Allergology International. 2007;56:285-291
DOI: 10.2332/allergolint.O-06-463

ORIGINAL ARTICLE

Inhalation and Incubation with Procaterol Increases Diaphragm Muscle Contractility in Mice

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ABSTRACT

Background: Although procaterol is used clinically as a β_2-adrenergic receptor agonist to relax airway smooth muscle, it has not yet been clarified whether procaterol has inotropic effects on respiratory muscles.
Methods: Three intervention groups were investigated: a procaterol inhalation only group; a procaterol inhalation plus endotoxin injection group (*in vivo*); and a procaterol incubation group (*in vitro*). The diaphragm muscle in all groups was dissected and measurements of its contractile properties were performed.
Results: The effects of procaterol inhalation shifted the force-frequency curves upward at 30 minutes after inhalation, and inhibited the decline of force-frequency curves due to endotoxin injection *in vivo*. *In vitro* administration of procaterol resulted in an increase in the force-frequency curves in a dose-dependent manner.
Conclusions: It can be concluded that procaterol has an inotropic effect on the diaphragmatic muscles taken from normal animals as well as on the diaphragm muscles in a septic animal model.

KEY WORDS

COPD, diaphragm muscle, endotoxin, respiratory muscle fatigue, β_2-agonists

INTRODUCTION

Procaterol is used clinically as a β_2-adrenergic receptor agonist (β_2-agonist) to relax airway smooth muscle and is a principal bronchodilator agent used to treat bronchial asthma by metered dose inhalation (MDI). Procaterol, salbutamol and tulobuterol are classified as short-acting β_2-agonists, and formoterol and salmeterol are long-acting β_2-agonists. The rank order of relaxation efficacy of these β_2-agonists seems to be as follows: isoproterenol = procaterol = formoterol > salbutamol > salmeterol >> tulobuterol.[1] Therefore, procaterol is classified as a selective full or strong β_2-adrenergic receptor agonist. Based on these relationships, bronchodilators are used to treat patients with bronchial asthma. Following a previous report showing that isoproterenol stimulates cAMP synthesis via β_2-adrenergic receptor in the skeletal muscle,[2] there have been several reports concerning the effects of β_2-agonists on the diaphragm muscle, namely, isoproterenol and aminophylline were shown to improve the contractility of fatigued canine diaphragms.[3] In addition, fenoterol improved fatigued canine diaphragms,[4] terbutaline activated contraction in isolated fast- and slow-twitch skeletal muscle fibers in rats,[5] and broxaterol increased the force output of fatigued canine diaphragm more than salbutamol.[6] From these reports, it seems likely that β_2-agonists increase contractility in normal and fatigued diaphragm muscles. However, it has not yet been clarified whether procaterol has inotropic effects on respiratory muscles, especially on diaphragm muscles.

In addition, it is known that the injection of endotoxin induces a decrement in diaphragm muscle contractility and that this deterioration may be caused by a network of cytokines such as tumor necrosis factor-α (TNF-α) and free radicals such as nitric oxide (NO) and oxygen derived intermediates, including superoxide and the hydroxyl radical.[7,8] We have recently reported that IL-10[9] and IL-13[10] play a protective role in diaphragm muscle after endotoxin injection into

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Received 23 August 2007. Accepted for publication 19 February 2007.

the peritoneal cavity. Because it has been reported that isoproterenol has protective effects on diaphragmatic contractility in septic peritonitis,[11] it is suggested that procaterol could prevent endotoxin-induced diaphragm muscle deterioration.

Accordingly, we first attempted to examine the effects of procaterol inhalation in normal animals and measured the diaphragm contractile properties. Secondly, we examined whether procaterol exerted any protective effects against endotoxin induced deterioration. Thirdly, to ascertain whether procaterol has any direct effects on diaphragm muscles, we incubated diaphragm muscle strips in three different concentrations of procaterol *in vitro*. We found that procaterol increases contraction in normal and endotoxin damaged diaphragm muscles (*in vivo* study) and increases the force-frequency curves in a dose-dependent manner (*in vitro* study).

