Salbutamol enhances isotonic contractile properties of rat diaphragm muscle

H. F. M. Van Der Heijden, W. Z. Zhan, Y. S. Prakash, P. N. R. Dekhuijzen, and G. C. Sieck. Salbutamol enhances isotonic contractile properties of rat diaphragm muscle. J. Appl. Physiol. 85(2): 525–529, 1998.—The effects of the β2-adrenoceptor agonist salbutamol (Slb) on isotonic and isometric contractile properties of the rat diaphragm muscle (Diamus) were examined. A loading dose of 25 µg/kg Slb was administered intracardially before Diamus excision to ensure adequate diffusion. Studies were then performed with 0.05 µM Slb in the in vitro tissue chamber. cAMP levels were determined by radioimmunoassay. Compared with controls (Ctl), cAMP levels were elevated after Slb treatment. In Slb-treated rats, isometric twitch and maximum tetanic force were increased by ~40% and ~20%, respectively. Maximum shortening velocity increased by ~15% after Slb treatment, and maximum power output increased by ~25%. During repeated isotonic activation, the rate of fatigue was faster in the Slb-treated Diamus, but both Slb-treated and Ctl Diamus fatigued to the same maximum power output. Still, endurance time during repetitive isotonic contractions was ~10% shorter in the Slb-treated Diamus. These results are consistent with the hypothesis that β2-adrenoceptor stimulation by Slb enhances Diamus contractility and that these effects of Slb are likely mediated, at least in part, by elevated cAMP.

β2-adrenoceptor agonist; skeletal muscle; velocity of shortening; fatigue; cAMP

PHARMACOLOGICAL IMPROVEMENT of diaphragm muscle (Diamus) contractility may be of clinical importance in the treatment of chronic obstructive pulmonary disease (COPD) when compromised Diamus function is a limiting factor. Recent in vitro studies in the rat Diamus have demonstrated an increase in isometric contractile force generation with either subcutaneous or in vitro administration of salbutamol (Slb), a β2-adrenoceptor agonist (24, 25). The ability of the Diamus to shorten during activation is also critically important in the generation of ventilatory pressure; however, to date, no study has examined the effects of Slb treatment on isotonic contractile properties of the Diamus. In limb muscles, acute administration of Slb has been reported to increase isometric force in predominantly fast-twitch muscles (type II fibers) and to decrease force production in predominantly slow-twitch muscles (type I fibers) (1). The differential effect of Slb on type I and II fibers may also be relevant in the Diamus. A selective effect on type II fibers might be expected to result in an increase in shortening velocity and/or power output of the Diamus.

The purpose of the present study was to investigate the effects of Slb treatment on the isotonic contractile properties of the rat Diamus. On the basis of the observations cited above, we hypothesized that Slb increases the maximum velocity of shortening (V_{max}) and power output of the Diamus. Furthermore, given the well-known transduction mechanisms associated with the β2-adrenoceptor pathway in smooth and cardiac muscles, we hypothesized that the effects of Slb on isotonic Diamus properties are mediated via an elevation in cAMP.

METHODS

Animals, treatment, and surgical procedures. All procedures used in this study were approved by the Institutional Animal Care and Use Committee of the Mayo Clinic and were in strict accordance with the American Physiological Society animal care guidelines. Adult male Sprague-Dawley rats (mean body weight 320–4 g) were divided into two groups: 1) saline-treated controls (Ctl; n = 14); and 2) salbutamol treated (Slb; n = 12). Animals were anesthetized by intramuscular administration of ketamine (60 mg/kg) and xylazine (2 mg/kg). To minimize potential Slb diffusion limitations, animals in the Slb group were intracardially administered a loading dose of 25 µg/kg Slb; Ctl animals were administered an equal volume of 0.9% NaCl (0.5 ml/kg). Within 5 min after intracardial infusion of Slb or NaCl, the Diamus was excised and transferred to oxygenated Rees-Simpson solution (Ctl) or Rees-Simpson solution containing 0.05 µM salbutamol (Glaxo-Wellcome). The concentration of Slb was calculated based on the mean human serum concentration after a single oral dose of 4 mg (10–20 µg/l or 0.03–0.07 µM) (12, 13).

cAMP measurements. In a subset of Ctl (n = 8) and Slb-treated (n = 8) animals, midcostal Diamus segments were dissected, weighed, and then incubated, in triplicate, for 15, 30, and 60 min in the presence (Slb group) or absence (Ctl group) of 0.05 µM Slb dissolved in oxygenated Rees-Simpson solution. This Rees-Simpson solution also contained 1 mM of the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (Sigma Chemical). Immediately after this incubation period, the muscle segments were frozen in melting isopentane cooled in liquid nitrogen and stored at −70°C.

