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 β_2-adrenoceptor agonist salbutamol (Slb) on isometric and isotonic 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 isotonic 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 isotonic 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.

β_2-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 β_2-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 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 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 β_2-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/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 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 extraction, 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}. Segments (~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 Ca^{2+}, 1 Mg^{2+}, 120 Cl^-, 25 HCO_3^-, 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%

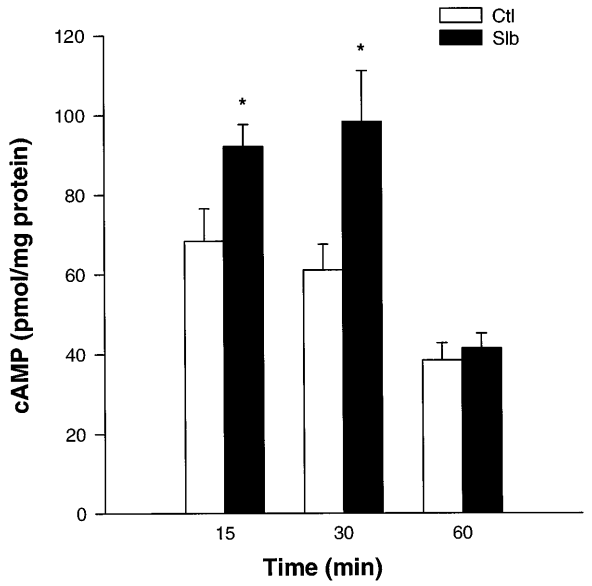

Fig. 1. Effect of acute administration of salbutamol (Slb) on cAMP levels in rat diaphragm muscle (Dia_{mus}). Values are means ± SE. After exposure to 0.5 μM Slb, cAMP levels were significantly elevated ($P < 0.05$). However, by 1 h after Slb exposure, cAMP levels decreased and were comparable between control (Ctl) and Slb-treated Dia_{mus}. * Significant difference between Ctl and Slb groups, $P < 0.05$.

O_2-5% CO_2, and temperature was maintained at 26°C. The origin of the muscle bundle at the costal margin was attached to a metal clamp mounted in series with a micromanipulator at the base of the tissue chamber. The central tendon was glued to a plastic holder that was firmly attached to the lever arm of a dual-mode length-force servo-control system (model 300B, Cambridge Technologies).

The muscle was stimulated directly by using platinum plate electrodes placed on either side of the muscle. Rectangular current pulses (0.5-ms duration) were generated by a Grass S88 stimulator and were amplified by using a current amplifier (Sect. of Engineering, Mayo Foundation). The stimulus intensity yielding the maximum twitch force response was determined, and the stimulus intensity was set at ~125% of this value for the remainder of the experiment (~220 mA). Muscle preload force was adjusted by using the micromanipulator until optimal fiber length for maximal twitch force (L_o) was achieved.

The Cambridge system was controlled by using commercial software (LabView, National Instruments) configured to meet the present experimental requirements and implemented on an IBM 486 personal computer. Length and force were independently controlled through the software, allowing the Cambridge system to operate either in isometric or isotonic modes, respectively. Length and force data outputs were digitized by using a data-acquisition board (AT-MIO-16-L9; National Instruments) at a sampling frequency of 500 Hz.

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).

After determination of isometric P_t and P_o, the Cambridge system was set for isotonic measurements. The muscle was stimulated at 75 Hz (600-ms-duration train) while force was clamped at different levels ranging from 3 to 100% of P_o. At least 1 min intervened between each force-clamp level. V_{max} at each clamp level was calculated as the change in muscle length during a 30-ms period and was expressed as muscle lengths per second (L_o/s). To eliminate the effect of muscle compliance, the time window for shortening velocity measurements was set to begin 10 ms after the first detectable change in length. V_{max} was calculated by extrapolating the force-velocity curve to zero load by using the modified Hill equation (15). Power output was calculated as the product of force and velocity, and the load clamp level yielding maximum power was determined from the force-power curve. The optimal work performed by the Dia_{mus} was calculated as the area under the curve relating force and power.

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/cm^3). 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_t 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_t/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

Table 1. *Effect of salbutamol on contractile properties of rat Da_{mus}*

Group	P_t, N/cm^2	P_o, N/cm^2	P_t/P_o, %	V_{max}, L_o/s	Max Power, W/m^2	Total Work, J/m^2
Ctl	8.3 ± 0.4	21.2 ± 0.5	39.1 ± 1.6	5.30 ± 0.21	1,811 ± 64	340.8 ± 24.6
Slb	11.5 ± 0.3*	25.6 ± 0.4*	45.1 ± 1.4*	5.98 ± 0.21*	2,275 ± 146*	463.7 ± 36.5*

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, $P < 0.05$.