METHODS

ANIMAL PREPARATION

Experiments were performed on 95 animals divided into three groups using BALB/c mice weighing 23.1 ± 0.4 *g* (Charles River Japan, Yokohama, Japan). (A) In the procaterol inhalation group, animals were given 2 inhalation puffs from a procaterol MDI (metered dose inhaler) via a 75-ml spacer, and then diaphragm muscles were dissected and the contractility was measured immediately (0 hours), 30 minutes, 1 hour, 2 hours and 4 hours later (n = 5 animals each). (B) In the endotoxin only groups, animals were given an intraperitoneal injection of *E. coli* endotoxin (20 mg/kg, 055: B5, Sigma Chemical Co., St. Louis, MO, USA) in 0.5 ml of saline, with measurement of muscle contractility immediately (0 hours), 2 hours and 4 hours later (n = 5 animals each). In the procaterol inhalation plus endotoxin groups, animals were initially given 2 puffs of inhalation from a procaterol MDI via a 75-ml spacer, followed 5 minutes later by an intraperitoneal injection of *E. coli* endotoxin (20 mg/kg) in 0.5 ml of saline. The diaphragm muscles were then dissected and measured as to muscle contractility immediately (0 hours), 2 hours and 4 hours later (n = 5 animals each). Because we previously showed that the force-frequency curves decreased maximally at 3–4 hours and then recovered at 6 hours after endotoxin injection,[8] we measured and analyzed diaphragm muscle contractility at 4 hours after endotoxin administration. (C) In the *in vitro* groups, diaphragm muscles taken from normal animals (n = 5 animals each) and other diaphragm muscles taken at 4 hours after intraperitoneal injection of *E. coli* endotoxin (20 mg/kg) in 0.5 ml of saline (n = 5 animals each) were incubated in an organ buffer with concentrations of 0, 10^{-5}, 10^{-6}, and 10^{-7} M of procaterol hydrochloride (Sigma Chemical Co.) for 1 hour each, and then their respective contractile properties were measured. Written approval was obtained from the Tohoku University Animal Facility.

MEASUREMENTS OF MUSCLE CONTRACTION

Muscle strips (width; 3–4 mm, length; 8–11 mm, weight; 0.008–0.010 g) were dissected from the right and left hemidiaphragm of each animal under diethyl ether anesthesia and mounted in separate organ baths containing Krebs-Henseleit solution oxygenated with a 95% O_2 –5% CO_2 gas mixture (37.0 ± 0.5℃, 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; Ca^{2+}, 5.0; Mg^{2+}, 2.0; Cl^-, 145.0; HCO_3^-, 15.0; HPO_4^{2-}, 1.9; SO_4^{2-}, 2.0; glucose, 110 mg%; d-tubocurarine, 10 μM; and regular crystalline zinc insulin, 50 U/liter. Both muscle strips were simultaneously stimulated with supramaximal currents of 200–250 mA (*i.e.*, 1.2 to 1.5 times the current required to elicit maximal twitch tension, pulse duration of 0.2 ms) by a constant current stimulus isolation unit (SS-302J, Nihon Kohden Co., Tokyo, Japan) driven by a stimulator (SEN-3201, Nihon Kohden Co.). The elicited tensions were measured by a force transducer (UL-100GR, Minebea Co., Fujisawa, Japan). 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, Japan) 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 the following measurements.

The diaphragm force-frequency relationship was assessed by sequentially stimulating muscles at 1, 10, 20, 30, 50, 70, 100 and 120 Hz, respectively. Each stimulus train was applied for approximately 1 second, 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 Co., Tokyo, Japan). The force-frequency curves obtained from the groups studied were displayed as elicited tensions (N/cm^2) on the Y-axis and stimulating frequencies on the X-axis.

Twitch contraction was elicited by a single pulse stimulation (0.2-ms duration of pulses), and the trace of the twitch contraction was recorded at high speed (10 cm/seconds). The twitch kinetics were assessed by twitch tension (TT, N/cm^2), 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 the analysis of contractile velocity of twitch contractions, TT/CT (slope during contraction time) and (TT/2)/HRT (slope during half relaxation time) were calculated from the curve of the twitch contraction trace. After

completion of this protocol, the muscle strip was removed from the organ bath and weighed.

