Effects of salbutamol on rat diaphragm contractility

H. F. M. VAN DER HEIJDEN, R. H. H. VAN BALKOM, H. T. M. FOLGERING, C. L. A. VAN HERWAARDEN, AND P. N. R. DEKHUIJZEN

Department of Pulmonary Diseases, University Hospital Nijmegen, 6500 HB Nijmegen, The Netherlands

Effects of salbutamol on rat diaphragm contractility. J. Appl. Physiol. 81(3): 1103–1110, 1996.—The aim of this study was to investigate 1) the effects and time course of single doses of salbutamol on isometric contractile properties of isolated rat diaphragm strips and 2) whether these effects were caused by a direct effect on the muscle. Two experiments were performed. In one, salbutamol was administered subcutaneously in doses of 12.5, 25, 50, or 100 μg/kg (25 and 50 μg/kg sc resulted in serum concentrations of 9 and 15 μg/l, respectively, 0.5 h after injection) and in vitro contractile properties were determined 0.5, 1, 2, or 4 h after administration; in the other, salbutamol was added to the tissue bath in a concentration of ≤2, ≤10, ≤20, and ≤80 μg/l. Twitch force, maximal tetanic force, and twitch force-to-tetanic force ratio all increased in a dose-dependent way in both experiments. The increases in force generation were slightly higher after subcutaneous administration. Force-frequency curves were shifted upward in both experiments. No significant effects of time of salbutamol administration were found, but the increase in force generation was most pronounced within 2 h after subcutaneous administration. In conclusion, in vitro force generation can be improved by low concentrations of salbutamol. The slightly higher increases in force generation after subcutaneous administration suggest that in vivo salbutamol may have additional positive inotropic actions on diaphragm contractility besides a direct β2-adrenergic effect on the muscle itself.

Several studies have indicated that the β2-adrenoceptor agonist salbutamol may improve skeletal and respiratory muscle function. In vitro experiments showed that salbutamol hyperpolarized membrane potential in both peripheral skeletal muscles and diaphragm (6, 36). Salbutamol increased subtetanic force generation in fast-contracting skeletal muscles and decreased force in slow-contracting muscles (1). This is consistent with the in vivo effects of catecholamines on skeletal muscles (4). Experiments in dogs showed no significant increase in transdiaphragmatic pressure (Pdi) or twitch force (Pt) in fatigued diaphragm after short-term intravenous salbutamol administration (11, 29). In contrast, salbutamol did increase diaphragm Pt during compensated metabolic acidosis (12) and increased shortening of the canine diaphragm (16). Contradicting effects of salbutamol on respiratory muscle function have also been described in humans. Two studies showed no improvement of Pdi (14) or maximal inspiratory mouth pressure (Ptmax) (35) in normal subjects. In contrast, long-term salbutamol administration in healthy volunteers increased Ptmax by 7 and 15% after 14 and 21 days of oral treatment, respectively (23).

Although several of these studies indicate that salbutamol may increase diaphragm force generation under specific circumstances, the exact effects of salbutamol on diaphragm contractile properties and its mechanism of action have not been fully clarified yet. To our knowledge, the in vitro effects of salbutamol on diaphragm contractile properties have not been studied. The aims of the present study were therefore to 1) investigate the dose-response effects and time course of effects of salbutamol on in vitro isometric contractile properties of rat diaphragm and 2) investigate whether these effects can be explained by a direct effect on the diaphragm. We aimed to study the effects of salbutamol in low concentrations, which were likely to be of clinical relevance.

METHODS

Study Design

Protocol A. Saline or salbutamol (Glaxo-Wellcome, The Netherlands) in a dose of 12.5, 25, 50, or 100 μg/kg was administered subcutaneously. Salbutamol was dissolved in 0.9% NaCl, and all rats received a volume of 0.5 ml/kg body wt. At 0.5, 1, 2, or 4 h after injection, the animals were killed and contractile properties of isolated diaphragm strips were determined.

