Salbutamol enhances isotonic contractile properties of rat diaphragm muscle

H. F. M. Van der Heijden,1 W. Z. Zhan,2 Y. S. Prakash,2 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 isotonic contractile properties of the rat diaphragm muscle (DiaRmus) were examined. A loading dose of 25 µg/kg Slb was administered intracardially before DiaRmus 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, isotonic 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 DiaRmus, but both Slb-treated and Ctl DiaRmus fatigued to the same maximum power output. Still, endurance time during repetitive isotonic contractions was ~10% shorter in the Slb-treated DiaRmus. These results are consistent with the hypothesis that β2-adrenoceptor stimulation by Slb enhances DiaRmus 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 (DiaRmus) contractility may be of clinical importance in the treatment of chronic obstructive pulmonary disease (COPD) when compromised DiaRmus function is a limiting factor. Recent in vitro studies in the rat DiaRmus have demonstrated an increase in isotonic contractile force generation with either subcutaneous or in vitro administration of salbutamol (Slb), a β2-adrenoceptor agonist (24, 25). The ability of the DiaRmus 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 DiaRmus. In limb muscles, acute administration of Slb has been reported to increase isotonic 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 DiaRmus. A selective effect on type II fibers might be expected to result in an increase in shortening velocity and/or power output of the DiaRmus.

The purpose of the present study was to investigate the effects of Slb treatment on the isotonic contractile properties of the rat DiaRmus. On the basis of the observations cited above, we hypothesized that Slb increases the maximum velocity of shortening (Vmax) and power output of the DiaRmus. 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 DiaRmus 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 DiaRmus 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 DiaRmus 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, DiaRmus cAMP levels were measured 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 DiaRmus 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 DiaRmus. Segments (~3 mm wide) of the DiaRmus 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 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.

To determine isotonic fatigue, the load clamp level was set for maximum power output (determined to be ~30% P o in both groups), and the muscle was stimulated at 75 Hz in 330 ms duration trains repeated every second. Stimulation continued until no muscle shortening could be observed, and this period was defined as the isotonic endurance time.

After contractile measurements, the length of the muscle segment was measured by using digital calipers. The muscle was freed from the ribs and tendon, and the segment was weighed. Muscle cross-sectional area (CSA) was estimated based on the following formula: CSA = muscle weight (g)/L o (cm)·1.056 (g/cm³). This estimated CSA was then used to determine specific force (i.e., force/area) of the muscle segment.

Statistics. Differences in most contractile parameters between the two treatment groups were analyzed by using a Student’s t-test. Repeated measurements during the fatigue test were analyzed by using a two-way ANOVA (repeated measurements design). For the cAMP data, treatment effects were assessed by using a two-way ANOVA with treatment group and incubation time as variables. Statistical significance was accepted at a P < 0.05 level. All values are reported as means ± SE.

RESULTS

cAMP measurements. In the presence of 0.05 µM Slb, cAMP levels in the Dia mus increased significantly compared with Ctl (P < 0.05; Fig. 1). The Slb-induced increase in Dia mus cAMP was time dependent, being elevated by ~65% compared with Ctl after 15 min incubation but elevated only by ~45% after 30 min. By 60 min after incubation, cAMP levels were not significantly different between Slb and Ctl animals.

Isometric 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 o was ~20% greater (P < 0.05; Table 1). As a result, the P o/P o ratio was also increased by ~15% in the Slb-treated Dia mus (P < 0.05; Table 1).

Isotonic contractile properties. In the Slb-treated Dia mus, the force-velocity relationship was shifted upward and to the right compared with Ctl (Fig. 2). The extrapolated V max of the Slb-treated Dia mus was ~15% faster than in Ctl (Fig. 2, Table 1; P < 0.05). Therefore, the proportionate effects of Slb on V max and P o were comparable.

The force-power curve of the Slb-treated Dia mus was shifted upward compared with Ctl (Fig. 3). Maximum
power output, observed at ~30% $P_o$ in both groups, was increased by ~25% after Slb treatment ($P < 0.05$; Table 1, Fig. 3). Total work performed by the Slb-treated Dia$_{mus}$ increased by ~36% compared with Ctl ($P < 0.05$; Table 1).