DATA ANALYSIS

The cross-sectional area of the strip was calculated by dividing the muscle mass by the product of the strip muscle length and muscle density (1.06 g/cm^3),[12] and tension was calculated as force per unit area (N/cm^2). Data obtained from both halves of the diaphragm in one animal were averaged; therefore, the number of samples used was n = 5 (animals) per treatment/time point for force-frequency curves, twitch kinetics and fatigability. The mean values of tensions for each frequency of force-frequency curves, twitch kinetics and fatigability were compared by Student's t -test. To compare the entire configuration of each force-frequency curve at 0 and 4 hours for each group, analysis of variance (ANOVA) with Fisher's protected least significant difference (PLSD) *post hoc* test was performed, and parameters of twitch kinetics were compared using two-way repeated-measures ANOVA with Fisher's PLSD *post hoc* test. Data are presented as means ± SEM (standard error of the mean). P -values < 0.05 were considered to indicate significant differences.

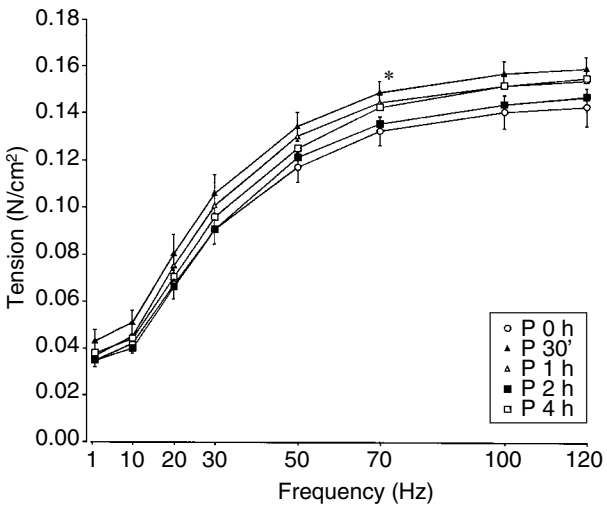

Fig. 1 Changes in the force-frequency curves in the procaterol inhalation group at 0 hours (open circle), 30 minutes (closed triangle), 1 hour (open triangle), 2 hours (closed square), and 4 hours (open square), respectively. * $p <$ 0.05, compared with 70 Hz at 0 hours.

RESULTS

CHANGES OF CONTRACTION PROPERTIES IN THE INHALATION GROUPS

In the inhalation group, the tensions of force-frequency curves at 30 minutes mostly shifted upward, and the force-frequency curves at 1, 2, and 4 hours were located between 30 minutes and 0 hours. There was a significant change only at 70 Hz for 30 minutes ($p < 0.05$), but not at frequencies of 1, 2, and 4 hours compared with those at 0 hours (Fig. 1). Because the shifts of force-frequency curves due to inhalation were small, there were no significant differences in TT, CT, and HRT at each inhalation time compared with those at 0 hours. Likewise, there were no significant differences in TT/CT and (TT/2)/HRT at each inhalation time compared with those at 0 hours (Table 1).

