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Preferable inotropic action of procaterol, a potent bronchodilator, on impaired diaphragmatic contractility in an intraabdominal septic model

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Abstract
Intraabdominal sepsis can lead to acute respiratory failure, and concomitant diaphragmatic dysfunction may be aggravated by sepsis-induced airway hyperreactivity. We previously reported that isoproterenol, a nonselective β-adrenoceptor agonist, increased diaphragmatic contractility and accelerated recovery from fatigue during sepsis. The purpose of this study was to demonstrate the direct inotropic effect of a potent bronchodilator and β_2-selective adrenoceptor agonist, procaterol, on fatigued diaphragmatic contractility in an intraabdominal septic model. Rats were divided into two groups: a cecal ligation and perforation (CLP) group and a sham group. CLP was performed in the CLP group whereas laparotomy alone was performed in the sham group. The left hemidiaphragm was removed at 16 h after the operation. The diaphragmatic tissues were exposed to procaterol (10^{-8}–10^{-6} M), and muscle contractility was assessed. Intracellular cyclic AMP levels were also measured in the CLP model. Procaterol caused an upward shift in the force–frequency curves in the CLP group whereas it had no effect on the curves in the sham group. Procaterol significantly increased cyclic AMP levels in the CLP model. We conclude that the potent bronchodilator procaterol had a direct and positive inotropic effect on the diaphragm in an intraabdominal septic model.

Key words Sepsis · Diaphragm · Bronchodilator · Muscle contraction

Diaphragmatic dysfunction plays an important role in the development of acute respiratory failure during sepsis [1,2]. Sepsis-induced airway hyperreactivity [3,4] and impairment of alveolar surfactant increase the work of breathing, resulting in further diaphragmatic dysfunction. We have recently demonstrated that intraabdominal sepsis is associated with diaphragmatic dysfunction [5,6], which is thought to result from impairment and/or fatigue of diaphragmatic muscle [1]. We have also reported that isoproterenol, a nonselective β-adrenoceptor agonist, increased diaphragmatic contractility and accelerated recovery from fatigue during intraabdominal sepsis [7]. Mammalian skeletal muscle adrenoceptors are predominantly of the β_2 subtype, and β_2-adrenoceptors have been found in the diaphragm [8]. The purpose of this study was therefore to demonstrate the direct inotropic effect of the potent bronchodilator procaterol, a selective β_2-adrenoceptor agonist, on impaired diaphragmatic contractility in an intraabdominal septic model.

With approval of the Institutional Committee on Animal Research, 24 male Wistar rats (weight, 250–300 g; 8–10 weeks old) were used in this study. Animals were randomly divided into two groups: a sham group ($n = 8$) and a cecal ligation and perforation (CLP) group ($n = 16$). The CLP group was also divided into two groups: a contraction test group ($n = 8$) and a cyclic AMP measurement group ($n = 8$). Intraabdominal sepsis was induced using the previously described CLP technique [9]. Briefly, under isoflurane anesthesia, we performed a laparotomy and ligated the cecum while maintaining intestinal continuity. The cecum was then perforated in two locations on its antimesenteric surface and squeezed to extrude a small amount of fecal material. The bowel was returned to the abdomen and the incision was closed. Rats were observed in a recovery cage for 2 h postoperatively; they were deprived of food but had free access to water. The control group (sham group) underwent similar laparotomy without CLP. In both groups, the left hemidiaphragm was removed at 16 h after the operation and tested for contraction and cyclic AMP levels as described next.

Muscle strips (10 mm in width) without phrenic nerves were dissected from the medial aspect of the left hemidiaphragm of each rat. The isolated strips were placed in an organ bath containing oxygenated Krebs–Ringer's solution (27°C) [5,6]. The strips were then

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mounted vertically in a tissue chamber with the central tendon positioned superiorly and attached to a Grass FT-10 force transducer (Grass Instruments, Quincy, MA, USA) and positioned between two platinum plates. The strips were stimulated with supramaximal currents delivered via platinum field electrodes. The current was supplied by an amplifier driven by a Grass S48 stimulator (Grass Instruments). Muscle contractility characteristics were then assessed from the force–frequency relationship. Force–frequency relationships were determined by stimulating the diaphragmatic strips tetanically at frequencies from 10 to 100 Hz (10-Hz increments). An interval of 10 s was used between stimuli, and pulses were 0.2 ms in duration with a train duration of 400 ms. Muscle tension ($kg \cdot cm^{2-1}$) was calculated as force (kg) per unit of cross-sectional area (cm^2), and the cross-sectional area was calculated by dividing muscle mass by length in centimeters, ($1.056\,mg \cdot cm^{3-1}$) [5,6]. Procaterol (10^{-8}, 10^{-7}, and 10^{-6} M) was cumulatively administered to the organ bath, and the muscle force at each stimulus frequency was measured and recorded in both the sham and CLP groups.

