transdermal treatment of tulobuterol would protect diaphragm contractility during administration of endotoxin in an animal model.

Accordingly, we first attempted to ascertain the direct effects of tulobuterol on the diaphragm muscle, which was excised at 4 h after intraperitoneal injection of endotoxin, by incubating diaphragm muscle strips in two different concentrations of tulobuterol in vitro. Then at 0, 12, and 24 h from the start of transdermal tulobuterol treatment, we examined the effects of intraperitoneal endotoxin injection at 0 and 4 h. We found diaphragm muscle contractility to be protected by the transdermal tulobuterol treatment at each time.

Measurement of Muscle Contraction

Muscle strips (3-4 mm wide) were dissected from the right and left hemidiaphragms of each animal under diethyl ether anesthesia and mounted in separate organ baths containing Krebs-Henseleit solution oxygenated with a 95% O₂ - 5% CO₂ gas mixture (37.0 ± 0.5°C, pH 7.40 ± 0.05). The composition of the aerated Krebs-Henseleit solution in mEq/L was as follows: Na⁺, 153.8; K⁺, 5.0; Ca²⁺, 5.0; Mg²⁺, 2.0; Cl⁻, 145.0; HCO₃⁻, 15.0; HPO₄²⁻, 1.9; SO₄²⁻, 2.0; glucose, 110 mg%; d-tubocurarine, 10 µM; and regular crystalline zinc insulin, 50 U/L. Both muscle strips were simultaneously stimulated with supramaximal currents of 200-250 mA (i.e., 1.2 to 1.5 greater than the current required to elicit maximal twitch tension, with a pulse duration of 0.2 milliseconds) by a constant-current stimulus isolation unit (SS-3021, Nihon Kohden, Tokyo) driven by a stimulator (SEN-3201, Nihon Kohden, Tokyo). The tensions elicited were measured by a force transducer (UL-100GR, Minebea Co., Fujisawa). The length of each muscle strip was changed by moving the position of the force transducer with a micrometer-controlled rack-and-pinion gear (accuracy of displacement, 0.05 mm; Mitsutoyo Co., Kawasaki) and measured with a micrometer in close proximity to the muscle. The optimal length of the muscle (Lo) was defined as the muscle length at which twitch tension development was maximal, and this Lo was maintained in subsequent measurements.

The diaphragm force-frequency relationship was assessed by sequentially stimulating muscles at 1, 10, 20, 30, 50, 70, 100, and 120 Hz. Each stimulus train was applied for approximately 1 s, and adjacent trains were applied at approximately 10-second intervals. The tensions of both muscle strips were recorded by a hot-pen recorder (RECTI-HORIZ-8K, San-ei, Tokyo). The force-frequency curves obtained from the groups studied were displayed as elicited tensions (N/cm²) on the Y-axis and stimulating frequencies on the X-axis.

Twitch contraction was elicited by single-pulse stimulation (with 0.2-ms pulse duration), and the trace of the twitch contraction was recorded at high speed (10 cm/s). The twitch kinetics were assessed based on twitch tension (TT, N/cm²), contraction time (CT, the time required to develop peak tension, milliseconds), and half-relaxation time (HRT, the time required for peak tension to fall by 50%, milliseconds) during a single muscle contraction. For analysis of the rate of twitch contractions, TT/CT (slope during contraction time) and (TT/2)/HRT (slope during half-relaxation time) were calculated from the