CHANGES OF CONTRACTION PROPERTIES IN THE ENDOTOXIN AND PROCATEROL INHALATION PLUS ENDOTOXIN GROUPS

In the endotoxin injection only group, the force-frequency curves shifted downward gradually from 0 hours (0.135 ± 0.004 N/cm^2 as a peak) to 4 hours (0.096 ± 0.006 N/cm^2 as a peak) (Fig. 2A). There were significant decreases at 100, and 120 Hz at 2 hours (each $p < 0.05$), and at 20 ($p < 0.05$), 30 ($p <$ 0.01), 50, 70, 100, and 120 Hz (each $p < 0.001$), respectively, at 4 hours compared with those immediately after endotoxin intraperitoneal injection (0 hours). This shows that the intraperitoneal administration of endotoxin deteriorated diaphragm muscle contraction in the 4-hour follow-up period. On the other hand, in the procaterol inhalation plus endotoxin administration group, the force-frequency curves shifted upward from 0 hours (0.136 ± 0.006 N/cm^2 as a peak) to 2 hours (0.163 ± 0.007 N/cm^2 as a peak), while those at 4 hours (0.147 ± 0.007 N/cm^2 as a peak) decreased to a level between those of 0 and 2 hours (Fig. 2B). The tensions of 50 ($p < 0.05$), 70 ($p <$ 0.01), 100, and 120 Hz (each $p < 0.05$) each significantly increased from the level at 0 hours.

There were no significant changes in TT, CT, and HRT in the endotoxin injection only and procaterol in-

Table 1 Changes in Twitch Kinetics and Fatigue Indices in the Procaterol Inhalation Groups.

	P 0 h	P 30'	P 1 h	P 2 h	P 4 h
TT (N/cm^2)	0.037 ± 0.004	0.044 ± 0.004	0.038 ± 0.004	0.036 ± 0.004	0.037 ± 0.003
CT (sec)	0.027 ± 0.002	0.028 ± 0.001	0.025 ± 0.002	0.026 ± 0.002	0.027 ± 0.001
HRT (sec)	0.040 ± 0.004	0.041 ± 0.004	0.040 ± 0.004	0.034 ± 0.004	0.037 ± 0.004
TT/CT (N/cm^2/sec)	1.37 ± 0.12	1.53 ± 0.10	1.49 ± 0.13	1.35 ± 0.15	1.37 ± 0.10
(TT/2)/HRT (N/cm^2/sec)	0.46 ± 0.03	0.54 ± 0.04	0.48 ± 0.04	0.53 ± 0.04	0.52 ± 0.04

TT denotes twitch tension, CT contraction time, HRT half relaxation time.

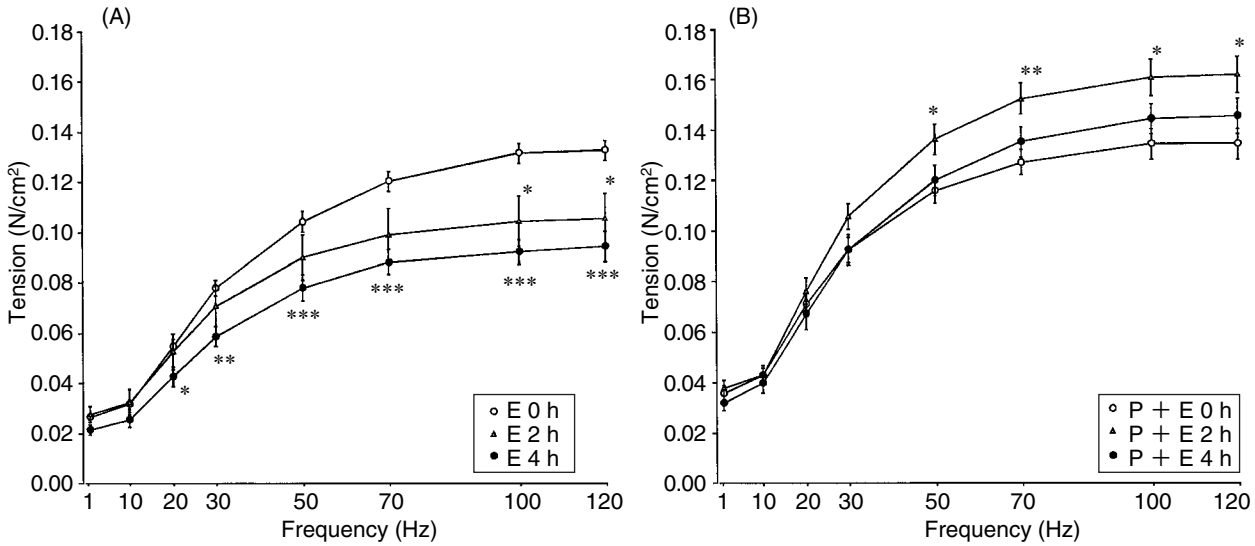

Fig. 2 Changes in the force-frequency curves in the endotoxin (E) injection (**A**) and procaterol inhalation plus endotoxin (P + E) injection (**B**) groups at 0 hours (open circle), 2 hours (open triangle), and 4 hours (closed circle), respectively. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with each frequency at 0 hours.