Protocol B. Saline or salbutamol in a concentration of 0.01 μM (~3 pg/l), 0.03 μM (~10 pg/l), 0.06 μM (~20 pg/l), or 0.3 μM (~80 pg/l) was dissolved in Krebs-Ringer solution before each experiment. Subsequently, diaphragm strips were mounted in the tissue baths containing this Krebs solution to determine direct effects of salbutamol on contractile properties.

Animals and Treatment

A total number of 125 adult male outbred Wistar rats, aged 17–20 wk and weight 556 ± 6 (SE) g, were used in these experiments. In both protocols, five animals were studied in each group. The rats were housed under standard conditions and were fed ad libitum. The study was approved by the Animal Experiments Committee of the University of Nijmegen.

The salbutamol concentration of 10 μg/l was based on the mean human serum concentration reached after a single oral dose of 4 mg, which was ~10–20 μg/l (10). The actual serum salbutamol concentrations reached in protocols A and B were measured in all subcutaneously treated rats and in five samples obtained from each in vitro concentration. Serum samples were obtained from all rats in the study. Blood samples were collected from the thoracic cavity immediately after excision of the diaphragm and dissection of the aorta and vena cava. The samples were subsequently centrifuged and stored at ~80°C until analysis. The analysis was performed by using high-performance liquid chromatography with fluorescence detection (Scotlab Analytical, Coatbridge, Scotland, UK) (3).
A pilot study showed no effects of any of the salbutamol concentrations used on the pH of the oxygenated Krebs solution. A maximal increase of $P_t$ was reached within 10 min after addition of salbutamol and lasted for ≥15 min. Therefore, a period of 15 min for thermoequilibration and diffusion of salbutamol (protocol B) was applied in both protocols.

The experiments with different doses and time groups were performed at random. The investigator (H. F. M. Van Der Heijden) was blinded with regard to dose and time of administration.

**Contractile Properties**

The rats were anesthetized with pentobarbital sodium (70 mg/kg ip). A tracheotomy was performed, and a polyethylene canula was inserted. The animals were mechanically ventilated with a gas mixture of 95% O₂-5% CO₂ (flow of 0.5 ml/g body wt⁻¹ min⁻¹; respiration frequency of 70 breaths/min).

The diaphragm was quickly cut from the ribs after combined laparotomy and thoracotomy and was immediately submersed in cooled oxygenated Krebs solution at a pH of 7.4. This Krebs solution consisted of (in mM) 137 NaCl, 4 KCl, 2.7 CaCl₂, 2 MgCl₂, 1 KH₂PO₄, 24 NaHCO₃, and 7 glucose and 25 mM D-tubocurarine (Sigma Chemical, The Netherlands). From the middle lateral costal region of each hemidiaphragm, a rectangular bundle was dissected parallel to the long axis of the muscle fibers. Silk sutures were tied firmly to both ends of the bundle. The bundles were suspended in two tissue baths containing Krebs solution, maintained at 37°C, and perfused with a 95% O₂-5% CO₂ mixture. One end was connected to a isometric force transducer (model 31/1437-10, Sensotec, Columbus, OH) mounted on a micrometer. Two platinum stimulating electrodes were placed parallel to the bundles. The bundles were placed at their optimal length ($L₀$), defined as the length at which peak $P_t$ was obtained. Stimuli were applied with a pulse duration of 0.2 ms and a train duration of 250 ms and were delivered by a stimulator (ID-electronics, University of Nijmegen, The Netherlands) activated by a personal computer. To ensure supramaximal stimulation, the bundles were stimulated at ~20% above the voltage at which maximal forces were obtained (~6 V over stimulating electrodes). Data acquisition and storage of the amplified signal were performed by using a Dash-16 interface on a personal computer (Twist-trigger software, ID-electronics, University of Nijmegen).