To determine the mechanism by which procaterol, a β_2-selective adrenoceptor agonist, acts on the septic rat diaphragm, intracellular cyclic AMP levels were measured with or without 10^{-6} M procaterol. Diaphragmatic strips in the CLP group were incubated in Krebs–Ringer's solution containing procaterol for 20 min at 27°C. After incubation, the strips were plunged into liquid nitrogen and stored at –80°C until assays. Diaphragmatic strips were homogenized in Hank's balanced salt solution, and 200 µl of the homogenized solution was used for measurement of protein concentrations [10]. The homogenized solution was then centrifuged at 1000 *g* for 10 min at 4°C. Extraction of cyclic AMP from the supernatant was performed by the solid-phase extraction method using Amprep SAX minicolumns (code RPN 1908). Cyclic AMP levels in the diaphragmatic tissues were measured by the Biotrak cyclic AMP enzyme immunoassay system (cAMP Biotrak EIA; Biotrak, Del Mar, CA, USA).

In all experiments, an antagonistic effect of the β-adrenoceptor was tested with 20-min pretreatment of 10^{-6} M propranolol. All drugs and chemicals used in this study were purchased from Sigma (St. Louis, MO, USA). Comparison of data in the groups was made by repeated-measures analysis of variance (ANOVA) with Scheffe's procedure for post hoc comparison of data sets. A P value of less than 0.05 was considered significant. Data are presented as means ± SD.

The effects of procaterol on diaphragmatic force–frequency curves in the sham and CLP groups are shown in Fig. 1. Muscle tensions at all frequencies tested (10–100 Hz) in the CLP group were significantly lower than those in the sham group ($P < 0.01$). Peak tetanic tension at 100 Hz was significantly lower (by 71%) in the CLP group (approximately $0.5\,kg \cdot cm^{-1}$) than in the sham group (approximately $1.7\,kg \cdot cm^{2-1}$, $P < 0.01$). Procaterol did not induce any significant changes in the force–frequency curves for the sham group, but it significantly increased force generation and caused upward shifts in the force–frequency curves for the CLP group between the range of 30 and 100 Hz by more than 300% (10^{-7} and 10^{-8} M; $P < 0.01$). The positive inotropic effects of procaterol on diaphragmatic contractility in the CLP rats were completely abolished following exposure to propranolol ($n = 3$ each; data not shown).

Figure 2 shows the changes in cyclic AMP levels during exposure to 10^{-6} M procaterol with or without 10^{-6} M propranolol in the CLP group. Procaterol significantly increased cyclic AMP level by 52% ($P < 0.05$). The effect of procaterol on cyclic AMP production was completely abolished by preincubation with propranolol.

The present study demonstrated that the potent bronchodilator procaterol, a β_2-selective adrenoceptor agonist, increased impaired diaphragmatic contractility and also increased cyclic AMP level in a rat

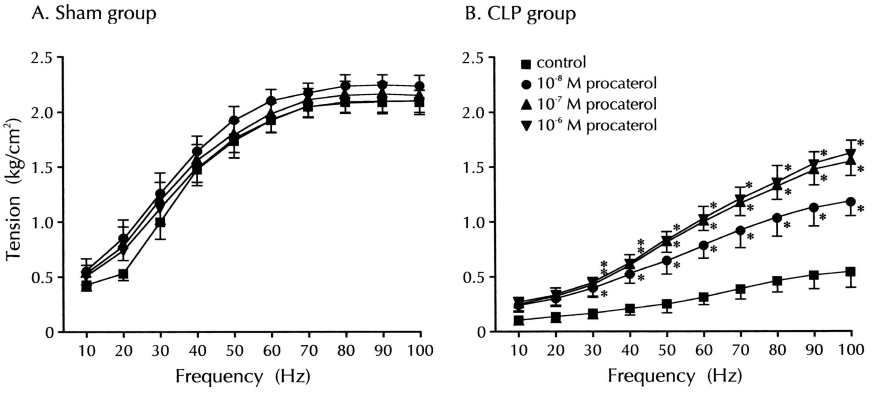

Fig. 1. Effects of procaterol on diaphragmatic force–frequency curves in the sham (**A**) and CLP (**B**) groups. Data are expressed as means ± SD, ($n = 8$ each). *CLP*, cecal ligation and perforation. Procaterol at any concentration tested (10^{-8}–10^{-6} M) caused significant upward shifts in the curves in the CLP group, whereas it had no effect on the curves in the sham group. *$P < 0.05$ vs control