Table 2 Changes of Twitch Kinetics and Fatigue Indices in the Endotoxin Injection and Procaterol Inhalation plus Endotoxin Groups.

	E 0 h	E 2 h	E 4 h	P + E 0 h	P + E 2 h	P + E 4 h
TT (N/cm²)	0.024 ± 0.002	0.029 ± 0.003	0.020 ± 0.002	0.033 ± 0.002	0.038 ± 0.003	0.035 ± 0.003
CT (sec)	0.030 ± 0.001	0.030 ± 0.002	0.031 ± 0.001	0.031 ± 0.002	0.028 ± 0.001	0.029 ± 0.001
HRT (sec)	0.039 ± 0.003	0.042 ± 0.005	0.037 ± 0.003	0.049 ± 0.005	0.038 ± 0.002	0.042 ± 0.004
TT/CT (N/cm²/sec)	0.83 ± 0.06	0.92 ± 0.08	0.67 ± 0.07	1.07 ± 0.07	1.36 ± 0.13	1.17 ± 0.09
(TT/2)/HRT (N/cm²/sec)	0.32 ± 0.02	0.35 ± 0.03 *	0.30 ± 0.03	0.35 ± 0.03	0.51 ± 0.05 ††	0.42 ± 0.02

TT denotes twitch tension, CT contraction time, HRT half relaxation time.
Significant differences compared with E 0 h: * $p < 0.05$, and that compared with P + E 0 h: †† $p < 0.01$.

halation plus endotoxin administration groups. The (TT/2)/HRT in the endotoxin only group was significantly increased at E 2 hours ($p < 0.05$), compared with that of E 0 hours, and that of the procaterol inhalation plus endotoxin injection group was significantly increased at P + E 2 hours ($p < 0.01$) compared with that of P + E 0 hours (Table 2). These data indicate that procaterol increases relaxation speeds more than endotoxin only does, especially at 2 hours after procaterol inhalation.

CHANGES OF CONTRACTION PROPERTES IN THE INCUBATION GROUPS

We performed an incubation study of procaterol *in vitro* to confirm the effects of procaterol observed in the *in vivo* study detailed above. At first, in the normal diaphragm groups, the force-frequency curves shifted upward in a manner dependent on the procaterol concentration of 10^{-7}, 10^{-6}, and 10^{-5} M (Fig. 3A), respectively. With 10^{-5} M of procaterol (P 10^{-5} M), there were significant increases at 1 ($p < 0.05$), 10 ($p < 0.01$), 30 ($p < 0.05$), 50 ($p < 0.01$), 70, 100, and 120 Hz (each $p < 0.001$), respectively, compared with each value of buffer only (B). Thus, the entire force-frequency curve at 10^{-5} M (0.189 ± 0.007 N/cm² as a peak) was significantly higher than that of 0 M (0.145 ± 0.005 N/cm² as a peak) ($p < 0.001$). Secondly, in the endotoxin plus incubation with procaterol groups, the force-frequency curves shifted upward in a dose-dependent manner (Fig. 3B). With 10^{-5} M of procaterol (E, P 10^{-5} M), there were significant increases at 1, and 20 Hz (each $p < 0.01$), 30 ($p < 0.05$), 50, 70, 100, and 120 Hz (each $p < 0.01$), respectively, compared with each value for the buffer only (E, B), and with 10^{-6} M of procaterol (E, P 10^{-6} M), there were significant increases at 20 ($p < 0.01$), 30 ($p < 0.01$), 50, 70, 100, and 120 Hz (each $p < 0.001$), respectively, compared with the respective values of the buffer only. Again, the entire force-frequency curve at 10^{-5} M (0.170 ± 0.010 N/cm² as a peak) was significantly higher than that for 0 M (0.127 ± 0.004 N/cm² as a peak) ($p < 0.001$).