After ethanol extraction, Diamus cAMP levels were measured by using a radioimmunoassay kit (Amersham). Muscle protein content was assessed by using a colorimetric protein concentration assay (Bio-Rad), and cAMP levels were normalized to protein content.

Measurement of Diamus contractile properties. On the basis of the time course of changes in cAMP levels in response to Slb (Fig. 1), all contractile measurements were completed within 30 min after excision of the Diamus. Segments (~3 mm wide) of the Diamus from the midcostal region were mounted vertically in a glass tissue chamber containing oxygenated Rees-Simpson solution with the following composition (in mM): 135 Na+, 5 K+, 2 Ca2+, 1.1 Mg2+, 120 Cl−, 25 HCO3−, 11 glucose, 0.3 glutamic acid, 0.4 glutamate, N,N-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid buffer, and 0.012 d-tubocurarine chloride (pH 7.4). The solution was oxygenated with 95%


O2-5% CO2, and temperature was maintained at 26°C. The levels in rat diaphragm muscle (Dia mus). Values are means ± SE. After exposure to 0.5 µM Slb, cAMP levels were significantly elevated (P < 0.05). However, by 1 h after Slb exposure, cAMP levels decreased and were comparable between control (Ctl) and Slb-treated Dia mus. *Significant difference between Ctl and Slb groups, P < 0.05.

After determination of isometric P o, the Cambridge system was first set for length control (isometric mode) such that the system acted purely as a force transducer. Peak twitch force (P t) was determined from a series of five single stimuli. At 26°C, we previously demonstrated that maximum tetanic force (P o) of the Dia mus is achieved at 75-Hz stimulation (in 600-ms-duration train) (18, 22).

Isotonic contractile properties. The mean Dia mus strip weight (Ctl: 27.9 ± 1.1 mg and Slb: 26.3 ± 1.3 mg) and L o (Ctl: 18.6 ± 0.5 mm and Slb: 19.2 ± 0.3 mm) were not different between Ctl and Slb groups. Between 15 and 30 min after incubation with 0.05 µM Slb, P o of the Dia mus increased by ~40% compared with Ctl (P < 0.05; Table 1), and P t was ~20% greater (P < 0.05; Table 1). As a result, the P t/P o ratio was also increased by 10.2 ± 0.3 on October 15, 2017 http://jap.physiology.org/ Downloaded from
power output, observed at ~30% Po in both groups, was increased by ~25% after Slb treatment (P < 0.05; Table 1, Fig. 3). Total work performed by the Slb-treated Diamus increased by ~36% compared with Ctl (P < 0.05; Table 1).

Isotonic fatigue. During repetitive isotonic contractions, maximum power output of the Diamus in both groups progressively declined (P < 0.05; Fig. 4). Accordingly, the work performed by the Diamus also progressively decreased with repetitive contractions. The rate of decrement in power output, and consequently the work performed, was significantly faster in the Slb-treated Diamus compared with Ctl (P < 0.05; Fig. 4). Isotonic endurance time was also ~10% shorter in the Slb-treated compared with Ctl (P < 0.05; Fig. 4).

DISCUSSION

The present study demonstrated that acute Slb treatment increases both isometric and isotonic contractility of the rat Diamus. The improved power output and work performance of the Slb-treated Diamus were associated with a more rapid rate of fatigue. However, both Slb-treated and Ctl Diamus fatigue to the same levels of optimal work performance and maximum power output. The endurance time during repeated isotonic shortening was slightly shorter in the Slb-treated Diamus compared with Ctl. It is likely that these changes in fatigability of the Slb-treated Diamus reflected the increased work performance of the muscle. Associated with the improved contractile performance of the Diamus, there was also a transient increase in cAMP levels. Although not conclusive, these results are consistent with the perspective that the Slb-induced enhancement of Diamus contractility is mediated, at least in part, by elevated cAMP.