**Methods**

**Animal Preparation**

Experiments were performed on 50 BALB/c mice weighing 21.7 ± 0.2 g (Charles River Japan), which were divided into 2 groups. (A) In the tulobuterol incubation group, diaphragm muscles excised from untreated animals (E0) and from animals after 4 h of intraperitoneal injection of endotoxin (E4) of Escherichia coli (E. coli) endotoxin (20 µg/kg, 055:B5, Sigma Chemical Co., St. Louis, MO, USA) were incubated in an organ bath without tulobuterol (n = 5 each). In addition, diaphragm muscles excised from animals after 4 h of intraperitoneal E. coli endotoxin (20 µg/kg) administration were incubated in an organ bath containing concentrations of 10⁻⁵ or 10⁻³ M of tulobuterol (kindly provided by Abbott Japan Co.) (n = 5 each) for 1 h, and then measured for contractility, termed T10⁻⁵ and T10⁻³ M, respectively. (B) In the transdermal tulobuterol treatment (in vivo) group, a small piece (4 × 4 mm²) of a 0.5 mg tulobuterol patch sheet (16 × 16 mm²) was affixed to the shaved back of each animal and covered with a small adhesive strip. To examine the effect of transdermal tulobuterol treatment, the diaphragm muscles of mice immediately after E. coli endotoxin (20 µg/kg) administration were dissected and measured for contractility at 0, 12, and 24 h (n = 5 each) after patch affixation, termed T0E0, T12E0, and T24E0, respectively; to examine such effect 4 h after E. coli endotoxin (20 µg/kg) injection, the diaphragm muscles of another group of mice were dissected and measured for contractility at 0 + 4, 12 + 4, and 24 + 4 h (n = 5 each) after patch affixation, termed T0E4, T12E4, and T24E4, respectively (Fig. 1). This study was approved by the Animal Ethics Committee of Tohoku University, Sendai, Japan. The study was performed from September 1, 2007 to January 31, 2008, and supported in part by a grant for physiological research on respiratory muscles.

**Transdermal Tulobuterol Treatment**

![Fig. 1. Experimental time courses of transdermal tulobuterol treatment and endotoxin injection. T0E0 and T0E4: Immediately after endotoxin administration (E0) and 4 hours after endotoxin administration (E4) at beginning of tulobuterol treatment (T0), respectively. T12E0 and T12E4: Immediately after endotoxin administration (E0) and 4 hours after endotoxin administration (E4) at 12 hours of tulobuterol treatment (T12), respectively. T24E0 and T24E4: Immediately after endotoxin administration (E0) and 4 hours after endotoxin administration (E4) at 24 hours of tulobuterol treatment (T24), respectively.](image-url)
curve of the twitch contraction trace.

Data Analysis

The cross-sectional area of the strip was calculated by dividing the muscle weight by the product of the strip muscle length and muscle density (1.06 g/cm$^3$) (Close, 1972), and tension was calculated as force per unit area (N/cm$^2$). Data were averaged by the number of muscle strips for force-frequency curves and twitch kinetics. Parameters were compared using the unpaired Student’s t-test and two-way repeated-measures ANOVA followed by Fisher’s protected least significant difference (PLSD). All data are expressed as means ± SEM (standard error of the mean). $p < 0.05$ was considered to be statistically significant.

Results

Changes in Contractility in the Tulobuterol Incubation Group

The force-frequency curves for the effects of intraperitoneal injection of endotoxin (20 mg/kg) and 1-h tulobuterol incubation on diaphragm muscle excised 4 h after the intraperitoneal injection of endotoxin are shown in Fig. 2. As shown in Fig. 2A, the mean peak tension of the force-frequency curves at E0 was 13.5 ± 0.8 N/cm$^2$, while that at E4 was 10.0 ± 0.9 N/cm$^2$, which was significantly decreased from E0 ($p < 0.001$); there were significant decreases at 30, 50, 70 ($p < 0.05$, each), 100, and 120 ($p < 0.01$, each) Hz for E4 compared with those for E0. The force-frequency curves for the effects of 1-h tulobuterol incubation on diaphragm muscle excised 4 h after the intraperitoneal injection of endotoxin (20 mg/kg) are shown in Fig. 2B. The peak tensions of the force-frequency curves at $10^{-5}$ M were 14.0 ± 1.1 N/cm$^2$ ($p < 0.001$), and mostly increased, while those at $10^{-7}$ M were 13.0 ± 1 N/cm$^2$ ($p < 0.01$) compared with E4 (10.0 ± 0.9 N/cm$^2$). As shown in Fig. 2B, there were significant increases at 1 ($p < 0.05$), 20, 30, 50 ($p < 0.01$, each), 70, 100, and 120 ($p < 0.05$, each) Hz for $10^{-5}$ M compared with those for E4.