The TT of both normal muscle ($p < 0.05$) and endotoxin damaged muscles ($p < 0.01$) incubated in a 10^{-5}

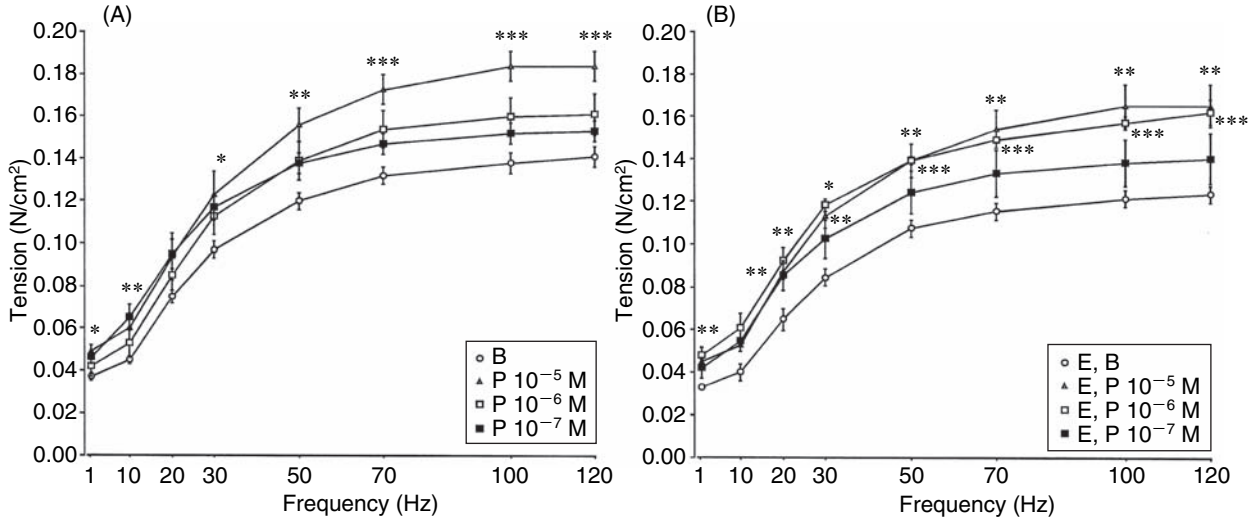

Fig. 3 Changes in the force-frequency curves in the procaterol incubation with normal diaphragm (**A**), and procaterol incubation with endotoxin administered diaphragm (**B**) groups at buffer only (B or E, B; open circle), procaterol 10^{-5} M (P or E, P 10^{-5} M; open triangle), 10^{-6} M (P or E, P 10^{-6} M; open square), and 10^{-7} M (P or E, P 10^{-7} M; closed square), respectively. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with each frequency at B or E, B.

Table 3 Changes of Twitch Kinetics and Fatigue Indices in the Incubation Groups.

	B 0 M	P 10^{-5} M	P 10^{-6} M	P 10^{-7} M
TT (N/cm²)	0.042 ± 0.002	0.056 ± 0.003 *	0.045 ± 0.006	0.050 ± 0.004
CT (sec)	0.032 ± 0.001	0.032 ± 0.001	0.032 ± 0.002	0.042 ± 0.004 **
HRT (sec)	0.044 ± 0.003	0.046 ± 0.006	0.044 ± 0.006	0.063 ± 0.006 *
TT/CT (N/cm²/sec)	1.31 ± 0.06	1.74 ± 0.11 *	1.39 ± 0.13	1.24 ± 0.16
(TT/2)/HRT (N/cm²/sec)	0.48 ± 0.03	0.64 ± 0.06 **	0.51 ± 0.03	0.41 ± 0.03
	E, B 0 M	E, P 10^{-5} M	E, P 10^{-6} M	E, P 10^{-7} M
TT (N/cm²)	0.038 ± 0.002	0.048 ± 0.001 †	0.047 ± 0.005	0.043 ± 0.006
CT (sec)	0.037 ± 0.002	0.035 ± 0.001	0.035 ± 0.002	0.041 ± 0.001
HRT (sec)	0.055 ± 0.007	0.057 ± 0.011	0.045 ± 0.007	0.058 ± 0.003
TT/CT (N/cm²/sec)	1.03 ± 0.05	1.38 ± 0.02 † †	1.34 ± 0.08 †	1.05 ± 0.12
(TT/2)/HRT (N/cm²/sec)	0.36 ± 0.03	0.47 ± 0.07	0.53 ± 0.02 †	0.37 ± 0.03