Isotonic fatigue. During repetitive isotonic contractions, maximum power output of the Dia$_{mus}$ in both groups progressively declined ($P < 0.05$; Fig. 4). Accordingly, the work performed by the Dia$_{mus}$ 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 Dia$_{mus}$ 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 Dia$_{mus}$. The improved power output and work performance of the Slb-treated Dia$_{mus}$ were associated with a more rapid rate of fatigue. However, both Slb-treated and Ctl Dia$_{mus}$ fatigued 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 Dia$_{mus}$ compared with Ctl. It is likely that these changes in fatigability of the Slb-treated Dia$_{mus}$ reflected the increased work performance of the muscle. Associated with the improved contractile performance of the Dia$_{mus}$, 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 Dia$_{mus}$ contractility is mediated, at least in part, by elevated cAMP.

The increase in Dia$_{mus}$ specific force (both $P_t$ and $P_o$) after acute Slb-treatment is consistent with previous studies on the Dia$_{mus}$ (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 Dia$_{mus}$ were restricted to type II fibers. Yet, the effects of Slb treatment on isotonic contractile properties of the Dia$_{mus}$ 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 Dia$_{mus}$, and, consequently, the amount of work per-

<table>
<thead>
<tr>
<th>Group</th>
<th>$P_t$, N/cm$^2$</th>
<th>$P_o$, N/cm$^2$</th>
<th>$P_t/P_o$, %</th>
<th>$V_{max}$, L$_o$/s</th>
<th>Max Power, W/m$^2$</th>
<th>Total Work, J/m$^2$</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. Dia$_{mus}$, diaphragm muscle; Ctl, control animals; Slb, salbutamol-treated (25 µg/kg intracardial loading dose and 0.05 µM in vitro) animals; $P_t$, peak twitch force; $P_o$, maximum tetanic force; $V_{max}$, maximum velocity of shortening; $L_o$, optimal fiber length; Max power, maximum power output. *Significant difference from Ctl, $P < 0.05$.  

Fig. 2. Effect of Slb on isotonic shortening velocity of rat Dia$_{mus}$. Values are means ± SE. Curves represent shortening velocities at different levels of absolute force at which muscle was clamped. In Slb-treated Dia$_{mus}$, force-velocity relationship was shifted upward and to the right compared with Ctl. $L_o$, optimal fiber length. Maximum shortening velocity, i.e., shortening velocity at zero load, was also significantly increased in Slb group ($P < 0.05$).

Fig. 3. Effect of Slb on power production in rat Dia$_{mus}$. Values are means ± SE. $P_o$, maximum tetanic force. Slb treatment significantly increased power production at different isotonic force levels as well as maximum power produced ($P < 0.05$).
formed by the Slb-treated Dia
sub increased. The increase in power output and work would be accompanied by an increase in energy consumption, which could underlie the greater susceptibility of the Slb-treated Dia
sub to isometric fatigue.

The increase in Dia
sub cAMP levels after Slb treatment is in agreement with previous results in both fast- and slow-twitch limb skeletal muscles (1). These results are also consistent with the elevation of cAMP levels in limb skeletal muscles induced by terbutaline, another β2-adrenoceptor agonist (5, 7, 8). It is likely that the increase in cAMP levels induced by β2-adrenoceptor stimulation in the Dia
sub involves G-protein activation and increased adenylate cyclase activity (3, 17). In isolated skeletal muscle fibers, the increase in force induced by terbutaline is mimicked by 8-bromoadenosine cAMP, a membrane-permeable analog of cAMP (5–7).

There are several potential mechanisms by which elevated cAMP might mediate an increase in Dia
sub specific force and a faster cross-bridge cycling rate. For example, it has been suggested that the β2-adrenoceptor agonist-induced elevation in cAMP in skeletal muscle fibers leads to an improvement of excitation-contraction (EC) coupling and an increase in Ca2+ release from the sarcoplasmic reticulum (5, 7, 8). This suggestion is supported by the fact that 1 mM caffeine, which stimulates sarcoplasmic reticulum Ca2+ release, prevents the inotropic effect of terbutaline on force generation (5, 7). The effect of cAMP on EC coupling could be mediated via the activation of cAMP-dependent protein kinases and the subsequent phosphorylation of either voltage-dependent dihydropyridine receptors in the T tubules or ryanodine-receptor Ca2+-release channels in the sarcoplasmic reticulum (14, 19, 20, 26). Indeed, both β-adrenergic receptors and adenylate cyclase activity have been detected in T tubules (9). Intracellular Ca2+ levels were not measured in the present study; therefore, it remains unclear to what extent a Slb-induced enhancement of EC coupling might have contributed to the observed improvements in Dia
sub contractility. It is also possible that other cAMP-dependent signaling cascades in skeletal muscle fibers could also have contributed to the Slb-induced improvements in Dia
sub contractility.

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

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