Table 1 summarizes the twitch kinetics at E0 and E4 of untreated diaphragm muscle, and such kinetics after the T10$^{-7}$ M and T10$^{-5}$ M 1-h tulobuterol incubation using E4 diaphragm muscle. The TT/CT at E4 (119 ± 8 N/cm$^2$/sec, $p < 0.05$) was significantly lower than that at E0, and the TT/2/HRT at E4 (49 ± 4 N/cm$^2$/sec, $p < 0.01$) was also significantly lower than that at E0. In the 1-h tulobuterol incubation using E4 diaphragm muscle, the twitch tension at $10^{-5}$ M (4.9 ± 0.6 N/cm$^2$, $p < 0.05$) was significantly higher than that at E4, and contraction time at $10^{-5}$ M (0.030 ± 0.001 sec) was significantly higher than that at E0 and E4 ($p < 0.05$, each). The half-relaxation time at $10^{-5}$ M (0.036 ± 0.003 sec) was significantly higher than that at E0. The TT/2/HRT at $10^{-7}$ M (74 ± 13 N/cm$^2$/sec, $p < 0.05$) was also significantly higher than that at E4. These results show that diaphragm muscle tension decreased due to endotoxin injection in both TT/CT and (TT/2)/HRT at E4; however, the TT after 1-h incubation with $10^{-5}$ M tulobuterol increased significantly compared with that at E0.

Changes in Contractility by Endotoxin Injection During the Tulobuterol Transdermal Treatment

The force-frequency curves of E0 and E4 at 0 (T0), 12 (T12) and 24 (T24) h after in vivo tulobuterol transdermal treatment are summarized in Fig. 3. There were no significant changes in peak tensions in the force-frequency curves at T0E0 (13.2 ± 0.9 N/cm$^2$) and T0E4 (14.1 ± 1.5 N/cm$^2$) nor any significant changes in tensions at each frequency (Fig. 3A). Again, there were no significant changes in peak tensions in the force-frequency curves at T12E0 (15.4 ± 1.3 N/cm$^2$) and T12E4 (14.4 ± 1.1 N/cm$^2$) nor any significant changes in tensions at each frequency (Fig. 3B). Finally, there were no significant changes in peak tensions in the force-frequency curves at T24E0 (16.1 ± 1.3 N/cm$^2$) and T24E4 (13.9 ± 0.8 N/cm$^2$) nor any significant changes in tensions at each frequency (Fig. 3C).

![Fig. 2. Changes in force-frequency curves for (A) intraperitoneal injection of endotoxin (20 mg/kg) at E0 (closed circles) and E4 (closed squares), (B) 1-hour tulobuterol incubation using diaphragm muscle excised 4 h after the intraperitoneal endotoxin injection, 10$^{-7}$ M (open triangles), and 10$^{-5}$ M (closed triangles). E4 (closed squares) is shown to be the same as that in (A). *$p < 0.05$, **$p < 0.01$, compared with each frequency of E0 or E4, respectively. ***$p < 0.01$, ****$p < 0.001$, compared between groups by ANOVA.](image-url)
Table 2 summarizes the twitch contraction kinetics for E0 and E4 at 0 (T0), 12 (T12), and 24 (T24) h after in vivo transdermal tulobuterol treatment. There were no significant changes in twitch kinetics parameters between E0 and E4 at T0, T12, and T24. There were significant changes in TT (4.7 ± 0.5 N/cm², \( p < 0.05 \)) and HRT (0.035 ± 0.004 sec, \( p < 0.05 \)) of T24E0 compared with those of T0E0. These results indicate that the transdermal tulobuterol treatment over a period of 28 h was able to prevent the depressive effect of endotoxin on force-frequency curves and twitch kinetics.

Table 2. Twitch kinetics of untreated diaphragm muscle immediately after (E0), 4 hours after (E4) endotoxin injection, and such kinetics after T10⁻⁷ M and T10⁻⁵ M of 1-h tulobuterol incubation using E4 diaphragm muscle.