In protocol A, the diaphragm strip dimensions at $L₀$ were 18.02 ± 0.18 × 1.59 ± 0.03 × 0.42 ± 0.01 (SE) mm (length × width × thickness) and the weight was 24.2 ± 0.6 mg. In protocol B, these dimensions were 16.67 ± 0.38 × 1.90 ± 0.07 × 0.59 ± 0.01 mm and their mean weight was 28.2 ± 1.0 mg. After a thermoequilibration period of 15 min, the following measurements were performed.

**Twitch characteristics.** Two twitches were recorded at $L₀$ to determine maximal $P₀$, contraction time (CT) (time to peak $P_t$), and half-relaxation time (RT₁/₂). Average values were used for further analysis (7).

**Maximal tetanic force ($P₀$).** The bundles were stimulated twice at 160 Hz to obtain a maximal plateau in force generation. The maximal force was defined as the $P₀$.

**Force-frequency characteristics.** The bundles were stimulated at 2-min intervals at frequencies of 25, 50, 80, 120, and 160 Hz.

After these measurements, length, thickness, and width of each muscle bundle were measured at $L₀$. The bundles were weighed, and the cross-sectional area was calculated by dividing diaphragm strip weight (in grams) by strip length (in centimeters) times specific density (1.056). $P_t$ and $P₀$ were expressed per cross-sectional area (in kilograms per square centimeter), and $P_t/P₀$ was calculated for each muscle bundle.

**Statistics**

The data obtained from the two diaphragm strips of each rat were averaged and used for further analysis. In protocol A, two-way analysis of variance (ANOVA) was performed using dose and time of administration as separate factors to examine salbutamol treatment and time of dosing effects and possible interaction between these factors. Interaction between these factors was not significant for any parameter. Therefore, no linear contrast analysis was performed and main effects of each factor were considered not to be confounded by interaction. The significance of each main effect is reported.

In protocol B, one-way ANOVA was performed to determine differences between separate concentrations using Duncan's multiple-range test. To evaluate force-frequency characteristics, a two-way ANOVA was performed using dose and stimulation frequency as separate factors to assess interaction between these factors. Pearson's correlation coefficients between salbutamol concentration and $P₀$, $P₀$, and force generation at 25-Hz stimulation frequency were computed. Finally, the salbutamol effects in protocols A and B were compared using t-tests for separate ranges of salbutamol concentrations.

The limit of statistical significance was set at $P < 0.05$. All analyses were performed with the SPSS/PC+ package (v5.01) (28). All values are means ± SE.

**RESULTS**

**Salbutamol Concentrations**

The mean serum salbutamol concentrations reached in protocol A are presented in Fig. 1. At 0.5 h after injection, salbutamol concentrations could be measured in all treated rats. Doubling the administered dose approximately doubled the serum concentration. At later time points, the concentrations were either below the lower limit of detection (2 μg/l) or blank. Only at a dose of 100 μg/kg could salbutamol be detected 2 h after injection. At 4 h after injection, no salbutamol could be measured.

---

**Fig. 1. Salbutamol concentration curves (protocol A). Values are means ± SE. Time is in hours. □, Dose of 12.5 μg/kg sc; ●, 25 μg/kg sc; ○, 50 μg/kg sc; ■, 100 μg/kg sc.**
Table 1. Protocol A (subcutaneous administration of salbutamol): $P_t$

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0.5 h</th>
<th>1 h</th>
<th>2 h</th>
<th>4 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>0.441 ± 0.037</td>
<td>0.420 ± 0.042</td>
<td>0.477 ± 0.041</td>
<td>0.431 ± 0.072</td>
</tr>
<tr>
<td>Salbutamol, µg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.5</td>
<td>0.553 ± 0.050</td>
<td>0.597 ± 0.066</td>
<td>0.515 ± 0.027</td>
<td>0.520 ± 0.025</td>
</tr>
<tr>
<td>25</td>
<td>0.588 ± 0.038</td>
<td>0.635 ± 0.044</td>
<td>0.541 ± 0.040</td>
<td>0.526 ± 0.041</td>
</tr>
<tr>
<td>50</td>
<td>0.609 ± 0.057</td>
<td>0.681 ± 0.082</td>
<td>0.631 ± 0.083</td>
<td>0.658 ± 0.038</td>
</tr>
<tr>
<td>100</td>
<td>0.736 ± 0.025</td>
<td>0.694 ± 0.074</td>
<td>0.686 ± 0.035</td>
<td>0.594 ± 0.067</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed as twitch force ($P_t$) in kilograms per square centimeter. Significant difference between treatments is found at $P < 0.001$ using 2-way analysis of variance (ANOVA). There are no significant differences between times of salbutamol administration.