TT denotes twitch tension, CT contraction time, HRT half relaxation time.
Significant differences compared with B 0 M: * $p < 0.05$; ** $p < 0.01$, and that compared with E, B 0 M: † $p < 0.05$; † † $p < 0.01$.

M concentration of procaterol increased significantly. Both CT and HRT increased at 10^{-7} M ($p < 0.01$ and $p < 0.05$, each) compared to those of B 0 M. Regarding TT/CT, there were significant increases at 10^{-5} M ($p < 0.05$), and endotoxin damaged muscle plus procaterol incubation at 10^{-5} and 10^{-6} M (each $p < 0.01$, $p < 0.05$), respectively. Similarly, as for (TT/2)/HRT, there were significant increases at 10^{-5} M ($p < 0.01$) in comparison with that of B 0M, and for endotoxin damaged muscle plus procaterol incubation at 10^{-6} M ($p < 0.05$) (Table 3).

DISCUSSION

In the present study, the inhalation of procaterol itself initially shifted force-frequency curves upward at 30 minutes after inhalation only at 70 Hz. Subsequently, however, there were no significant changes in the force-frequency curves. Secondly, in the endotoxin injection group, the force-frequency curves shifted downward in a time-dependent manner. On the other hand, in the procaterol inhalation plus endotoxin injection group, the force-frequency curves mostly shifted upward at 2 hours and decreased at 4 hours. Thirdly, in the incubation groups, procaterol shifted force-frequency curves upward in a dose-dependent manner in the normal diaphragm muscles. Even when using endotoxin damaged muscle incubated in procaterol, the force-frequency curves similarly shifted upward. From these results, procaterol can be considered to have a direct inotropic effect on the skeletal muscles such as diaphragm muscles, and, this finding in itself seems very important clinically

and pharmacologically.

It has been reported that salbutamol has inotropic effects on rat diaphragm contractility and that these effects are potentiated by foreshortening.[13] In addition, it has been reported that albuterol (salbutamol) inhalation enhances respiratory muscle output in patients with COPD primarily by improving the length-tension relationship of the diaphragm rather than by improving its contractility[14] and that albuterol reduces dynamic hyperinflation during exercise in patients with COPD.[15] Whether or not procaterol acts on the skeletal muscles such as diaphragm muscles has not been previously examined. Our results indicate that procaterol has inotropic effects on diaphragm muscles in mice and that these effects may be additive with primary bronchodilator activity. Concerning the relatively slight augmentation of force-frequency curves by procaterol inhalation *in vivo*, we speculate that this is due to parasympathetic activities which might antagonize the effects of β_2-agonists. Even when we administered β_2-agonists such as procaterol, therefore, the force-frequency shift was depressed compared with that in the *in vitro* study.

A previous study found that adrenaline acts directly through cyclic AMP to produce an increase in twitch tension.[16] The force potentiating effect is considered to occur via binding to membrane-bound β-receptors, and adrenergic stimulation exerts its effect via a stimulatory G-protein which activates adenylate cyclase resulting in formation of cyclic adenosine monophosphate (cAMP), and cAMP acts as a second messenger and activates protein kinases which catalyze protein phosphorylation.[17] It has been also reported that force is potentiated following β-adrenoceptor activation by a cyclic AMP-dependent phosphorylation of Ca^{2+} release channels to facilitate sarcoplasmic reticulum (SR) calcium release during titanic stimulation.[18] From these previous reports, it may be concluded that the augmented force-frequency curves in our *in vitro* study might have been exerted via cAMP increments by procaterol dose-dependent administration.