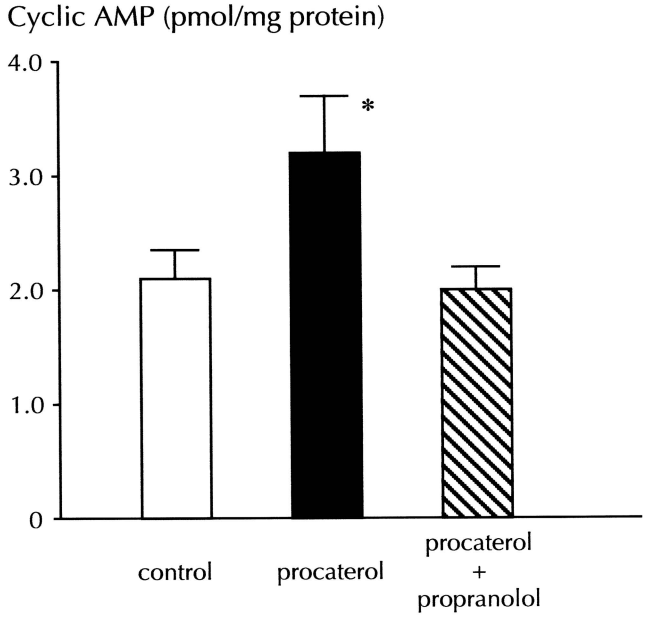

Fig. 2. Effects of procaterol with or without propranolol on cyclic AMP levels in the CLP group. Data are expressed as means ± SD, ($n = 8$ each). *CLP*, cecal ligation and perforation. Procaterol significantly increased cyclic AMP level by 52% ($P < 0.05$), and the effect of procaterol on cyclic AMP production was completely abolished by preincubation with 10^{-6} M propranolol. *$P < 0.05$ vs control

intraabdominal septic model. These changes induced by procaterol were completely blocked by preincubation with propranolol, a nonselective β-adrenoceptor antagonist. These findings suggest that activation of the adenylate cyclase system via stimulation of β_2-adrenoceptors plays an important role in the effect of the bronchodilator procaterol on impaired diaphragmatic contractility in CLP rats. These results are consistent with results of previous studies in which fatigued canine diaphragmatic muscle was used [11,12]. Procaterol binds membrane-bound β-receptors and exerts its effect via a stimulatory G protein that activates the adenylate cyclase system, resulting in the formation of cyclic AMP [13]. Cyclic AMP subsequently activates protein kinase A (PKA), which controls many biochemical events through phosphorylation of target proteins [14,15]. In a previous study, we demonstrated, using dibutyryl cyclic AMP, that an increased myoplasmic cyclic AMP level was responsible for the positive inotropic effects of sympathomimetic amines on diaphragmatic contractility in CLP rats [7].

Procaterol did not have any effect on the muscle contractility in the sham group. Recent evidence has shown the mechanisms underlying such differences between type I and type II muscle fibers [16]. β_2-agonists increase peak twitch tension in type II fibers but decrease it in type I fibers of mammalian skeletal muscle [17]. Moreover, the positive inotropic effect of sympathomimetic amines on type II muscle fibers is much greater in fatigued than in nonfatigued fibers, and their negative inotropic effect on type I muscle fibers is not potentiated after fatigue [17]. Further studies, however, are needed to clarify this point.

In conclusion, activation of β_2-adrenoceptors might be responsible for the positive inotropic effects of sympathomimetic amines on the CLP rat diaphragm. Administration of the potent bronchodilator procaterol may help to improve respiratory failure associated with diaphragmatic fatigue during sepsis. The potent bronchodilator may also inhibit sepsis-induced bronchoconstriction, reducing work of breathing and improving diaphragmatic fatigue.

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