In protocol B, the in vitro salbutamol concentrations were below the lower limit of detection in four out of five samples at 0.01 µM. In the other groups, salbutamol concentrations of 10.1 ± 0.8, 22.2 ± 1.6, and 78.3 ± 4.0 µg/l were measured.

Contractile Properties

Protocol A. Twitch Characteristics and $P_0$. Salbutamol increased $P_t$ in a dose-dependent fashion ($P < 0.001$). No significant effects of time of administration were found, and no interaction between these factors was found. The effects of salbutamol treatment were most pronounced at 0.5 and 1 h after administration (Table 1). For CT, significant effects were found for dose ($P < 0.01$) and time of administration ($P < 0.001$). The values ranged from 27.6 ± 0.5 to 31.7 ± 0.8 ms. CT was increased at 2 h after administration in all salbutamol groups except the 50 µg/kg group. RT$_{1/2}$ ranged from 22.0 ± 0.9 to 26.7 ± 1.4 ms and was not changed by salbutamol.

A significant effect of dose on $P_0$ was found ($P < 0.01$). $P_t$ increased in a dose-dependent way in all groups (Table 2). No effect of time of administration or interaction between these factors was observed. For $P_t/P_0$, significant effects of dose and time of administration (both $P < 0.001$) were found without interaction. $P_t/P_0$ values ranged from 0.233 ± 0.009 to 0.310 ± 0.006. Greatest increases in $P_t/P_0$ were observed 0.5 and 1 h after administration of salbutamol. At 4 h after administration, no effects of salbutamol treatment were found, which is in agreement with the absence of salbutamol in serum (Fig. 1).

FORCE-FREQUENCY CHARACTERISTICS. Significant dose effects were found for force generation at stimulation frequencies of 25 ($P < 0.001$), 50 ($P < 0.01$), and 80 Hz ($P < 0.05$). No significant effects of time of administration or interaction were found. Further two-way ANOVA of force-frequency characteristics, with salbutamol dose and stimulation frequency as factors, showed significant effects of salbutamol treatment ($P < 0.01$ at 0.5, 2, and 4 h and $P < 0.05$ at 1 h after administration). No interaction was present between stimulation frequency and dose, suggesting that force generation was influenced by salbutamol in a similar way at all stimulation frequencies studied. The force-frequency curves obtained 0.5 h after subcutaneous administration are shown in Fig. 2.

Protocol B. Twitch Characteristics and $P_0$. $P_t$ increased in the presence of salbutamol in a concentration-dependent fashion (Table 3). This effect was significant at a concentration of ~10 µg/l and higher. CT was increased at all concentrations; RT$_{1/2}$ did not change at any concentration studied. The values ranged from 23.8 ± 1.1 to 25.8 ± 0.7 ms. $P_0$ was increased by salbutamol in concentrations of ~10 µg/l or higher; $P_t/P_0$ was not changed.