<table>
<thead>
<tr>
<th></th>
<th>Untreated diaphragm muscle</th>
<th>E4 diaphragm muscle</th>
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<tbody>
<tr>
<td></td>
<td>E0</td>
<td>E4</td>
</tr>
<tr>
<td>TT (N/cm²)</td>
<td>3.8 ± 0.5</td>
<td>2.8 ± 0.2</td>
</tr>
<tr>
<td>CT (sec)</td>
<td>0.023 ± 0.001</td>
<td>0.024 ± 0.001</td>
</tr>
<tr>
<td>HRT (sec)</td>
<td>0.026 ± 0.002</td>
<td>0.030 ± 0.002</td>
</tr>
<tr>
<td>TT/CT (N/cm²/sec)</td>
<td>167 ± 23</td>
<td>119 ± 8*</td>
</tr>
<tr>
<td>(TT/2)/HRT (N/cm²/sec)</td>
<td>72 ± 5</td>
<td>49 ± 4*</td>
</tr>
</tbody>
</table>

TT, Twitch tension; CT, Contraction time; HRT, Half-relaxation time.

Significant difference compared with E0: \( \hat{p} < 0.05 \); \( \hat{p} < 0.01 \)

Significant difference compared with E4: \( p < 0.05 \)

In Fig. 4, the force-frequency curves are replotted for a combination of normal diaphragm muscles and transdermal treatment groups. The force-frequency curves of both T12E0 (\( p < 0.01 \)) and T24E0 (\( p < 0.001 \)) shifted significantly upward from that of E0 (Fig. 4A). Because the force-frequency curve of T0E0 was measured immediately after transdermal path affixation, it was not significantly different from that of E0. There were significant increases at 50 (\( p < 0.05 \)) Hz for T12E0, and at 20, 30, 50 (\( p < 0.05 \),
Protective Effects of Transdermal Tulobuterol Treatment

Discussion

In the present study, we found that tulobuterol increased contraction of the diaphragm muscles excised at 4 h after intraperitoneal endotoxin injection in a dose-dependent manner in the incubation group. In agreement with the results for the incubation group, we also found that at 0, 12, and 24 hours after transdermal tulobuterol treatment, such treatment protected against a decrease in force-frequency curves of diaphragm muscles excised 4 h after intraperitoneal endotoxin injection. Taken together, these results indicate that tulobuterol improves the contractility of diaphragm muscle damaged by endotoxin and that clinical application of a transdermal tulobuterol patch once a day can provide a defense against the effects of endotoxin administration for over 24 hours.

As proof of the direct effect of tulobuterol on endotoxin-damaged diaphragm muscle, in the incubation group, the force-frequency curves of endotoxin-administered diaphragm muscle shifted upward with tulobuterol treatment, indicating that tulobuterol was able to increase the force-frequency curves of diaphragm muscle damaged by endotoxin. Even though the diaphragm muscle suffered cellular damage by superoxides or free radicals, which was induced by endotoxin (Shindoh et al. 1990, 1992), tulobuterol was able to inhibit the decrease of contractility via \( \beta_2 \)-adrenergic receptors to increase cAMP and subsequent \( \text{Ca}^{2+} \) kinetics.