The increase in Diamus specific force (both Pt and Po) after acute Slb-treatment is consistent with previous studies on the Diamus (24, 25) as well as in limb muscles (1). However, in limb muscles, it was suggested that the positive inotropic effect of Slb was limited to fast-twitch muscles, comprising type II fibers, whereas force decreased in response to Slb treatment in slow-twitch muscles comprising type I fibers (1). In the present study, it was not possible to discern whether the positive inotropic effects of Slb on the Diamus were restricted to type II fibers. Yet, the effects of Slb treatment on isotonic contractile properties of the Diamus are consistent with a selective effect on type II fibers. Both V max and maximum power output were increased after Slb treatment. The force-power curve was significantly shifted upward in the Slb-treated Diamus, and, consequently, the amount of work performed was increased by 10.2 ± 0.3% compared with Ctl (P < 0.05; Table 1).

### Table 1. Effect of salbutamol on contractile properties of rat Diamus

<table>
<thead>
<tr>
<th>Group</th>
<th>Pt, N/cm²</th>
<th>Po, N/cm²</th>
<th>Pt/Po, %</th>
<th>V max, L₀/s</th>
<th>Max Power, W/m²</th>
<th>Total Work, J/m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ctl</td>
<td>8.3 ± 0.4</td>
<td>21.2 ± 0.5</td>
<td>39.1 ± 1.6</td>
<td>5.30 ± 0.21</td>
<td>1,811 ± 64</td>
<td>340.8 ± 24.6</td>
</tr>
<tr>
<td>Slb</td>
<td>11.5 ± 0.3*</td>
<td>25.6 ± 0.4*</td>
<td>45.1 ± 1.4*</td>
<td>5.98 ± 0.21*</td>
<td>2,275 ± 146*</td>
<td>463.7 ± 36.5*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Diamus, diaphragm muscle; Ctl, control animals; Slb, salbutamol-treated (25 µg/kg intracardial loading dose and 0.05 µM in vitro) animals; Pt, peak twitch force; Po, maximum tetanic force; V max, maximum velocity of shortening; L₀, optimal fiber length; Max power, maximum power output. *Significant difference from Ctl, P < 0.05.
Adrenoceptor stimulation in the dia mus involves elevated cAMP, which might mediate an increase in dia mus contractility (5–7). 8-bromoadenosine cAMP, a membrane-permeable analog of cAMP (3, 17), increases in force induced by terbutaline is mimicked by 8-bromoadenosine cAMP. In isolated skeletal muscle fibers, the G-protein activation and increased adenylate cyclase activity (3, 17) underlie the greater susceptibility of the Slb-treated dia mus to isotonic fatigue. These fiber types produce low amounts of force and are not fatigable (4). Accordingly, the transient effect of Slb on dia mus contractility is unlikely to be physiologically significant in the normal animal. However, under conditions such as COPD, increased resistance to breathing may necessitate recruitment of motor units consisting of type I and IIa fibers, which produce greater force but are more fatigable. Slb treatment in such situations would enhance contractility and thus add to the inspiratory pressure generating capacity of the dia mus. During fatigue, β2-adrenoceptor agonist treatment may also increase dia mus contractility, as other in vivo studies have shown by using terbutaline (2), fenoterol (23), and broxaterol (10).

In conclusion, the present study demonstrated that acute Slb treatment increases cAMP levels and improves both isometric and isotonic contractility of the rat dia mus. The rate of fatigue during repeated isometric contractions was faster in the Slb-treated dia mus, but both Slb-treated and Ctl dia mus fatigued to the same maximum power output. These results are consistent with the hypothesis that β2-adrenoceptor stimulation by Slb enhances dia mus contractility and that these effects of Slb are likely mediated, at least in part, by cAMP-dependent mechanisms.

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