FORCE-FREQUENCY CHARACTERISTICS. Force generation was increased at all stimulation frequencies (Fig. 3). This effect was concentration dependent. In a salbutamol concentration of ~80 µg/l, significant increases in force production were observed at all stimulation frequencies used. At 25-Hz stimulation, force generation was also significantly increased at ~10 µg/l salbutamol. Two-way ANOVA of force-frequency characteristics with

Table 2. Protocol A (subcutaneous administration of salbutamol): $P_0$

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0.5 h</th>
<th>1 h</th>
<th>2 h</th>
<th>4 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>1.855 ± 0.130</td>
<td>1.815 ± 0.213</td>
<td>1.898 ± 0.147</td>
<td>1.810 ± 0.187</td>
</tr>
<tr>
<td>Salbutamol, µg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.5</td>
<td>2.056 ± 0.165</td>
<td>2.166 ± 0.231</td>
<td>2.051 ± 0.092</td>
<td>2.198 ± 0.171</td>
</tr>
<tr>
<td>25</td>
<td>2.304 ± 0.197</td>
<td>2.314 ± 0.093</td>
<td>2.039 ± 0.116</td>
<td>2.158 ± 0.173</td>
</tr>
<tr>
<td>50</td>
<td>2.114 ± 0.236</td>
<td>2.292 ± 0.264</td>
<td>2.448 ± 0.252</td>
<td>2.398 ± 0.202</td>
</tr>
<tr>
<td>100</td>
<td>2.375 ± 0.112</td>
<td>2.320 ± 0.205</td>
<td>2.448 ± 0.153</td>
<td>2.353 ± 0.223</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed as maximal tetanic force ($P_0$) in kilograms per square centimeter. Significant difference between treatments is found at $P < 0.01$ using 2-way ANOVA. There are no significant differences between times of salbutamol administration.
salbutamol concentration and stimulation frequency as factors showed significant effects of salbutamol concentration ($P < 0.001$). No interaction was found between stimulation frequency and concentration, suggesting that force generation was influenced by salbutamol in a similar way at all stimulation frequencies studied.

Comparison between protocols A and B. Pearson’s correlation coefficients between salbutamol concentration and contractile properties ($P_{i}$, $P_{0}$, and force generation at 25 Hz) are summarized in Table 4. Significant correlations for all these parameters were found in protocol B and at 0.5 h after injection in protocol A. In the overall analysis in protocol A, only $P_{i}$ showed a significant correlation with salbutamol concentration. At 1 and 2 h after injection, the correlation coefficients were reduced because the number of samples in which salbutamol could be detected was reduced.

In Figs. 4 and 5, the effects of specific ranges of salbutamol are compared between protocols A and B. These ranges were set at the lower limit of detection (2 pg/L) and the upper limit of the therapeutical concentration after oral administration (20 pg/L). In the saline-treated animals, no significant differences in $P_{i}$ and $P_{0}$ between both protocols were observed. In the bundles exposed to salbutamol with concentrations <2 pg/L, $P_{i}$ and $P_{0}$ were significantly higher in the in vivo experiment (protocol A). Increases in force generation were similar at salbutamol concentrations of 2–20 pg/L, but at concentrations >20 pg/L group $P_{i}$ and $P_{0}$ were again significantly higher in the in vivo experiment (protocol A).

DISCUSSION

The present study shows that salbutamol improves in vitro force generation in rat diaphragm bundles. Increases in force generation were most prominent within 2 h after subcutaneous administration and were dose dependent. Increases in $P_{i}$ and $P_{i}/P_{0}$ were found at clinically relevant salbutamol concentrations. Comparable results were found both after subcutaneous injection and in a strictly in vitro experiment, although increases in force generation were highest after subcutaneous administration at salbutamol concentrations <2 pg/L and >20 pg/L.

Based on body weight and systemic availability, a dose of ~25 μg/kg sc used in protocol A is comparable to a single oral dose of 4 mg for a person weighing 70 kg (57 pg/kg), since absolute systemic availability in humans is ~50% after oral administration due to first pass elimination in the intestinal wall (24). This dose resulted in a plasma concentration of ~10–20 pg/L in