It is known that adrenaline and other sympathomimetic amines increase the maximal twitch tension of unfatigued skeletal muscles (Bowman and Zaimis 1958) and that this effect on muscle contractility may arise through activation of the adenyl cyclase/cyclic 3', 5'-AMP system, which in turn leads to changes in the rates of uptake of \( \text{Ca}^{2+} \) by the sarcoplasmic reticulum (Bowman and Nott 1969). At concentrations which produce effects on muscle contractility, salbutamol (a \( \beta_2 \)-adrenoceptor agonist) has been found to...
significantly elevate cyclic AMP (cAMP) concentrations in isolated fast- and slow-contracting muscles of the guinea pig (Al-Jeboory and Marshall 1978). Thus, it is currently considered that \( \beta_2 \)-adrenergic agonists stimulate adenyl cyclase, elevating cAMP, which activates cAMP-dependent protein kinase (PKA) and which phosphorylates several key proteins, including 1) troponin I (reducing Ca\(^{2+}\) affinity of TnC), 2) sarcolemmal Ca\(^{2+}\) channels (increasing \( I_{Ca} \)), 3) phospholamban (increasing SR Ca\(^{2+}\) pump rate) and 4) SR Ca\(^{2+}\) release channels (modifying RyR gating) (Bers 2001). We speculate that the augmentation of contractility by tulobuterol observed in the diaphragm muscle damaged by endotoxin is an overall effect of changes in the contractile and relaxation rates due to Ca\(^{2+}\).

Since the discovery in 1986 of a plasma protein termed LPS binding protein (LBP) (Tobias et al. 1986) led to the discovery of unanticipated mechanisms of LPS-induced cell activation, CD14 has been found to be a soluble serum protein or a glycosylphosphatidylinositol (GPI)-anchored protein of myeloid lineage cells. It is now known that CD14 plays a key role in LPS-induced cell activation and that a functional LPS receptor of myeloid cells comprised of GPI-anchored CD14 together with a presently unidentified transmembrane protein binds LPS and initiates cell activation via kinase cascades (Ulevitch and Tobias 1995). Kurt-Jones et al. (2000) have reported that CD14 and Toll-like receptor (TLR) pattern recognition receptors are essential for the innate immune response to components of Gram-negative and Gram-positive bacteria, mycobacteria, spirochetes and yeast, and that the innate immune response to the fusion protein of an important respiratory pathogen of humans, respiratory syncytial virus (RSV), is mediated by TLR4 and CD14. Thus, a common receptor activation pathway can initiate innate immune responses to both bacterial and viral pathogens.

Concerning TLR4, it has been reported that the codominant \( Lps^2 \) allele of C3H/Hej mice, which renders them highly susceptible to Gram-negative infection, corresponds to a missense mutation in the third exon of the TLR4, predicted to replace proline with histidine at positions 712 (His\(^{712}\) (CAT) and Asn\(^{719}\) (AAC)) of the polypeptide chain (Qureshi et al. 1999). On the other hand, C57BL/10ScCr (Pro\(^{712}\) (CCT) and Asn\(^{719}\) (AAC)) mice are homozygous for a null mutation of TLR4 (Poltorak et al. 1998). Thus, the mammalian TLR4 protein has been adapted primarily to subserve the recognition of LPS, and it transduces the LPS signal across the plasma membrane. Destructive mutations of TLR4 predispose to the development of Gram-negative sepsis, leaving most aspects of immune function intact. In the present study, we used BALB/c J (Pro\(^{712}\) (CCT) and Asn\(^{719}\) (AAT)) mice (Qureshi et al. 1999), which have no point mutation of TLR4 and react to LPS, but which are not highly susceptible to Gram-negative infection.

Concerning CD14, recent studies have also shown that CD14-159C→T single nucleotide polymorphism (SNP) has an inverse correlation with serum levels of IgE (Koppelman et al. 2001; Vercelli et al. 2001) and that increasing endotoxin exposure is associated with a reduced risk of allergic sensitization and eczema but with an increased risk of nonatopic wheeze in children with the CC genotype at -159 of the CD14 gene (Simpson et al. 2006). In addition, the C allele of CD14-260 has also been reported to be associated with higher levels of both total and specific serum IgE to aeroallergens in children with regular contact with pets, whereas an association in the opposite direction was found in children with regular contact with stable animals. This modifying effect of animal exposure was not explained by levels of house dust endotoxin (Eder et al. 2005), and it was hypothesized that a common polymorphism in the endotoxin receptor, CD14 C-260T, and endotoxin levels in the home might interact to influence total serum IgE levels into adulthood (Williams et al. 2006). These genetic factors seem to modify LPS responsiveness, which may influence susceptibility to the development of allergy and/or asthma.