Endotoxin, a cell wall component of all gram-negative bacteria, is well known to be responsible for a large part of the toxic manifestations associated with infections by such bacteria and for a generalized and potentially lethal inflammatory reaction, known as the generalized Schwartzman reaction.[19] In particular, IFN-γ is more important in the pathogenesis of the generalized Schwartzman reaction, specifically by its interaction with other cytokines, namely, THF-α, IL-6, and IFN-α and/or -β. Based on the findings by the present authors showing that TNF-α mRNA is expressed in diaphragm muscle cells after endotoxin administration,[8] we also speculate that TNF-α and NO may contribute to endotoxin induced diaphragm muscle contractile deterioration. We also previously reported that IL-10[9] and IL-13[10] have protective effects against endotoxin induced diaphragm muscle deterioration due to blockage of NO production. From these previous studies, the results of the present experiment might suggest possibilities that procaterol inhibits cytokines such as TNF-α and NO production, and that procaterol directly affects muscle contractility, even if a muscle suffers cellular damage.

Recently, it has been reported that isoproterenol (which has both β_1 and β_2-agonist activity) increased diaphragmatic contractility with an increment of cyclic AMP (cAMP). However, in the same study using cecal ligation and perforation (CLP), measured at 16 hours after the operation, diaphragmatic contractility was still depressed compared with that of the sham-operated animals in the septic peritonitis model of Wister rats.[11] It was speculated that there might be a diaphragm muscle injury and/or dysfunction in the adenylate cyclase system elicited by oxygen-derived free radicals. On the other hand, we have found that in diaphragm muscles subjected to endotoxin injection, force-frequency curves decreased, but then increased with the incubation of procaterol in a dose-dependent manner at 4 hours. There are several differences in the experimental designs, namely with regard to the animals (rats *vs*. mice), the surgery (CLP) or endotoxin intraperitoneal injection, and the waiting times (16 or 4 hours). We suggest that diaphragm muscle is probably still able to respond to β_2-agonists such as procaterol in the early period after endotoxin injection, and thus force-frequency curves recover in the manner revealed by the present study. Even though the cellular damage by free-radicals and/or cytokines might begin relatively early after injection of endotoxin observed in our previous data, in the early stage of endotoxin injection β-adrenoreceptors are not fully damaged and are thus able to stimulate G-protein, resulting in activation of adenylate cyclase and the consequent formation of cyclic adenine monophosphate (cAMP).

β_2-agonists induce inhibition of airway smooth-muscle cell proliferation and inflammatory mediator release, as well as nonsmooth-muscle effects, such as stimulation of mucociliary transport, cytoprotection of the respiratory mucosa, and attenuation of neutrophil recruitment and activation.[20] It has also been reported that procaterol has inhibitory effects on antigen-induced airway microvascular leakage and bronchoconstriction,[21] that the combination of theophylline and procaterol has inhibitory effects on the function of eosinophil,[22] and that procaterol inhibits IL-1β- and TNF-α-mediated epithelial cell eosinophil chemotactic activity.[23] From these reports, it seems that procaterol has an inhibitory effect on cytokine production in eosinophils, and we speculated that there are similar effects in diaphragm muscle tissue. Previous finding coupled with our results indicate that procaterol affects not only the bronchial smooth muscles and immunological activities, but also the diaphragm skeletal muscles.

In conclusion, procaterol has an inotropic effect on the diaphragmatic muscles taken from normal animals as well as on diaphragm muscles in a septic animal model. Even if the upward shift of the force-frequency curves is relatively small, it may be said that procaterol inhalation results in increases in the force-frequency curves. As a clinical application of the experiment, it is suggested that procaterol, by increasing diaphragm contractility, may relieve dyspnea and thus improve quality of life in patients with respiratory diseases.

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