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$P_{i}$, kg/cm²</th>
<th>CT, ms</th>
<th>$P_{0}$, kg/cm²</th>
<th>$P_{i}/P_{0}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>0.424 ± 0.027</td>
<td>25.6 ± 1.1</td>
<td>1.568 ± 0.047</td>
<td>0.270 ± 0.011</td>
</tr>
<tr>
<td>Salbutamol, pg/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤2</td>
<td>0.400 ± 0.028</td>
<td>28.8 ± 1.0*</td>
<td>1.491 ± 0.110</td>
<td>0.271 ± 0.009</td>
</tr>
<tr>
<td>10</td>
<td>0.538 ± 0.032†</td>
<td>28.6 ± 0.8*</td>
<td>1.897 ± 0.102†</td>
<td>0.385 ± 0.011</td>
</tr>
<tr>
<td>20</td>
<td>0.534 ± 0.059†</td>
<td>29.3 ± 0.5*</td>
<td>1.966 ± 0.172†</td>
<td>0.271 ± 0.012</td>
</tr>
<tr>
<td>80</td>
<td>0.611 ± 0.026†</td>
<td>28.9 ± 1.2*</td>
<td>2.096 ± 0.098†</td>
<td>0.393 ± 0.009</td>
</tr>
</tbody>
</table>

Values are means ± SE. CT, contraction time. Significantly different ($P < 0.05$) using 1-way ANOVA compared with: *saline; †≤2 pg/L salbutamol.
Table 4. Pearson’s correlation coefficients between salbutamol concentrations and contractile properties in protocols A and B

<table>
<thead>
<tr>
<th></th>
<th>Ft</th>
<th>F0</th>
<th>FF 25 Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>r</td>
<td>P</td>
</tr>
<tr>
<td>Protocol B (in vitro)</td>
<td>25</td>
<td>0.60</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Protocol A (in vivo)</td>
<td>100</td>
<td>0.41</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>0.5 h</td>
<td>25</td>
<td>0.68</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1 h</td>
<td>25</td>
<td>0.36</td>
<td>NS</td>
</tr>
<tr>
<td>2 h</td>
<td>25</td>
<td>0.38</td>
<td>NS</td>
</tr>
</tbody>
</table>

n = no. of rats. FF, force frequency; NS, not significant.

Fig. 4. Relationship between salbutamol concentration range and twitch force (Pt). Values are means ± SE. Open bars, values from protocol B (strictly in vitro experiment); solid bars, values from protocol A (subcutaneous salbutamol administration). **Significantly different at P < 0.01 using Student’s t-test.

Fig. 5. Relationship between salbutamol concentration range and maximal tetanic force (F0). Values are means ± SE. Open bars, values from protocol B; solid bars, values from protocol A. Significantly different using Student’s t-test at: *P < 0.05; **P < 0.01.

activation in the rat diaphragm coincides with the maximal concentration reached in this muscle, which explains the time pattern found in F0/P0.

The forces obtained in the saline bundles in protocol B were slightly lower than those obtained in protocol A, although these differences were not statistically significant (see Figs. 4 and 5). This may raise concerns about the stability of the diaphragm strips during the data collection. When we examined the data in protocols A and B and expressed the force produced in the final 160-Hz stimulation in the force-frequency protocol as a percentage of initial F0, the saline-treated rats reached levels of 100–103% of F0. In the salbutamol-treated animals, these percentages were slightly reduced, e.g., in the 100 µg/kg group these values were 85–102% and in protocol B they were 91–103%. This indicates that at the end of all measurements the diaphragm strip force production was approximately equal to F0 values measured at the start, reensuring the stability of the diaphragm strips. Furthermore, the diaphragm strip dimensions in both protocols were within the critical radius for O2 diffusion into skeletal muscles at 37°C (~0.6 mm) (31). Therefore, we assume that hypoxia of the diaphragm strips did not influence force generation.