From these reports concerning \( \beta_2 \)-adrenergic receptors and CD14 and TRL4 receptors mentioned above, tulobuterol is considered to increase the tension of diaphragm muscle contractility via \( \beta_2 \)-adrenergic receptors. On the other hand, endotoxin decreases its contractility via CD14 receptors in cardiomyocytes (Comstock et al. 1998) and TRL4 receptors in skeletal muscle (Lang et al. 2003). Therefore, we speculate that these two effects in the transdermal tulobuterol treatment groups may counterbalance each other, resulting in maintenance of diaphragm contractility for 24 hours, as observed in the present study.

Although the present experiment was done by intraperitoneal injection of endotoxin, for clinical application in patients with bronchial asthma, if such patients are treated with transdermal tulobuterol patches once a day, they may be able to avoid diaphragm muscle weakness resulting from inhalation of endotoxin. Because endotoxin injected into the intraperitoneal cavity or inhaled into the lung is absorbed by the veins and circulated in the body, it is suggested that patients may be protected from severe bronchial asthma attack due to the mitigation of diaphragm muscle weakness. Furthermore, diaphragm muscle weakness is considered to be critical in patients with COPD (Decramer et al. 1994; Kim et al. 2008). The \( \beta_2 \)-adrenergic agent salbutamol has inotropic effects on rat diaphragm contractility, these effects being potentiated by foreshortening (Heijden et al. 1997), and the \( \beta_2 \)-adrenergic agent albuterol enhances respiratory muscle output in patients with COPD, primarily by improving the length-tension relationship of the diaphragm muscle rather than by improving its contractility (Hatipoglu et al. 1999). Therefore, it is suggested that tulobuterol is also clinically applicable in patients with COPD due to its inotropic effect on diaphragm muscle.

Our previous report (Shindoh et al. 2007) showed that transdermal tulobuterol treatment exhibited inotropic effects on diaphragm muscle over a 24-hour period and that the
time course of the effects of the tulobuterol patch in the in vivo study was consistent with that of blood concentration in the case of transdermal tulobuterol application. Uematsu et al. (1993) have reported that tulobuterol was well absorbed after transdermal administration, with a lag-time of about 4 h, that $C_{\text{max}}$ and AUC increased linearly with dose, and that $t_{\text{max}}$ was about 9-12 h. They also reported that the mean percentage of drug absorbed during transdermal treatment for 24 h was 82-90% after a single dose (Uematsu et al. 1993). In the transdermal tulobuterol treatment in the present study, the differences of force-frequency curves between E0 and E4 at 0, 12, and 24 h were not significant; however, those at 24 h were wider than those of at 0 and 12 h. We speculate that these findings are closely related to the blood concentration of tulobuterol, which may be less at 24 h than at 0 and 12 h. Therefore, the degree of protective action of tulobuterol against endotoxin administration at 24 h might be weaker than at other times.

Concerning comparisons of E0, as shown in Fig. 3A, the force-frequency curve of T0E0 was not significantly different from that of E0 because it was immediately after transdermal tulobuterol treatment and transdermal tulobuterol absorbance had just begun. On the other hand, the force-frequency curves of T12E0 and T24E0 were significantly shifted upward from E0, which seems to support the existence of a trophic effect of transdermal tulobuterol treatment on the diaphragm muscle over a period of 24 hours. Furthermore, concerning comparisons of E4, the force-frequency curves of T0E4, T12E4, and T24E4 were significantly different from that of E4 as shown in Fig. 4B. We speculate that as 4 hours had passed since the beginning of transdermal treatment, the force-frequency curve of T0E4 was sufficiently and competitively increased against endotoxin and that the force-frequency curves of T12E4 and T24E4 were also sufficiently protected and competitive against endotoxin.

In conclusion, transdermal tulobuterol treatment has protective effects for over 24 hours on diaphragm muscle contractility when patients with bronchial asthma or COPD suffer from endotoxin exposure attacks and that it facilitates treatment of asthma and COPD due to its long effectiveness.

References