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} 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 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-

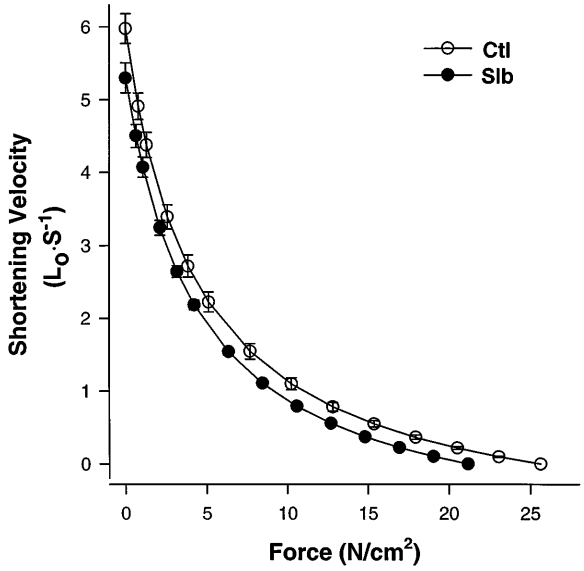

Fig. 2. Effect of Slb on isotonic shortening velocity of rat Dia_{mus}. Values are means ± SE. Curves represent shortening velocities at different levels of absolute force at which muscle was clamped. In Slb-treated Dia_{mus}, force-velocity relationship was shifted upward and to the right compared with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

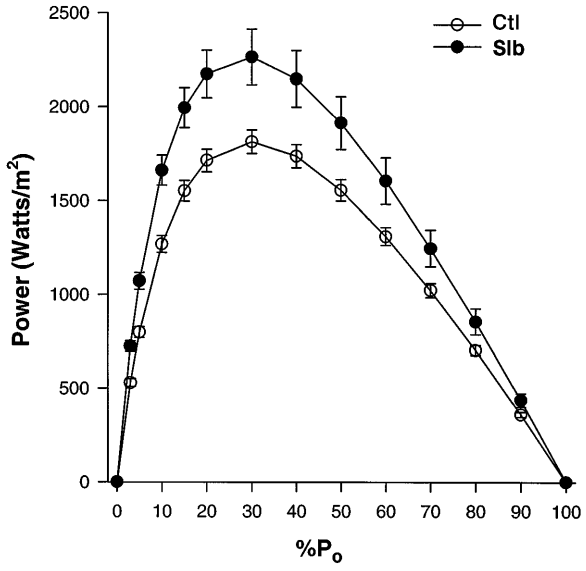

Fig. 3. Effect of Slb on power production in rat Dia_{mus}. Values are means ± SE. P_o, maximum tetanic force. Slb treatment significantly increased power production at different isotonic force levels as well as maximum power produced ($P < 0.05$).

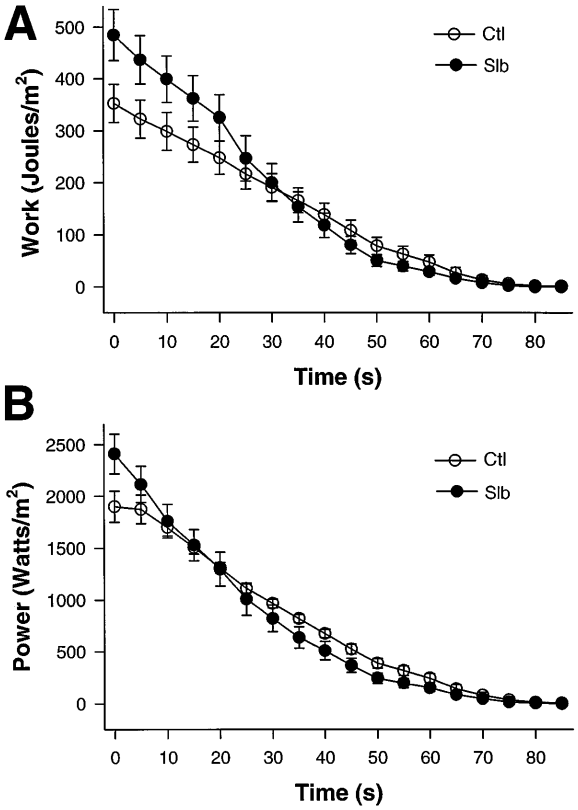

Fig. 4. Effect of Slb on work performed (*A*) and power output (*B*) during repetitive isotonic contractions of the rat Dia_{mus}. Values are means ± SE. Load clamp level was set for maximum power output (see Fig. 3), and muscle strips were directly stimulated at 75 Hz for 330 ms repeated every second. 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 Dia_{mus} 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 Dia_{mus} 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_{mus} to isotonic fatigue.

The increase in Dia_{mus} 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 β_2-adrenoceptor agonist (5, 7, 8). It is likely that the increase in cAMP levels induced by β_2-adrenoceptor stimulation in the Dia_{mus} 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_{mus} specific force and a faster cross-bridge cycling rate. For example, it has been suggested that the β_2-adrenoceptor agonist-induced elevation in cAMP in skeletal muscle fibers leads to an improvement of excitation-contraction (EC) coupling and an increase in Ca^{2+} release from the sarcoplasmic reticulum (5, 7, 8). This suggestion is supported by the fact that 1 mM caffeine, which stimulates sarcoplasmic reticulum Ca^{2+} 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 Ca^{2+}-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 Ca^{2+} 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_{mus} 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_{mus} contractility. For example, phosphorylation of the regulatory myosin light chain and/or troponin I can affect Ca^{2+} sensitivity and cross-bridge cycling kinetics.

The present study utilized a low, clinically relevant concentration of Slb. The results may be interpreted as a transient effect of Slb on Dia_{mus} contractility, especially on type IIx and IIb fibers. During normal ventilatory maneuvers of the Dia_{mus}, motor units consisting of type I and IIa fibers are predominantly recruited (21). 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 IIx and IIb 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, β_2-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 isotonic 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 β-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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