No significant differences in Pt and F0 between the two protocols were found at salbutamol concentrations of 2–20 µg/L. However, at concentrations >20 µg/L Pt and F0 were significantly higher in the in vivo experiment (protocol A). Also, when we selected the data only on salbutamol zero concentrations in the salbutamol-treated animals, Pt and F0 were both significantly higher in the in vivo experiment. These data, however, include the serum samples of rats that had salbutamol concentrations below the limit of detection (0–2 µg/L), which may have influenced the results. It could also indicate that the effect of salbutamol on rat diaphragm contractile properties is not solely a direct β2-adrenergic effect on the muscle but is also mediated by indirect effects. Subcutaneous administration of salbu-
tamol may stimulate cardiac β₂-adrenoceptors, which may increase heart rate (26). In addition, activation of β₂-adrenoceptors in skeletal muscle resistance arterioles may dilate diaphragm vessels (20). These effects may lead to an increased diaphragm blood flow, as was reported in rats after subcutaneous administration of the β₂-adrenoceptor agonist clenbuterol (30). These alterations may explain the additional effects found in vivo but were not specifically explored in the present study.

Data on the diffusion rate of salbutamol into diaphragm muscle strips are not available to our knowledge. In a pilot study, we found that an increase in P₀ was present after a few minutes and that the maximum effect was reached within 10 min after addition of salbutamol. The maximal effect of the β₂-adrenoceptor agonist terbutaline was also found within 10–15 min in a study investigating its in vitro effects on skeletal muscle contractile properties (5). Therefore, the thermoequilibration and diffusion period was set at 15 min in both protocols. In protocol A, there may have been some back diffusion from the diaphragm strip into the Krebs solution, since salbutamol is hydrophilic and rapidly dissociates from β₂-adrenoceptors in rat lung preparations (27). We did not try to prevent this back diffusion by adding salbutamol to the Krebs solution because data on the concentration reached in the rat diaphragm after subcutaneous administration are not available. Besides, addition of salbutamol in protocol A would interfere with our goal to study the mechanism of action.

Catecholamines exert opposing effects on fast- and slow-contracting skeletal muscles in vivo (1, 4). In cat soleus muscle, which contains predominantly type I (slow-contracting) muscle fibers, intravenously administered adrenaline depressed P₀, CT, RT₁/₂, and incomplete tetanic force but did not alter P₀. However, in tibialis anterior muscle, a type II (fast-contracting) muscle, P₀, CT, RT₁/₂, and incomplete tetanic force were increased (4). In guinea pig soleus and extensor digitorum longus muscles, similar effects were found with salbutamol and isoprenaline in vitro (1). A different response was observed in a study on the effects of terbutaline on skeletal muscles (5). This in vitro study reported an increase in P₀ both in slow and in fast skeletal muscle fibers after addition of 10⁻⁵ M terbutaline. P₀ increased in slow but not in fast muscle fibers, and RT₁/₂ was decreased in both muscle types (5). Increased force production and CT were also found in the present study. RT₁/₂ was not altered in our study, but force generation at low stimulation frequencies was increased significantly. This indicates that summation of force was enhanced. These effects could indicate an increased activation of type II muscle fibers in rat diaphragm. The diaphragm is a mixed skeletal muscle and contains ~40% type I, 30% type IIa, and 30% type IIb fibers (7). In studies investigating myosin heavy chain (MHC) distribution, the mature rat diaphragm consisted of ~30% MHC-slow, ~30% MHC-2A, ~30% MHC-2X, and ~10% MHC-2B fibers (15). In rat skeletal muscles, a strong correlation was found between β-adrenoceptor density and percentage of type I muscle fibers (22) and for β-adrenoceptor density and succinic dehydrogenase activity (37). In contrast, ¹²⁵I-labeled cyanopindolol-binding affinity was significantly higher in type IIb muscle fibers (22). In view of the results found in the present study, we speculate that β₂-agonist treatment predominantly affects type II fibers in the rat diaphragm, and thus the diaphragm could be considered as a fast skeletal muscle in this respect.

