Salbutamol enhances isotonic contractile properties of rat diaphragm muscle

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Van der Heijden, H. F. M., 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 (Dia mus) were examined. A loading dose of 25 µg/kg Slb was administered intracardially before Dia mus 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 isometric activation, the rate of fatigue was faster in the Slb-treated Dia mus, but both Slb-treated and Ctl Dia mus fatigued to the same maximum power output. Still, endurance time during repetitive isometric contractions was ~10% shorter in the Slb-treated Dia mus. These results are consistent with the hypothesis that β-adrenoceptor stimulation by Slb enhances Dia mus 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 (DIA mus) CONTRACTILITY MAY BE OF CLINICAL IMPORTANCE IN THE TREATMENT OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD) WHEN COMPROMISED DIA mus FUNCTION IS A LIMITING FACTOR. RECENT IN VITRO STUDIES IN THE RAT DIA mus 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 DIA mus 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 DIA mus. IN LIMB MUSCLES, ACUTE ADMINISTRATION OF Slb HAS BEEN REPORTED TO INCREASE ISOMETRIC FORCE IN PROMINENTLY FAST-TWITCH MUSCLES (TYPE II FIBERS) AND TO DECREASE FORCE PRODUCTION IN PROMINENTLY SLOW-TWITCH MUSCLES (TYPE I FIBERS) (1). THE DIFFERENTIAL EFFECT OF Slb ON TYPE I AND II FIBERS MAY ALSO BE RELEVANT IN THE DIA mus. A SELECTIVE EFFECT ON TYPE II FIBERS MIGHT BE EXPECTED TO RESULT IN AN INCREASE IN SHORTENING VELOCITY AND/OR POWER OUTPUT OF THE DIA mus.

THE PURPOSE OF THE PRESENT STUDY WAS TO INVESTIGATE THE EFFECTS OF Slb TREATMENT ON THE ISOTONIC CONTRACTILE PROPERTIES OF THE RAT DIA mus. 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 DIA mus. 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 DIA mus 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 DIA mus 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/kg) OR 0.03–0.07 µM (12, 13). cAMP MEASUREMENTS. IN A SUBSET OF Ctl (n = 8) AND Slb-treated (n = 8) animals, midcostal Dia mus 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 EXTRACT, DIA mus 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 DIA mus 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 DIA mus. SECTIONS (~3 mm wide) OF THE DIA mus 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 glutamine, 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 remained comparable between control (Ctl) and Slb-treated Dia mus. *Significant difference between Ctl and Slb groups, P < 0.05.

![Graph](http://jap.physiology.org/)  
**Fig. 1.** Effect of acute administration of salbutamol (Slb) on cAMP 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.

Isometric contractile properties. The mean Dia mus strip weight (Ctl: 27.9 ± 1.1 mg and Slb: 26.3 ± 1.3 mg) and L₀ (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, P₀ of the Dia mus increased by ~40% compared with Ctl (P < 0.05; Table 1), and P₁ was ~20% greater (P < 0.05; Table 1). As a result, the P₁/P₀ 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 V₉₀ 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 V₉₀ and P₁ 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 Diaatus increased by ~36% compared with Ctl ($P < 0.05$; Table 1).

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

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

<table>
<thead>
<tr>
<th>Group</th>
<th>$P_t$, N/cm²</th>
<th>$P_o$, N/cm²</th>
<th>$P_t/P_o$, %</th>
<th>$V_{max}$, $L_o$/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. Diaatus, 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 Diaatus. Values are means ± SE. Curves represent shortening velocities at different levels of absolute force at which muscle was clamped. In Slb-treated Diaatus, 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 Diaatus. 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 ($P < 0.05$).
adrenoceptor stimulation in the Diaphragn involves elevated cAMP might mediate an increase in Diaphragn contractility (5–7).

8-bromoadenosine cAMP, a membrane-permeable analog of cAMP (5–7), increases in force induced by terbutaline is mimicked by an increase in energy consumption, which could enhance contractility and addition to the inspiratory pressure generating capacity of the Diaphragn. During fatigue, β2-adrenoceptor agonist treatment may also increase Diaphragn 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 enhances Diaphragn contractility and improves both isometric and isotonic contractility of the rat Diaphragn. The rate of fatigue during repeated isometric contractions was faster in the Slb-treated Diaphragn, but both Slb-treated and Ctl Diaphragn fatigued to the same power level. Isotonic endurance time was shorter in the Slb group.

Fig. 4. Effect of Slb on work performed (A) and power output (B) during repetitive isotonic contractions of the rat Diaphragn. Values are means ± SE. Load clamp level was set for maximum power output (see Fig. 3), and muscle strips were directly stimulated at 75 Hz for 330 ms repeated every second. Time at which the muscle no longer shortened was defined as the isotonic endurance time. Power output was calculated as the product of force and shortening velocity, and work performed was calculated as the product of force and the time integral of the length curve. Rate of decrement in power output was significantly faster in Slb-treated Diaphragn compared with Ctl (P < 0.05), but both groups fatigued to same power level. Isotonic endurance time was shorter in the Slb group.

formed by the Slb-treated Diaphragn 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 Diaphragn to isotonic fatigue.

The increase in Diaphragn 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 Diaphragn involves G-protein activation and increased adenylyl 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 Diaphragn contractility. 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 sarcoplasmatic reticulum (5, 7, 8). This suggestion is supported by the fact that 1 mM caffeine, which stimulates sarcoplasmatic 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 sarcoplasmatic reticulum (14, 19, 20, 26). Indeed, both β-adrenergic receptors and adenylyl 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 Diaphragn 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 Diaphragn contractility.
REFERENCES