The design of the present study does not allow a direct comparison with previous animal studies (2, 11, 12, 29). In these in vivo studies in anesthetized dogs, salbutamol increased twitch Pdi only during compensated metabolic acidosis (12). In fatigued diaphragm, Pdi was not improved (11, 29). Salbutamol was applied intravenously in a bolus of 2.5–20 μg/kg (29) or as a 15-min intravenous infusion in a relatively high dose of ~0.6–2.2 μg·kg⁻¹·min⁻¹ (recommended human dose is 0.04–0.3 μg·kg⁻¹·min⁻¹ iv) (11, 12). However, terbutaline, in a dose of ~25 μg/kg iv administered in 5 min, did increase Pdi in fatigued but not in fresh canine diaphragm both after direct and phrenic nerve stimulation (2). This effect was blocked by the β₂-adrenoceptor antagonist propranolol (2). Likewise, fenoterol and broxaterol potentiated Pdi in fatigued but not in fresh canine diaphragm (8, 34). In fresh diaphragm, predominantly slow motor units are recruited for normal ventilatory maneuvers (32). In this situation, the lack of effect of β-agonist treatment is in line with our speculation that the β-agonist treatment predominantly affects type II muscle fibers. Our results might also suggest improvement of diaphragm contractility in fatigued diaphragm after β₂-adrenoceptor agonist treatment, as indeed was found for terbutaline (2) and fenoterol (34). However, salbutamol increased Pdi only during compensated metabolic acidosis (12) and not in fatigue canine diaphragm (11, 29). Why salbutamol treatment failed in this respect remains unclear.

The effects of salbutamol on diaphragm function in healthy humans are contradicting. Javaheri et al. (14) found no effects of salbutamol on maximal Pdi in fresh or fatigued diaphragm after 3 days of oral treatment (4 mg 3 times/day). Violante et al. (35) reported that intravenous administration of ~0.05 μg·kg⁻¹·min⁻¹ had no effect on Pimax and ventilatory endurance, but the duration of treatment was not mentioned (35). In contrast, Martineau et al. (23) reported a significant increase in Pimax after 14–21 days of oral treatment (8 mg 2 times/day). In studies investigating other β₂-adrenoceptor agonist effects on nonfatigued diaphragm, similar effects are reported. In patients with chronic obstructive pulmonary disease (COPD), oral terbutaline administration (2.5 mg 3 times/day for 7 days) (33) as well as terbutaline inhalation (7 days 500–1,500 μg 4 times/day) (13) did not improve Pdi (33) or Pimax. In healthy adults, a single oral dose of 7.5 or 15 mg terbutaline (n = 4) or 4 mg tulobuterol (n = 5) also did not improve Pimax (17). Finally, studies with broxaterol in COPD patients reported an increase in Pimax after intravenous administration (200 μg in 10 min) (9) but no increase in Pimax after 7 days of oral treatment.
(0.5 mg 3 times/day) (25). However, this last study did not report an increased endurance time (25). In contrast to the animal studies, all human studies used maximal voluntary contractions. Furthermore, the human diaphragm has a slightly different fiber type composition, containing 55 ± 5% type I, 21 ± 6% type IIA, and 24 ± 3% type IIB muscle fibers (19), but the total amount of oxidative fibers (type I and IIA) is approximately equal (~70% in rats and ~75% in humans) (7, 19). Further research is needed to determine the effects of salbutamol on the human diaphragm in patients with COPD and in the complex situation of respiratory muscle failure.

In conclusion, in vitro force generation of rat diaphragm bundles can be improved by the β2-adrenergic agonist salbutamol. These effects are present after low subcutaneous doses and in clinically relevant concentrations in vitro and appear to be concentration dependent. Overall, the increases in force generation were slightly higher after subcutaneous administration, suggesting that in vivo salbutamol may have additional positive inotropic actions on diaphragm contractility besides a direct β2-adrenergic effect on the muscle itself.

The authors thank Y. Brom for expert biotechnical assistance. This study was supported by a grant from Glaxo-Wellcome BV, The Netherlands. Address for reprint requests: H. F. M. Van Der Heijden, Dept. of Pulmonary Diseases, Univ. Hospital Nijmegen, PO Box 9101, 6500 HB Nijmegen, The Netherlands.

Received 4 April 1995; accepted in final form 2 April 1996.

REFERENCES


